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CYTOSINE ARABINOSIDE:

AN EVALUATION OF ITS USE IN THE TREATMENT OF ACUTE LEUKAEMIA AND NON HODGKIN'S LYMPHOMA

Being a Thesis Submitted for the Degree of

Doctor of Medicine

in the University of Cape Town

by

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University of Cape Town

To

Dave and Nita

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On a personal note, I am indebted to my parents, David and Nita Slevin, for so very many things, and to my wife Cherry and daughters Lindi and Amy from whom I took the time.

ABSTRACT

The treatment of the acute leukaemias and lymphomas has undergone a revolution over the past 20 years. In patients with acute leukaemia and extensive high grade non hodgkin's lymphoma, survival was usually measured in months and death was almost inevitable within a year of diagnosis. The use of cytotoxic chemotherapy has resulted in the achievement of complete remission in a substantial proportion of these patients with a small but ever increasing population obtaining long term survival and possibly cure.

Cytosine arabinoside has made a major contribution to the treatment of acute myeloblastic leukaemia and plays a part in the treatment of acute lymphoblastic leukaemia and non hodgkin's lymphoma, particularly in the treatment of central nervous system disease. This thesis describes a study of the use of this drug in the treatment of acute leukaemia and non hodgkin's lymphoma. The importance of the schedule of cytosine arabinoside on its anti-leukaemic effect in the treatment of acute myelogenous leukaemia is uncertain. A study of 72 patients with acute myelogenous leukaemia receiving combination chemotherapy in the Department of Medical Oncology at St. Bartholomew's Hospital has been conducted to examine the efficacy and toxicity of different schedules of cytosine arabinoside when used as part of combination chemotherapy.

An investigation of high dose cytosine arabinoside in the treatment of relapsed and resistant acute leukaemia and non hodgkin's lymphoma has been conducted in 40 patients. In view of the high incidence of central nervous system toxicity resulting from this therapy, a dose of $2g/m^2$ twice daily rather than the conventional $3g/m^2$ was used.

Alongside the clinical investigations a systematic evaluation of the clinical pharmacology of cytosine arabinoside was undertaken. The concentrations of cytosine arabinoside in plasma, cerebrospinal fluid and saliva were measured using a specific and sensitive radioimmunoassay. The effect of the dose, schedule and route of administration on the concentration versus time profile in both plasma and cerebrospinal fluid has been evaluated. In addition an attempt was made to create a delayed release formulation of cytosine arabinoside and the relationship between protein binding and extravascular drug concentrations was examined.

The studies reported in this dissertation were all designed and carried out by myself and I was personally responsible for the clinical management of the majority of patients who were studied.

These data have been presented at chemotherapy meetings both in the United Kingdom and the United States. A list of these meetings and of the publications arising from this work is provided. It is hoped that these data will be of value to the clinician using cytosine arabinoside to treat patients with haematological malignancy.

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In Press: Investigational New Drugs. The Journal of New Anticancer Agents.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW.

Introduction

Cytosine Arabinoside is an effective anti leukaemic agent, having been repeatedly demonstrated to clear blast cells from both bone marrow and cerebrospinal fluid when administered parenterally. Unlike the majority of cytotoxic agents it may be injected intravenously, intramuscularly, subcutaneously and intrathecally without local excoriation or inflammation provided thrombocytopenia is not profound. While the apparent S phase specificity of cytosine arabinoside has made continuous intravenous infusion the generally preferred schedule of administration, the clinical evidence supporting this is sometimes contradictory.

There are practical reasons for exploring alternatives to continuous intravenous infusion, provided they are clinically appropriate to the situation in hand. Continuous infusion chemotherapy requires more nursing attention and is usually, although not always, carried out in hospitals. Twice daily intravenous bolus and subcutaneous bolus injections of cytosine arabinoside have been used as compromise methods of administration. High dose infusions of cytosine arabinoside have recently been investigated and may be effective therapy in patients with relapsed acute myelogenous leukaemia, but the schedules of administration vary and remain empirical.

Intrathecal chemotherapy, both for the prevention and treatment of meningeal leukaemia is at best inconvenient and at worst

most unpleasant, and it would clearly be to the advantage of the patients were it possible to achieve prolonged cytotoxic levels of cytosine arabinoside in the cerebrospinal fluid with high dose intravenous injections which were part of systemic therapy, thereby preventing the necessity for lumbar punctures.

The following studies were undertaken to complement the established information concerning the clinical use and the pharmacokinetics of cytosine arabinoside, in the hope that the information might lead to its more rational use . In the following introduction the literature pertinent to this dissertation will be reviewed.

Clinical Evaluation of the Dose, Schedule and Anti-tumour Activity of Cytosine Arabinoside

In experimental systems the biological activity of cytosine arabinoside is markedly dependent upon the schedule of administration. There is extensive data suggesting that cytosine arabinoside kills cells only in the DNA synthetic phase (S phase) of the cell cycle (Chu et al 1962,Kim et al 1965 and Pizer et al 1960). Employing a spleen colony forming assay in mice, Bruce et al (1966) showed that cytosine arabinoside given over 24 hours caused a progressive decrease in surviving bone marrow stem cells with increasing dose, to 20% of control levels. After this a plateau was reached and further increase in dose did not result in greater stem cell

destruction, presumably because the remaining cells were non proliferating. Skipper et al (1967) investigated different schedules of cytosine arabinoside in mice with L1210 leukaemia and found that a constant infusion or 3 hourly doses over 24 hours resulted in much greater cell kill than single large doses. If this 24 hours schedule was repeated every 4 days the majority of the mice were cured.

These data led the South West Oncology Group (Frei et al 1969) to investigate the dose and schedule of cytosine arabinoside on normal bone marrow function in man.(Figure 1) Eighty eight patients with metastatic cancer were randomly allocated to 4 schedules of drug administration given every 2 weeks. With single intravenous bolus cytosine arabinoside injection virtually no bone marrow toxicity was observed at doses as high as 4,200 mg/m^2. With 24 hour continuous infusion, however, white cell and platelet depression began to appear at doses of 600 mg/m^2, and myelosuppression increased until doses of 1,500 mg/m^2. From 1,500 mg/m^2 to 3,200mg/m^2, a plateau occurred and there was no further increase in myelosuppression. The dose response curves for the 48 hour and 96 hour infusions show that myelosuppression began to appear at 450 mg/m^2, and thereafter increased in a linear fashion and was dose limiting at 800 to 1,000 mg/m^2. The lack of myelosuppression following single intravenous injection was consistent with the assumption that a brief exposure to cytosine arabinoside could only damage the minority of cells that were synthesizing DNA at that time. The plateau in

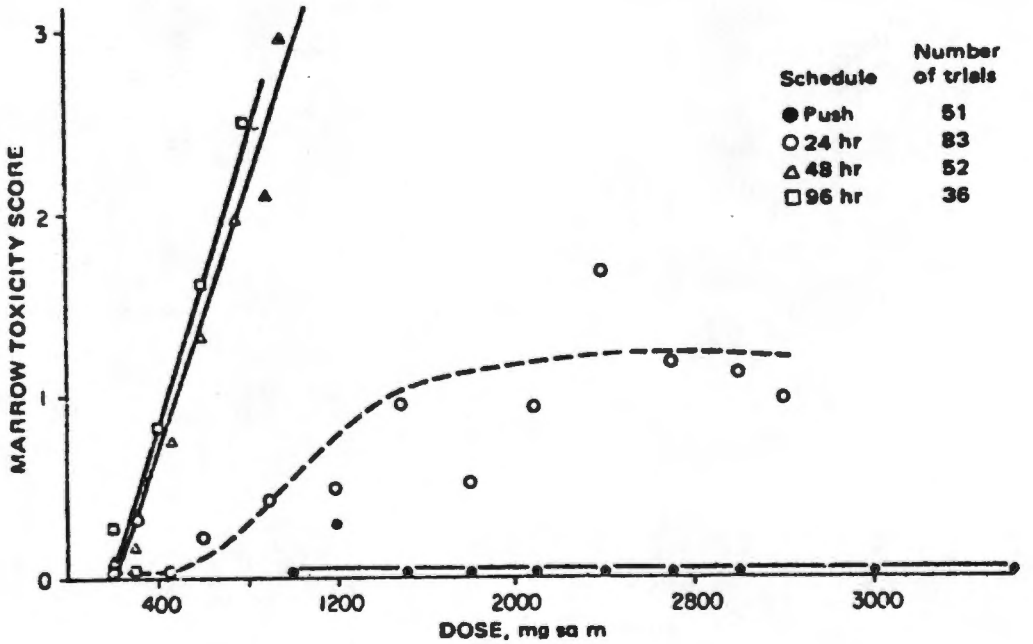


Figure 1

The effect of cytosine arabinoside schedule on bone marrow toxicity. *(Frei et al 1969)*

myelosuppression following a 24 hour infusion was similar to that seen with animal studies (Bruce et al 1966). The most likely explanation for this is that a proportion of the marrow stem cells are in a non-proliferating state, and are therefore not damaged by 24 hour exposure to cytosine arabinoside , and can re-enter the proliferating pool. On the other hand the steep and linear dose response for the 48 and 96 hour schedules, without a plateau, can be attributed to cells moving from the non-proliferating to the proliferating pool during this time period, thus becoming susceptible to cytosine arabinoside.

Talley et al (1967) treated 89 patients, most of whom had metastatic solid tumours, with cytosine arabinoside given either as a daily intravenous bolus for 3 to 5 days or a single large intravenous bolus (30 to 50 mg/kg) every 7 to 10 days. Thirty one patients received the daily intravenous regimen and in 20 patients a white blood count less than 3 x 10^9/l and a platelet count less than 100 x10^9/l was seen. In contrast, of the 38 patients who received large boluses of cytosine arabinoside every 7 to 10 days, in only 6 was the white count less than 3 x 10^9/l and in 10 the platelet count less than 100 x10^9/l. In the majority of these patients there was very little suppression of the white count or platelet count, and in no patient was the white count less than 1 x10^9/l. The ability to give several grams of cytosine arabinoside by intravenous bolus every week, with very little

myelosuppression, is of particular interest in relation to the recent studies of similar doses of cytosine arabinoside given twice a day for 4 to 6 days, which resulted in severe and prolonged myelosuppression lasting several weeks (Rudnik et al 1979 and Karanes et al 1979).

In a study designed to compare the bone marrow toxicity of continuous intravenous infusion and single daily injections of cytosine arabinoside given over a 5 day period, Burke et al (1968) showed that with a daily dose of 100 mg/m^2 continuous infusions produced far greater bone marrow depression than single daily injections. However, at doses of 150 mg/m^2 the same degree of myelosuppression was obtained with both the daily intravenous bolus and the continuous infusion schedules, and further dose increase did not intensify the degree of myelosuppression. Unfortunately, this important and much quoted study suffers from the severe limitation that very few comparisons were done between the intravenous bolus and infusion above 100 mg/m^2. Only 1 patient was treated with the infusion schedule at 150 and 250 mg/m^2 and 3 patients received the infusion schedule at 300 mg/m^2. This plateau in the degree of myelosuppression in patients receiving continuous infusions, is in sharp contrast to the data from Frei et al (1969) where a total of 88 trials were done in patients receiving continuous infusions of cytosine arabinoside for greater than 48 hours.

The Effect of Dose and Schedule of Cytosine Arabinoside when used as a Single Agent in the Treatment of Acute Leukaemia.

The Acute Leukaemia Group B (Ellison et al 1968) conducted 2 studies comparing different methods of cytosine arabinoside administration. In the first study adults with acute leukaemia were randomised to receive between 10 mg/m^2 per day and 30 mg/m^2 per day to be given as a 24 hour infusion until remission or bone marrow hypoplasia resulted. Once the patients entered remission they were randomised to receive no maintenance therapy or cytosine arabinoside 30 mg/m^2 every week by subcutaneous injection. A total of 98 evaluable patients with acute myeloblastic leukaemia were randomised to the two induction regimens and 16 out of 98 (16%) achieved complete remission. The results of this study are difficult to assess because the low dose arm was discontinued prematurely and half the patients received the infusion over 12 hours. However three times as many days of infusion were necessary at the lower dose to produce an effect equal to the higher dose. Those patients receiving maintenance therapy (13 patients) had a median duration of remission of 30 weeks compared with 5 weeks median remission duration in the unmaintained group (10 patients).

In the next study patients were randomised between a dose of 30 mg/m^2 per day given over 1 hour or 4 hours. When it was found that the 1 hour infusion delayed the appearance of marrow hypoplasia the randomisation was changed to three

groups receiving either 50 or 100 mg/m^2 per day over 1 hour or 30 mg/m^2 per day over 4 hours. The complete remission rate in the four groups ranged from 11% to 30% but the small numbers of patients in each group do not permit any comment to be made as to which is the optimal schedule of administration. Once in remission the patients were further randomised to two different schedules of cytosine arabinoside maintenance: twice weekly subcutaneous cytosine arabinoside (13 patients); or intensive intravenous treatment 5 days per month (10 patients). The median duration of remission was 11.5 weeks for the twice weekly administration group and 14 weeks for the 5 days per month group.

These two studies confirmed that cytosine arabinoside was an active drug in acute leukaemia, but because of several randomisations, the small number of patients in each group, and the premature discontinuation of some of the randomised arms, the study failed to achieve its major objective which was to determine the most effective schedule of administration of cytosine arabinoside.

More useful information can be obtained from a study with a similar objective carried out by the South-West Oncology Group (1974). Two hundred and forty seven adults, of which 185 were assessable, were randomised to receive cytosine arabinoside by one of two schedules: 800 mg/m^2 as an intravenous infusion over 48 hours or 1000 mg/m^2 as an intravenous infusion over 120 hours repeated every 2 weeks. The 120 hour schedule was

substantially more effective in inducing complete remission (38%) than the 48 hour schedule (20%) ($P < 0.025$). The median duration of remission was the same in both arms. However, 26% of the patients receiving the 120 hour schedule were alive at 1 year, compared with 17% of the patients receiving the 48 hour schedule.The 120 hour schedule consistently cleared the peripheral blood of blasts, whereas blasts frequently persisted in the 48 hour schedule.

Bodey et al (1969) treated 44 adults with acute myeloblastic leukaemia with a 5 day course of cytosine arabinoside at a dose of 200 mg/m^2 per day by continuous intravenous infusion. Nine out of thirty six (25%) of the evaluable patients achieved a complete remission.Of the patients who completed 3 courses of treatment 82% achieved a complete remission.This was compared with a previous study in which 8 patients had been treated at a dose of 150 to 450 mg/m^2 per day for 5 days by intravenous bolus. Only 1 of the 5 assessable patients achieved a complete remission. The authors concluded that continuous infusion of cytosine arabinoside was superior to rapid intravenous injection in patients with acute leukaemia. Once again the number of patients treated by the intravenous bolus schedule is insufficient to come to this conclusion.

The Childrens' Cancer Study Group A (Howard et al 1968) treated 57 children of whom 43 were assessable with cytosine arabinoside given as a daily intravenous bolus injection at a dose of 3 mg/kg per day for 10 days. Eleven out of 43 (26%)

achieved complete remission. In contrast infusions of cytosine arabinoside were given to 46 children with acute leukaemia (34 with acute lymphocytic leukaemia and 12 with acute myelocytic leukaemia) by Wang et al (1970a). They received 150 to 200 mg/m^2 per day for 5 days repeated every 2 weeks. Only 3 out of the 32 assessable patients achieved complete remission. One of the complete remitters was a patient with acute lymphatic leukaemia and 2 had acute myelogenous leukaemia. In view of the low complete remission rate obtained in the latter study, and the apparently superior results reported by Howard et al (1968) using daily intravenous bolus cytosine arabinoside, the authors (Wang et al 1970a) concluded that continuous intravenous infusions of cytosine arabinoside should be abandoned in the treatment of childhood acute leukaemia. In both the above studies cytosine arabinoside was given to children with acute leukaemia who were refractory to conventional chemotherapy, including prednisolone, vincristine, 6 mercaptopurine, methotrexate, cyclophosphamide and daunomycin. It is thus possible that better results could be obtained if the patients were treated with cytosine arabinoside as first line therapy.

Armentrout et al (1974) reported 31 patients with acute myeloblastic leukaemia treated with daily infusions of cytosine arabinoside for 8 hours per day at a dose of 4 mg/kg with a mean duration of 7 treatment days. Eleven out of 31 (30%) of all patients and 11 out of 24(46%) of the assessable patients achieved complete remission. The mean duration of remission was 5 months, and 4 patients remained in complete remission for more than 30 months after completing therapy.

Similarly Henderson et al (1968) treated 34 patients with 4 hour cytosine arabinoside infusions per day for 4 days. Eleven out of 25 adults and 7 out of 9 children achieved complete remission.

Recently massive doses of cytosine arabinoside have been used in patients with refractory acute leukaemia in an attempt to overcome resistance to this drug (Rudnik et al 1979 and Karanes et al 1979). The rationale for this therapy was based on the assumption that resistance to cytosine arabinoside may be mediated by decreased production or increased destruction of cytosine arabinoside triphosphate (araCTP). These mechanisms of resistance could potentially be overcome by increasing the amount of cytosine arabinoside available to the myeloblasts .

Momparler(1974) treated a single adult patient with acute leukaemia with five 1.7 g/m^2 doses of cytosine arabinoside over 36 hours, and obtained a two log reduction in bone marrow and peripheral blasts. Rudnick et al (1979) recently reported a phase 1 study in which 13 adults with acute leukaemia, who had failed conventional therapy, were treated with cytosine arabinoside at a dose of 3 to 7.5 g/m^2. Six patients had either one or two doses at weekly intervals and no response was obtained in this group. The next 7 patients were treated with three or four doses of 3 g/m^2 at 12 hour intervals. In this group there was one complete response and 2 partial responses. The most important aspect of this paper was the

finding that patients could tolerate cytosine arabinoside given in these doses .

Karanes et al(1979) reported 21 patients with acute myeloblastic leukaemia, who had relapsed and were refractory to conventional doses of cytosine arabinoside . They were treated with cytosine arabinoside at a dose of 3 g/m^2 every 12 hours as a 2 hour infusion for 4, 8 or 12 doses. The response to the high dose cytosine arabinoside was related to the number of doses. Neither of the 2 patients treated with 4 doses had a complete or partial response, whereas 3 out of 10 patients treated with 8 doses achieved complete response as did 6 out of 9 patients treated with 12 doses.

Similar results have been reported by Herzig et al (1982) and Early et al (1982 a, b) who obtained 10 out of 19 complete responders and 7 out of 10 complete responders respectively in patients with acute myelogenous leukaemia refractory to conventional dose cytosine arabinoside therapy. In both studies $3g/m^2$ cytosine arabinoside for 12 doses (6 days) was administered. Significant toxicity was found with cerebellar ataxia occurring in 22% of patients reported by Herzig et al (1982) and 13% of those reported by Early et al (1982a). Conjunctivitis and photophobia were also frequent complications but responded to local and systemic steroids.

The majority of the studies reviewed suggest that cytosine arabinoside is both more effective and toxic when given as

prolonged intravenous infusion rather than repeated intravenous bolus injection, however, the evidence for this is sometimes contradictory. Problems of study design often make assessment of the data difficult and the different patient populations in the reported studies preclude direct comparison between studies conducted at different institutions. Thus despite much effort by many investigators the most effective schedule of cytosine arabinoside remains controversial.

The Effect of the Schedule of Cytosine Arabinoside Administration when used as Part of Combination Chemotherapy to Treat Patients with Acute Myeloblastic Leukaemia.

The advent of combination chemotherapy has resulted in cytosine arabinoside being administered in combination with other cytotoxic drugs such as anthracycline antibiotics (daunorubicin and doxorubicin), 6 thioguanine and vincristine (Rees et al 1977, Gale et al 1977 and Bell et al 1982). The effect of different cytosine arabinoside schedules may be "masked" by these drugs and very few trials have been carried out to explore this question.

Preisler et al (1979) compared 41 patients with acute myeloblastic leukaemia treated with an intravenous infusion of 100 mg/m^2 of cytosine arabinoside per day for 7 days and doxorubicin ($25mg/m^2$) for 3 days with 36 patients treated with

a 10 day infusion of cytosine arabinoside and 3 days of doxorubicin at the same dose. The complete response rate with the 10 day cytosine arabinoside infusion (72%) was similar to the 7 day cytosine arabinoside infusion (67%). However, 94% of those patients achieving complete remission with the 10 day infusion did so after one course of treatment compared with 72% of those receiving the 7 day infusion ($P < 0.05$). This was achieved without any increase in toxicity or time to bone marrow recovery. The failure to achieve an increase in complete response rate with the 10 day infusion is not surprising as the majority of deaths were related to infection and bleeding, rather than resistant leukaemia. The shorter time to complete remission with the 10 day infusion was regarded as an index of the greater anti-leukaemic effect of this schedule of administration.

A recent study from the Cancer and Leukaemia Study Group B (Rai et al 1979, 1981a and b) is the only prospectively randomised trial designed to investigate the importance of the schedule of cytosine arabinoside administration when used in combination chemotherapy. Three hundred and fifty two patients were randomised to receive one of four schedules of cytosine arabinoside (Figure 2). They received 7 days of cytosine arabinoside either by continuous intravenous infusion or by intravenous bolus injection every 12 hours, or 5 days of cytosine arabinoside by continuous infusion or bolus intravenous injection every 12 hours. The patients receiving the 7 day infusion also received daunorubicin 45 mg/m^2 per day

CALGB STUDY
(RAI et al 1981)

REMISSION INDUCTION THERAPY FOR ACUTE MYELOGENOUS LEUKAEMIA

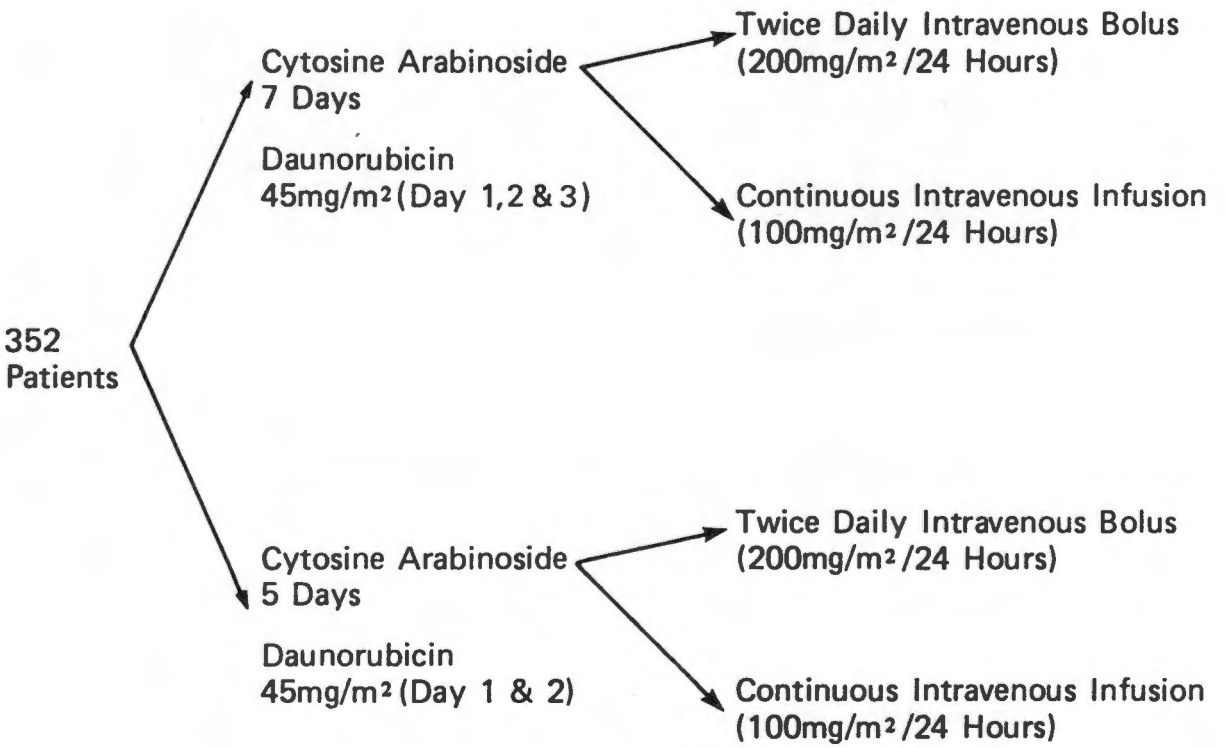


Figure 2

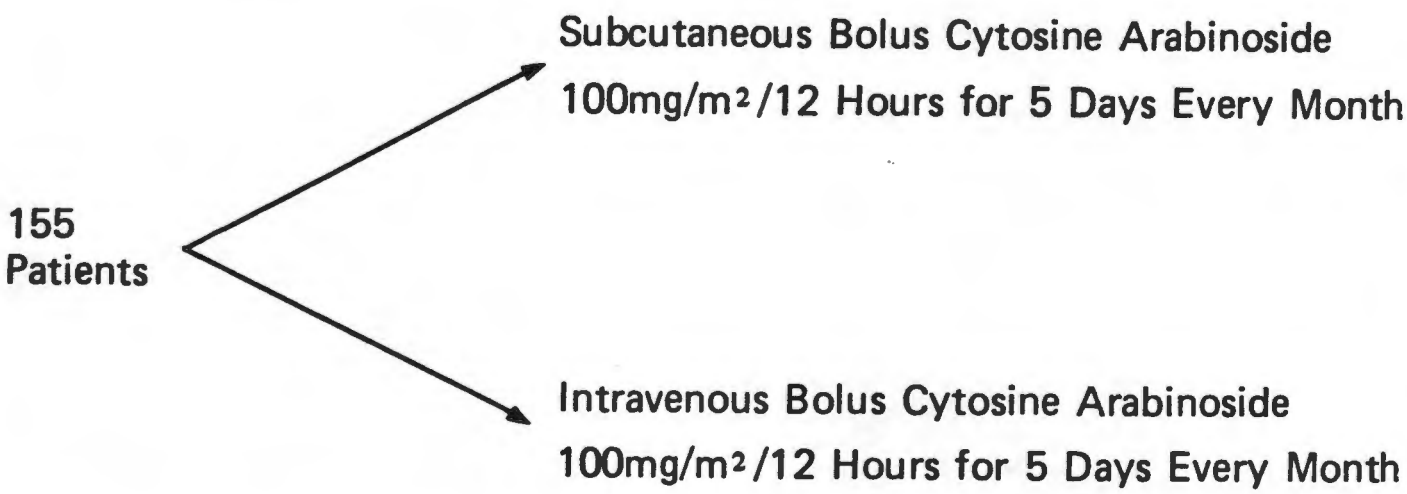
for 3 days and with the 5 day infusion they received daunorubicin 45 mg/m^2 per day for 2 days. One hundred and fifty five patients who achieved complete remission were randomly allocated to maintenance therapy with cytosine arabinoside (100 mg/m^2 twice daily) for 5 days each month, given either as an intravenous or subcutaneous bolus (Figure 3). They also received one of four drugs each month added on a cyclical rotational basis (6 thioguanine, cyclophosphamide, lomustine or daunorubicin).

Fifty five percent of patients receiving 7 day cytosine arabinoside infusions achieved complete remission, compared to 40% of those who received bolus injections over 7 days. Thirty nine percent of those receiving 5 day infusions of cytosine arabinoside and 29% receiving 5 days of cytosine arabinoside by intravenous bolus injection achieved complete remission. The 7 day regimen was significantly better than the 5 day regimen ($P < 0.01$). It was also of interest that 71% of the patients receiving the 7 day regimen achieved complete remission after one course, compared with 36% of those receiving a 5 day schedule ($P < 0.001$). The method of administration (bolus or intravenous infusion) did not influence the time to remission. Complications of therapy (haemorrhage and infection) were similar in the four regimens.

Of particular importance is the duration of response and survival of the patients. Those patients who were randomised to receive maintenance cytosine arabinoside by subcutaneous

CALGB STUDY
(RAI et al 1981)

MAINTENANCE THERAPY FOR COMPLETE REMITTERS



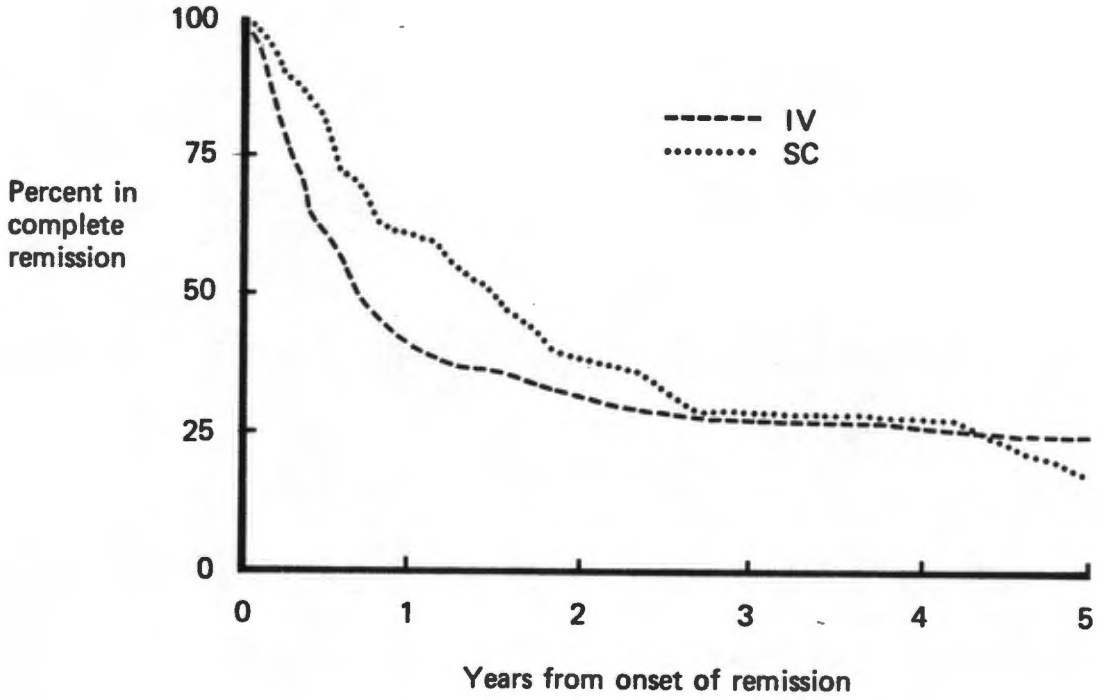
All Patients Also Received 6 Thioguanine, Cyclophosphamide, CCNU, and Daunorubicin.

Figure 3

bolus had a median duration of remission of 18 months versus 8 months for the intravenous regimen(Figure 4). In patients over the age of 60 the median duration of remission for subcutaneous bolus maintenance was 31 months versus 9 months for the intravenous bolus maintenance. The duration of remission for those patients who had intravenous infusion cytosine arabinoside for induction therapy and subcutaneous bolus for maintenance therapy was significantly longer than any of the other three groups ($P < 0.01$), and the survival data paralleled the remission data (Figure 5).Those patients who had intravenous infusion cytosine arabinoside for remission induction and subcutaneous bolus for maintenance therapy had, however, more haematological toxicity during maintenance therapy, and many could not tolerate the full dose treatment. The results of this very important study show that even when cytosine arabinoside is used in combination with other active drugs, the schedule of cytosine arabinoside administration in both induction and maintenance therapy can substantially influence the percentage of patients entering complete remission and the duration of remission and survival of these patients.

The Treatment of Central Nervous System Leukaemia with Cytosine Arabinoside.

Acute leukaemia involves the meninges in up to 25% of patients and the central nervous system was the major site of relapse in patients with acute lymphoblastic leukaemia who had

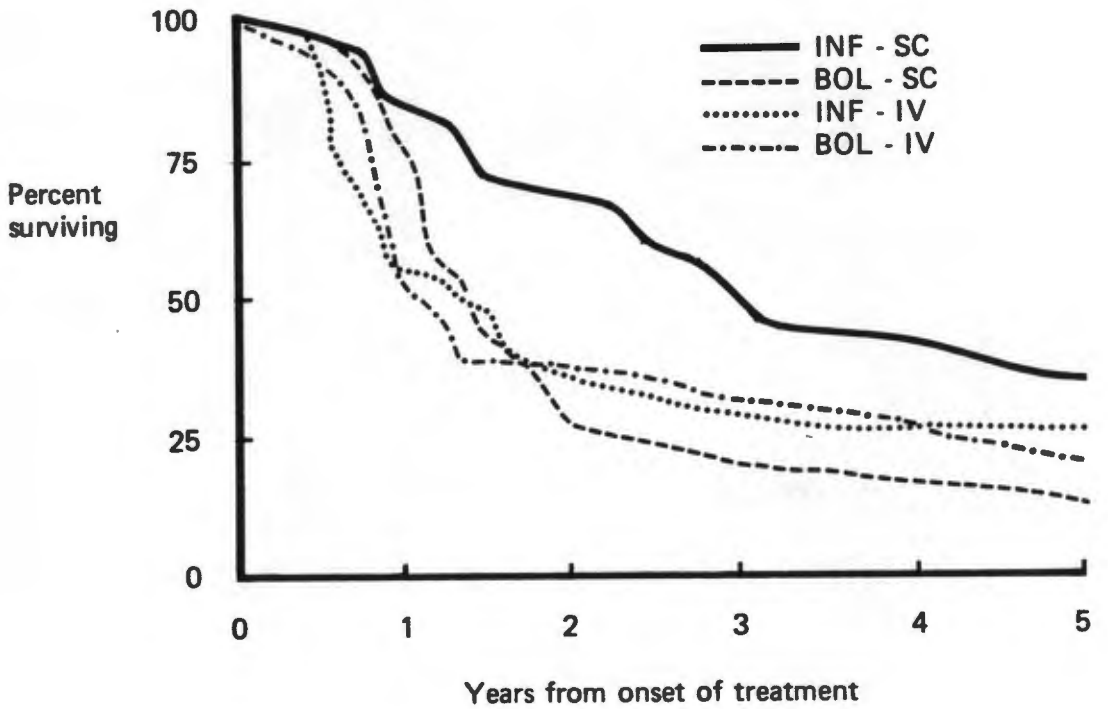


Cancer and Leukemia Group B AML study

Duration of remission according to method of administration of maintenance Cytosine Arabinoside

Figure 4

(Rai et al 1981)



Cancer and Leukemia Group B AML study

Survival according to method of Cytosine Arabinoside administration

Figure 5

(Rai et al 1981 a & b)

INF – SC : Cytosine arabinoside infusion for induction and subcutaneous bolus maintenance

BOL– SC : Cytosine arabinoside intravenous bolus for induction and subcutaneous bolus maintenance

INF – IV : Cytosine arabinoside infusion for induction and intravenous bolus maintenance

BOL– IV : Cytosine arabinoside intravenous bolus for induction and intravenous bolus maintenance

achieved complete remission but had not received central nervous system prophylaxis (Simone et al 1979 and Pinkel 1971). Intrathecal aminopterin (Nies et al 1965), and methotrexate (Sullivan et al 1969 and Hyman et al 1965) were shown to have activity in central nervous system leukaemia in the 1960's. Responses to this therapy were generally of short duration.

The activity of cytosine arabinoside in inducing remission in patients with acute myeloblastic and lymphoblastic leukaemia led to its use as intrathecal chemotherapy for central nervous system leukaemia. There are only two papers describing the use of intrathecal cytosine arabinoside as a single agent in the treatment of central nervous system leukaemia (Wang et al 1970b and Band et al 1973). Most patients were children with acute lymphoblastic leukaemia and a minority were adults with acute myeloblastic leukaemia. Doses of 4 to 75 mg/m^2 were administered intrathecally and were repeated once or twice per week. This therapy resulted in a dramatic decrease in the cerebrospinal fluid blasts in most patients but cleared the cerebrospinal fluid of leukaemia in a minority. The duration of these responses was short lived. In neither study could a dose response to cytosine arabinoside be demonstrated. Most of these patients had been previously treated with intrathecal aminopterin, methotrexate and radiotherapy and had relapsed or were resistant to this therapy. The treatment was generally well tolerated but meningeal irritation, thought to be attributable to the buffered diluent, was noted in a few children. In those patients who were not receiving concurrent

systemic chemotherapy no haematological toxicity was encountered.

Although these studies involved only 20 patients, most of whom had been previously treated, they demonstrated definite therapeutic activity against meningeal leukaemia and resulted in the incorporation of cytosine arabinoside together with methotrexate and cranial irradiation into the prophylaxis and treatment of central nervous system leukaemia (Simone et al 1979).

The Pharmacology of Cytosine Arabinoside in Man

Methods of Assay

The clinical interest in cytosine arabinoside has resulted in many attempts to produce sensitive and specific methods for measuring its concentrations in body fluids. Several of the early assays for cytosine arabinoside were performed using tritiated drug. Cytosine arabinoside was separated from its inactive metabolite uracil arabinoside by chromatography and the level of radioactivity determined. Some investigators (Finklestein et al 1970) had difficulty separating plasma cytosine arabinoside from uracil arabinoside by paper chromatography and therefore measured only total radioactivity. This makes interpretation of the data difficult as over 90% of radioactivity is made up of the inactive metabolite. Ho et al(1971) were however able to separate tritiated cytosine arabinoside from uracil arabinoside and could measure drug and metabolite for 3 hours after intravenous bolus administration to patients. Another approach is the use of high performance liquid chromatography (HPLC). Wan et al (1974) compared HPLC with tritiated drug separated by paper chromatography and found good correlation between the two methods. HPLC was however faster and more convenient and had the advantage that no tritiated drug need be given to the patient.

Cytosine arabinoside can also be detected using biological assays such as those developed by Pitillo et al (1967) and Hanka et al (1970), using a strain of Streptococcus faecalis. These early assays were not sensitive enough for studies of plasma concentrations of cytosine arabinoside in man and could theoretically be interfered with by antibiotics which are commonly administered to patients with acute leukaemia. Baguley et al (1971) reported an assay using mouse L-cells or mouse spleen cells and measured inhibition of incorporation of tritiated thymidine into DNA. This assay was sensitive to 40 ng/ml. They later utilised L1210 leukaemia cells and improved the sensitivity of the assay still further. More recently a bio-assay that was based on inhibition of tritiated thymidine incorporation into DNA of rat bone marrow cells, and was sensitive to cytosine arabinoside concentrations as low as 3 ng/ml,was reported by van Prooijen et al(1977a).

Some of the problems associated with bio-assays are illustrated by Harris et al (1979) using a similar bio-assay to that described by Baguley et al (1971). Inhibition of tritiated thymidine incorporation into transformed human lymphocytes was measured during incubation with cytosine arabinoside. This assay was compared with gas chromatography and gas chromatography/mass spectrometry and plasma concentrations of cytosine arabinoside measured by bio-assay showed poor correlation with the other two methods. In some instances plasma taken after cytosine arabinoside injection or during

cytosine arabinoside infusion stimulated the uptake of tritiated thymidine rather than having the opposite effect. It was thought that this might be due to the release of nucleotides from leukaemic blasts after exposure to cytosine arabinoside. Two hours after intravenous bolus cytosine arabinoside a plateau was found with the bio-assay but not with gas chromatography/mass spectrometry.

A specific and sensitive radioimmunoassay for cytosine arabinoside has recently been described by Okabayashi et al (1977). An antibody was raised in rabbits using cytosine arabinoside conjugated to human serum albumin and injected together with Freunds' adjuvant. There was very little cross reaction with uracil arabinoside, other nucleotides, antibiotics and other commonly used cytotoxic drugs, and the assay was sensitive to 20 ng/ml. Increasing the pH at which the radioimmunoassay was performed resulted in the antibody recognising uracil arabinoside and not cytosine arabinoside, and thus by performing the assay at two different pH's, both cytosine arabinoside and uracil arabinoside could be measured simultaneously. A disadvantage of this procedure was that it was necessary to extract the cytosine arabinoside into ethanol and the separation of the free from antibody bound cytosine arabinoside required the use of micropore filters.A radioimmunoassay which is sensitive to 1ng/ml and can be completed in 3 hours has been reported by Piall et al(1979).This assay does not cross-react with uracil arabinoside or with drugs commonly coadministered with cytosine

arabinoside. It has the advantage that no extraction procedure is required and separation of bound from free cytosine arabinoside is rapidly performed with dextran coated charcoal.

Clinical Pharmacology of Cytosine Arabinoside in Man.

Oral Administration

Ho et al (1971) showed that cytosine arabinoside was slowly absorbed from the gastro-intestinal tract reaching a plateau after 6 hours. Only 14% of the administered radioactivity appeared in the urine and less than 3% was cytosine arabinoside. Poor absorption has also been demonstrated by Finklestein et al (1970) and is consistent with the evidence from animal experiments that the oral dose required to exert an equivalent effect is 3 to 10 fold greater than the parenteral dose.(Goldenberg et al 1968).

Intravenous Bolus Administration

Studies using tritiated cytosine arabinoside showed that following an intravenous bolus total plasma radioactivity fell rapidly with a half life of 30 to 60 minutes (Creasey 1966). Ho et al (1971) using tritiated cytosine arabinoside and paper chromotography separation showed that all radioactivity could be accounted for by cytosine arabinoside and uracil arabinoside

and that when plotted on a semilog scale the disappearance of both cytosine arabinoside and uracil arabinoside was biphasic. There was a rapid initial phase (mean half life 12 minutes) and a slower second phase (mean half life 111 minutes). The half life of cytosine arabinoside was independent of the dose administered. Two patients were studied during the first and sixteenth dose of cytosine arabinoside and the results were similar on both occasions. Van Prooijen et al (1977 a) demonstrated no difference in the kinetics of cytosine arabinoside during the first and twentieth dose in 3 patients with acute myeloblastic leukaemia. The biphasic distribution has been confirmed by Wan et al (1974), van Prooijen et al (1977a) and Harris et al (1979).

These studies also confirmed the wide variation between patients. Harris et al (1979) found shorter initial half lives than previously described by Ho et al (1971) and a triphasic distribution in 2 patients. While van Prooijen (1977a) also found a biphasic decline in cytosine arabinoside concentrations, the mean initial and terminal plasma half lives were only 1.6 and 18.9 minutes respectively. Very frequent sampling was performed in the first 10 minutes (every 2 minutes) and samples were taken for only 60 minutes. It is likely therefore that in this study the initial phase was divided into 2 phases and that the longer terminal phase was not detected.

Constant Rate Infusions of Cytosine Arabinoside.

Plasma cytosine arabinoside concentrations were measured during constant rate infusions of different amounts of cytosine arabinoside in 3 patients by van Prooijen et al (1977 a). Concentrations were directly proportional to dose and plateau levels were reached at about 60 minutes. Harris et al (1979) measured plasma concentrations during cytosine arabinoside infusions at doses ranging from 10 to 160 mg/24 hours in 14 patients. All samples were taken after at least 48 hours of infusion and should therefore represent steady state concentrations. However, in any one patient there was up to a twofold variation in plasma concentrations during the same infusion. When infusion doses were doubled the plasma concentrations doubled in some cases but in others there was a less than 2 fold increase in plasma cytosine arabinoside. These authors suggested that this wide within-patient variation during infusions could be explained by changes in patient posture resulting in alterations of hepatic blood flow and have calculated the hepatic extraction ratio for cytosine arabinoside at 0.76. Wilkinson et al(1975) have demonstrated that hepatic clearance of drugs with high hepatic extraction ratios is related to hepatic blood flow.This may be markedly dependent on posture (Culbertson et al 1951), and may double on lying down.

In an attempt to achieve a plateau rapidly Wan et al(1974) gave a loading dose of 10mg/m^2 followed by an infusion of 40 mg/m^2 over 4 hours. Steady state levels were not obtained until 3 to 4 hours after the initiation of the infusion, and it was felt that the loading dose was inadequate. It is suggested that a loading dose of three times the hourly rate of infusion is needed to achieve rapid steady state concentrations. A loading dose of this order was used by Ho et al (1971) and a plateau was achieved almost immediately. There was good correlation between the dose of cytosine arabinoside administered and the plasma concentrations obtained.

Subcutaneous and Intramuscular Administration of Cytosine Arabinoside.

Only one study investigating subcutaneous and intramuscular administration of cytosine arabinoside has been published (Finklestein et al 1970). Tritiated cytosine arabinoside was used and the levels of radioactivity were not sufficient to permit separation of cytosine arabinoside from the inactive metabolite uracil arabinoside. Over 90% of the measured radioactivity represents uracil arabinoside and the kinetics described are therefore more representative of the elimination of uracil arabinoside rather than cytosine arabinoside. This is particularly important as the data from Ho et al (1971) show that uracil arabinoside declined with a half life which is much longer than that of cytosine arabinoside (Figure 6).

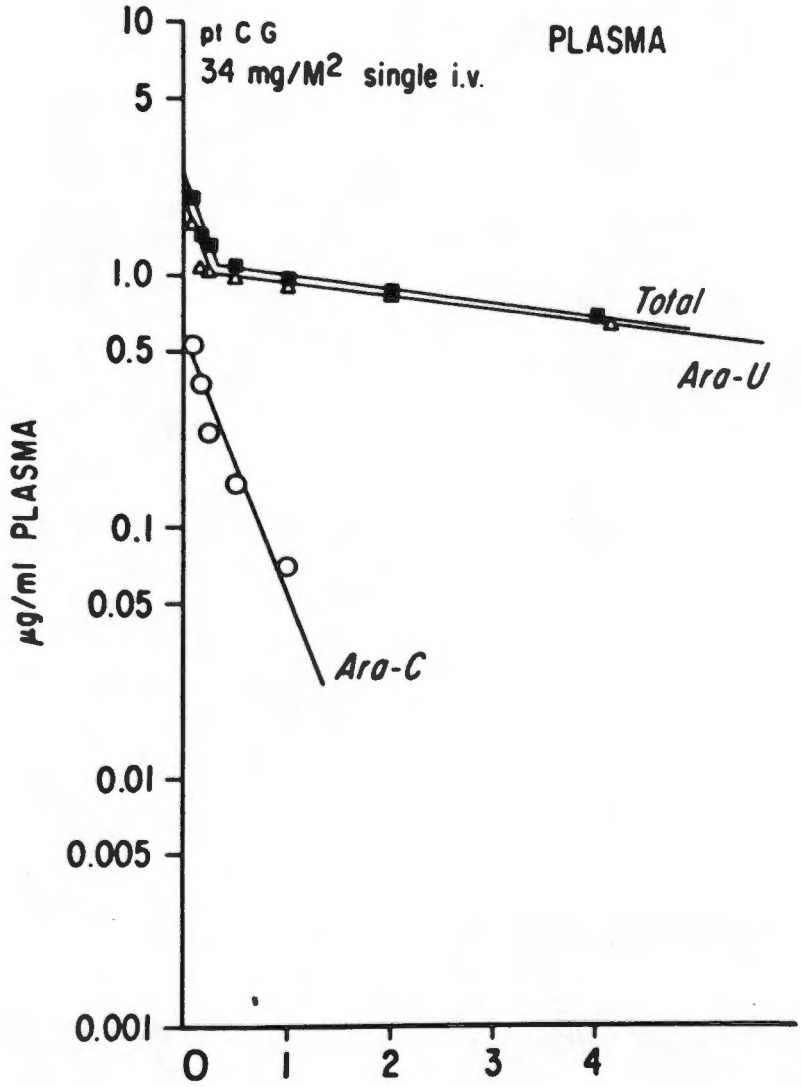


Figure 6

Pharmacokinetics of cytosine arabinoside and uracil arabinoside after intravenous bolus injection.
(Ho et al 1971)

Furthermore only 2 patients were studied by the intramuscular and subcutaneous routes and doses of cytosine arabinoside varied from 24 to 219 mg/m^2, containing variable amounts of radiolabelled drug. The data suggest that following subcutaneous injection plasma levels reached a peak at 1 hour and then declined with a half life of several hours (Figure 7). After intramuscular injection peak radioactivity was demonstrated between 25 minutes and 1 hour and declined rapidly in 1 patient but with a half life of several hours in the other. The area under the curve for the different methods of administration varied considerably despite the normalisation of the dose . These problems make the assessment of this data very difficult.

Intrathecal Administration of Cytosine Arabinoside

The intrathecal administration of cytosine arabinoside (50 mg/m^2) to a single patient with an Ommaya reservoir resulted in very large concentrations of cytosine arabinoside (500 mcg/ml) which then declined with a single exponential half life of 2 hours (Ho et al 1971). The ratio of cytosine arabinoside to uracil arabinoside (6:1) is the reverse of that found in plasma and is probably due to the very low cytidine deaminase levels in the cerebrospinal fluid and brain. Samples of cerebrospinal fluid were taken for 7 hours. Assuming a continued exponential decrease in cytosine arabinoside it was calculated that levels greater than 100 ng/ml would persist for approximately 24 hours(Figure 8).

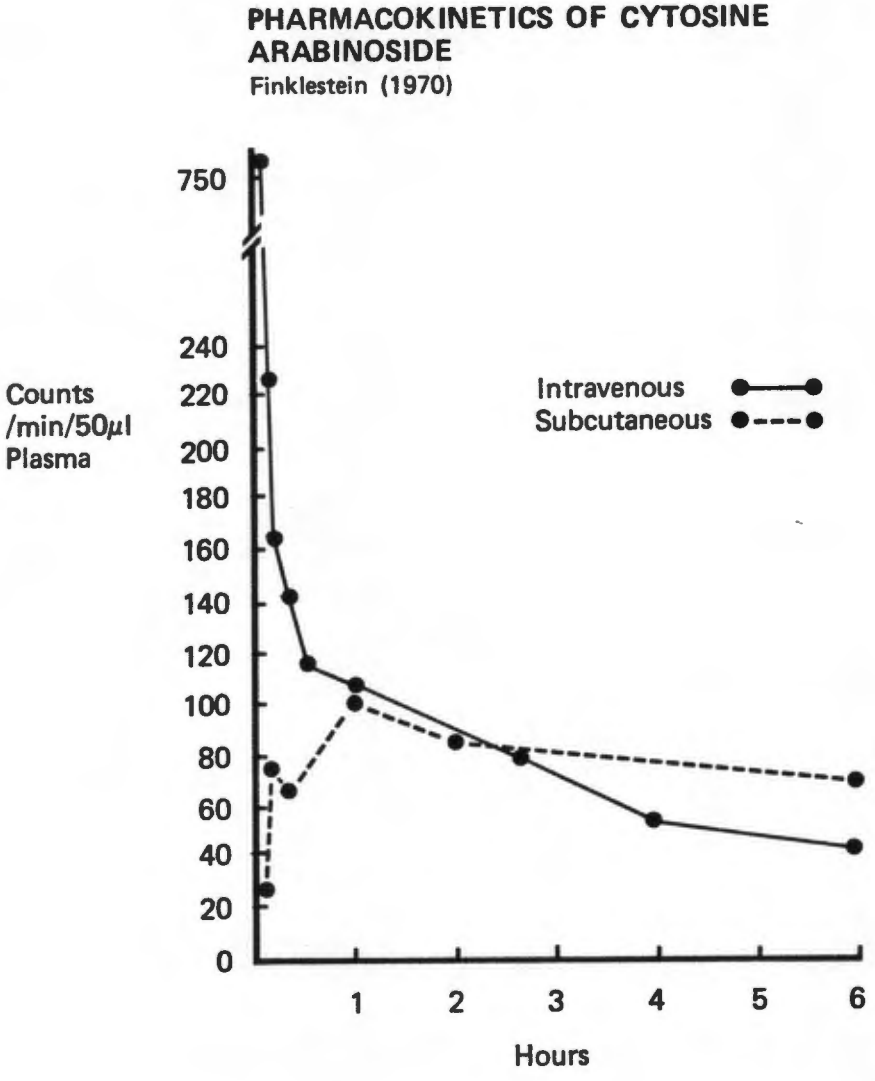


Figure 7

The pharmacokinetics of subcutaneous and intravenous bolus cytosine arabinoside. *(Finklestein et al 1970)*

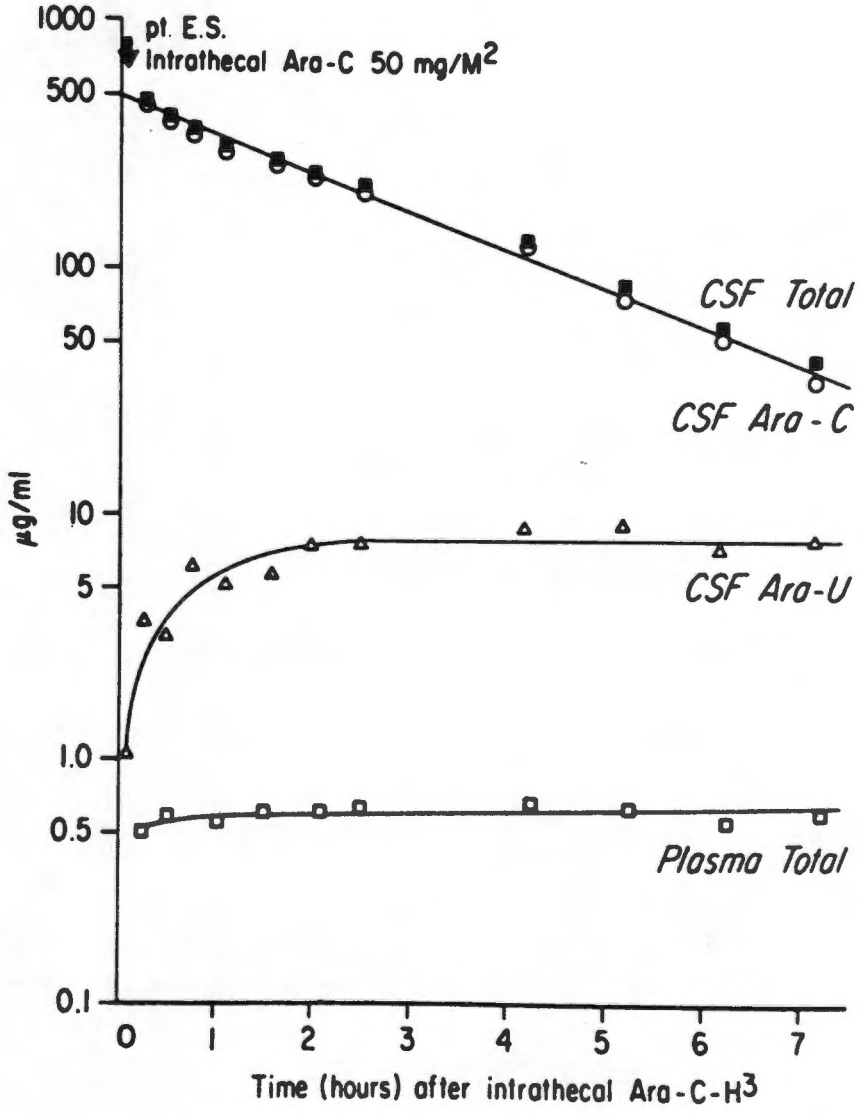


Figure 8

Cerebrospinal fluid pharmacokinetics of cytosine arabinoside and uracil arabinoside after injection of 50mg/m² into ommaya reservoir.
(Ho et al 1971)

Cerebrospinal Fluid Concentrations of Cytosine Arabinoside during Intravenous Infusions.

The first indication that cytosine arabinoside crossed the blood brain barrier came from a study by Howard et al(1968) who showed that single daily intravenous injections of cytosine arabinoside resulted in a reduction in the cerebrospinal fluid blasts in patients with central nervous system leukaemia. This suggested that cytosine arabinoside crossed the blood brain barrier. A single cerebrospinal fluid sample taken after an intravenous bolus of cytosine arabinoside in man revealed a very low concentration of the drug (Ho et al 1971). However, when cytosine arabinoside was given to a patient with an Ommaya reservoir as an intravenous bolus (56 mg) followed by an infusion (58 mg), over 2 hours, there was rapid equilibration between the plasma and cerebrospinal fluid ,and cerebrospinal fluid cytosine arabinoside levels were 40% of simultaneous plasma levels (Figure 9). Canellos et al (1979) measured simultaneous plasma and cerebrospinal fluid levels of cytosine arabinoside during continuous infusions of 200 mg/m^2 per day. The samples were taken on the second and subsequent days of the infusion to allow equilibration to occur. Cerebrospinal fluid:plasma ratios ranged from 0.26 to 0.91 in 7 patients.

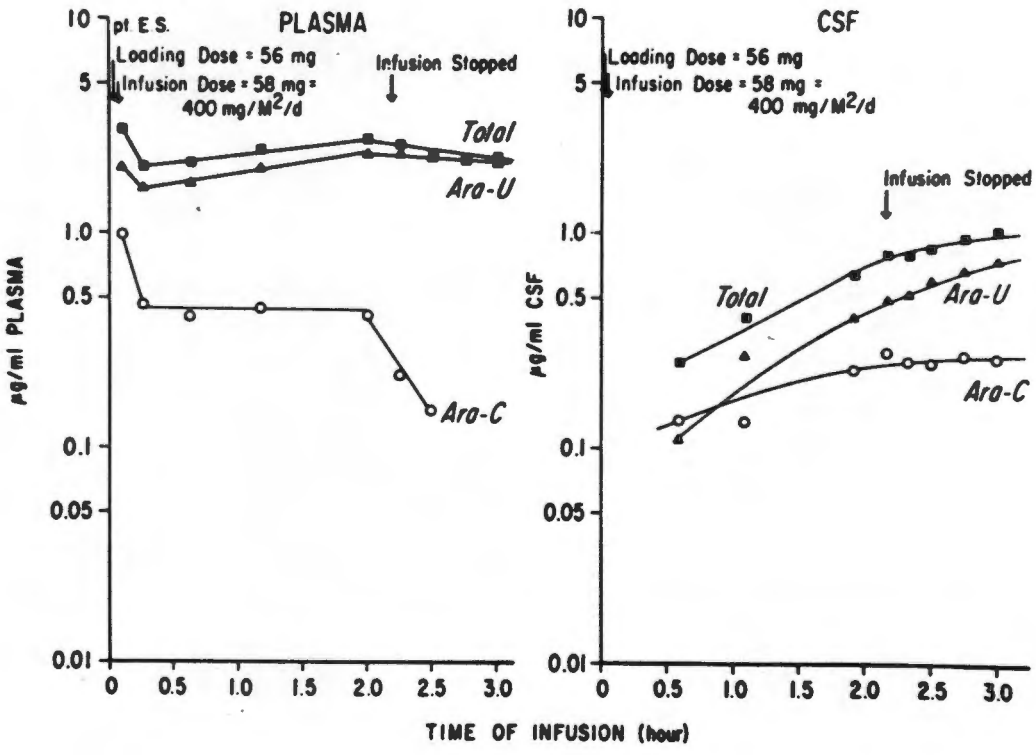


Figure 9

Plasma and cerebrospinal fluid cytosine arabinoside concentrations during an intravenous infusion preceded by a loading dose. *(Ho et al 1971)*

Delayed Release Formulations of Cytosine Arabinoside.

Continuous intravenous infusion of cytosine arabinoside suffers from the disadvantage of requiring patients to be in hospital and have repeated intravenous cannulation. The inconvenience of continuous intravenous infusion has resulted in several attempts to produce a delayed release formulation of cytosine arabinoside over the past 10 years.

The 5` esters of cytosine arabinoside have been studied in both animals and man (Ho et al 1977). The rate of release of cytosine arabinoside from these esters is dependent on their water solubility and the rate of hydrolysis in plasma. Single injections of cytosine arabinoside 5` palmitate and cytosine arabinoside 5` benzoate, were effective treatment for L1210 leukaemia in mice and provided sustained levels of cytosine arabinoside of greater than 100 ng/ml in the mouse plasma. In man, however, absorption was very slow and only 5 to 6% of total radioactivity was excreted in 24 hours. This was consistent with the lack of myelosuppression in patients treated with cytosine arabinoside 5` palmitate in a phase 1 study (Ho et al 1977). In 1 patient who came to post mortem 4 days after injection of cytosine arabinoside 5` palmitate, 92% of radioactivity was recovered from the site of injection. Surgically implantable subcutaneous sustained release cytosine arabinoside capsules have been used in animals, and have resulted in prolongation of survival compared with bolus

cytosine arabinoside in mice with L1210 leukaemia (Fu et al 1978). The necessity for a surgical approach, both to initiate and to terminate treatment, does not make this an attractive proposal for use in man.

Cyclocytidine is a water soluble drug which is hydrolysed to cytosine arabinoside and subsequently deaminated to uracil arabinoside. This hydrolysis proceeds at a slow but measurable rate after single dose intravenous and subcutaneous administration in man. The terminal half life of an intravenous bolus of cyclocytidine is 8 hours, and the drug is well absorbed following intramuscular or subcutaneous administration with peak plasma levels being reached at 1 hour. Phase 1 and 2 studies have been conducted in man (Sakai et al 1972, Lokich et al 1975, Burgess et al 1977 and Finklestein et al 1979), and myelosuppression similar to that with continuous infusions of cytosine arabinoside was found. The limiting toxicity was induction of severe postural hypotension which was not dose related. Parotid pain was also a frequent complication. Further studies with this drug are awaited, but these side effects may limit its usefulness as a repository form of cytosine arabinoside.

Liposomes (phospholipid vesicles) have recently been tested as biological carriers of cytotoxic drugs (Kaye 1981). Despite the fact that preferential tumour uptake has not been adequately demonstrated, liposomes possess the ability to profoundly affect the pharmacokinetics of the entrapped drugs. Kobayashi

et al (1977), Mayhew et al (1980) and Rustum et al (1979a) have demonstrated that liposome encapsulated cytosine arabinoside prolongs the survival of mice with L1210 leukaemia when compared with free drug, and is as effective as cytosine arabinoside given by continuous infusion. Before liposomes can be accepted as a repository form of cytosine arabinoside more work on the stability and toxicity in vivo is required. Furthermore large scale production of these vesicles is at present impractical (Kaye 1981).

The short half life of cytosine arabinoside in man is due to its rapid deamination by the enzyme cytidine deaminase, predominantly in the liver. The most potent deaminase inhibitor both in vitro and in vivo is tetrahydrouridine (Hanze 1967),and in the doses used in both animals and man it appears to be non toxic. Kreis et al (1977) studied the effect of tetrahydrouridine on the pharmacology of cytosine arabinoside. Tetrahydrouridine given 15 minutes before cytosine arabinoside produced a twofold increase in plasma concentrations. The initial half life and the terminal half life were both increased. In patients tetrahydrouridine in combination with cytosine arabinoside produced myelosuppression equivalent to that produced by an approximately 50 fold higher dose of cytosine arabinoside alone (Wong et al 1979). Further studies of the effect of tetrahydrouridine on the pharmacokinetics of cytosine arabinoside are indicated, but difficulties in producing tetrahydrouridine of sufficient purity for use in man have limited further studies (Ho 1981).

The Relationship between Cytosine Arabinoside Pharmacokinetics and Therapeutic Response in Patients with Acute Leukaemia.

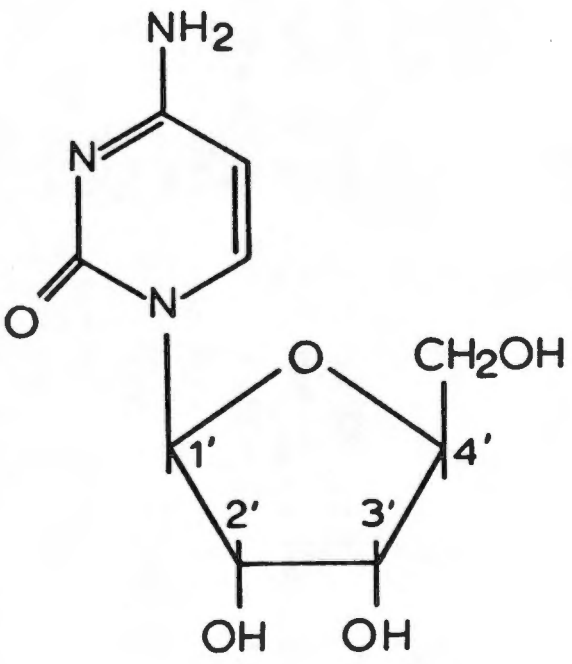
Baguley et al (1971) and van Prooijen et al (1977a) reported an association between a short half life of cytosine arabinoside and failure to achieve complete remission of acute leukaemia. However, Baguley et al(1971) used only 1 point to calculate the half life, and in the study reported by van Prooijen et al (1977a) 3 of the 5 patients who did not remit were relapsed patients as compared with 1 out of 9 patients who did respond. These studies also suffer from the severe limitation that they involve very small numbers of patients.

Harris et al (1979) used the time taken to reduce the peripheral blast count by 95% as a measure of the anti-leukaemic effect of cytosine arabinoside infusions. The mean plasma cytosine arabinoside concentration during the infusions multiplied by the number of days of infusion was calculated and was regarded as an "exposure index".Although this index showed wide variation between patients it did not correlate with the time taken to reduce the peripheral blasts by 95% and it was concluded that plasma concentrations of cytosine arabinoside during infusions do not correlate with response to this drug. This study however, involved only 12 patients and makes the unsubstantiated assumption that the time to decrease peripheral blood blasts by 95% is a reliable indicator of the anti-leukaemic effect of cytosine arabinoside.

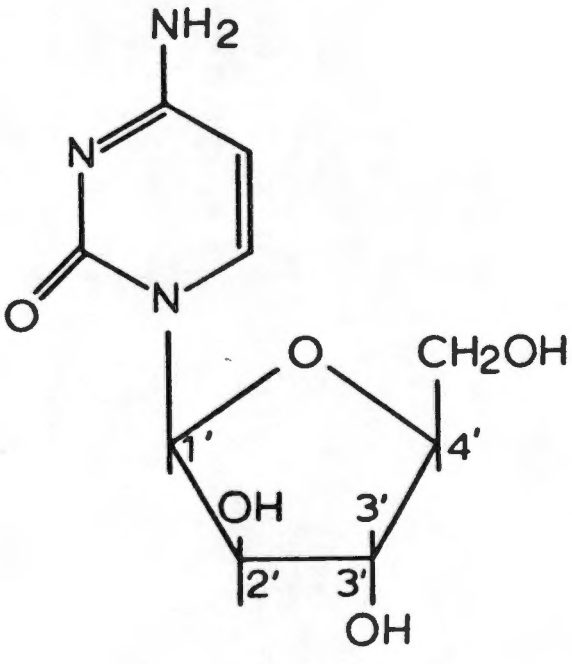
The Intracellular Metabolism of Cytosine Arabinoside

Cytosine arabinoside (araC) is a synthetic analogue of the naturally occurring nucleoside cytidine (Figure 10). It is transported into the cell from the extra-cellular fluid by a carrier mediated diffusion process (Kessel et al 1967 and 1969 and Mulder et al 1975). The drug is metabolised intracellularly along the same metabolic route followed by cytidine. The first phosphorylation step converts cytosine arabinoside to cytosine arabinoside 5` monophosphate (araCMP) using the enzyme deoxycytidine kinase and this enzyme appears to be the rate limiting step in the conversion of cytosine arabinoside to cytosine arabinoside triphosphate (araCTP)(Durham et al 1969 and Momparler et al 1968). The monophosphate is further phosphorylated to the diphosphate(araCDP) by deoxycytidine monophosphokinase (dCMP Kinase) (Schrecker 1970), and to the triphosphate by non specific nucleoside diphosphokinase (NDP Kinase) (Nakamura et al 1966).

Alternatively cytosine arabinoside can be deaminated by cytidine deaminase to the inactive metabolite uracil arabinoside (Steuart et al 1971a). AraCMP may also be deaminated by deoxycytidine monophosphate deaminase (dCMP deaminase) to uridine arabinoside monophosphate (araUMP) (Chabner et al 1979). The intracellular metabolism of cytosine arabinoside is illustrated in Figure 11.



Cytidine



Cytosine-Arabinoside

Figure 10

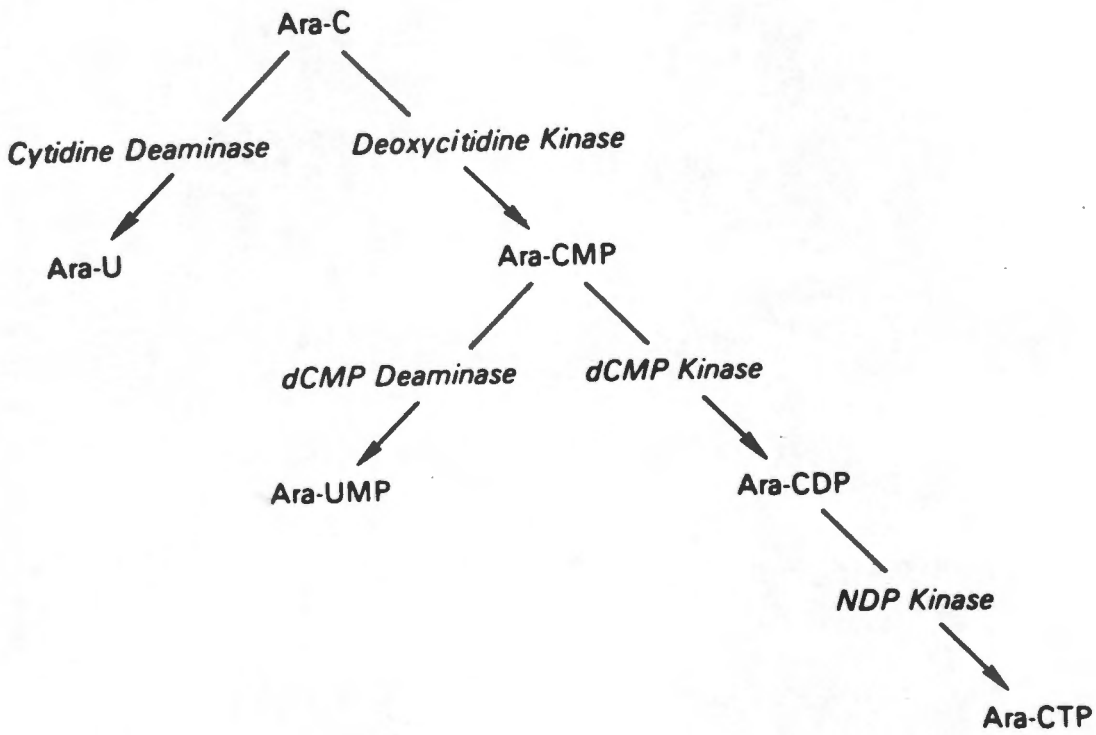


Figure 11

The intracellular metabolism of cytosine arabinoside.
(Chabner et al 1979)

The Mechanism of Action of Cytosine Arabinoside

Cytosine arabinoside has been reported to inhibit DNA synthesis in many systems including cell cultures (Silagi 1965) and intact human myeloblasts (Harris et al 1980).However the mechanism by means of which this inhibition takes place has been the subject of intensive debate over the past 2 decades.

Inhibition of Ribonucleoside Diphosphate Reductase.

Early biochemical studies (Chu et al 1962) suggested that inhibition of ribonucleoside diphosphate reductase by phosphorylated cytosine arabinoside was responsible for inhibition of DNA synthesis. This enzyme is responsible for the reduction of cytidine diphosphate (CDP) to deoxycytidine diphosphate (dCDP) which is an essential step in the synthesis of deoxycytidine triphosphate (dCTP). However, other investigators (Moore et al 1967 and Skoog et al 1971) consistently found only slight or no reduction in the production of dCTP by the phosphorylated metabolites of cytosine arabinoside. Thus it is reasonable to conclude that inhibition of this enzyme is almost certainly not a major factor in the cytotoxic action of cytosine arabinoside. Attention has therefore been focused onto other possible sites: inhibition of DNA polymerase and incorporation of cytosine arabinoside into nucleic acid.

Inhibition of DNA Polymerase by Cytosine Arabinoside

DNA polymerase enzymatically controls the incorporation of the triphosphate nucleotides into the newly formed DNA strand. In crude preparations of DNA polymerase, extracted from Ehrlich ascites cells, it was found that the enzyme was inhibited by araCTP (Kimball et al 1968). Similar results were found with DNA polymerase extracted from human leukaemic leucocytes (Inagaki et al 1969), calf thymus (Furth et al 1968 a and b) and Mouse L-cells (Graham et al 1970). The affinity of the enzyme for dCTP and araCTP was equal. Furthermore in all of these studies the inhibition of DNA polymerase by araCTP could be prevented by dCTP.

Although inhibition of DNA polymerase is undoubtedly a major site of action of cytosine arabinoside, the existence of irreversible damage resulting from exposure to cytosine arabinoside (Silagi 1965, Chu et al 1968) is difficult to explain on the basis of competitive inhibition alone. In addition the demonstration by Graham et al (1970) that Mouse L-cells incubated with cytosine arabinoside remained viable over a period of 14 hours despite a 97% reduction in DNA synthesis, suggests that cell death may not directly relate to inhibition of DNA synthesis. For this reason attention has been focused on the question of incorporation of cytosine arabinoside into nucleic acid.

Incorporation of Cytosine Arabinoside into DNA and RNA.

Cytosine arabinoside is rapidly phosphorylated to its triphosphate and incorporation into nucleic acid might thus be expected. Small amounts of radioactivity have been found in the acid insoluble fraction when various cell lines were incubated with tritiated cytosine arabinoside (Chu et al 1965 and Silagi 1965). However, the tritium associated with the nucleic acid could have resulted from contamination by metabolites of cytosine arabinoside from the acid soluble fraction. Chu et al (1968) and Momparler (1972) were able to isolate phosphorylated metabolites of cytosine arabinoside after degrading the nucleic acid suggesting that the incorporated radioactivity really originated from cytosine arabinoside. Several studies have demonstrated the incorporation of cytosine arabinoside into both DNA and RNA (Graham et al 1970, Chu et al 1968 and Grant et al 1980). These studies were performed under alkaline conditions which can degrade nucleic acid (Kufe et al 1980 and 1982) and this may lead to difficulty separating incorporation into DNA from that in RNA. Zahn et al (1972) and more recently Kufe et al (1980 and 1982) used the non-degrading cesium sulphate density gradient to separate RNA from DNA and demonstrated specific incorporation of cytosine arabinoside into DNA with less than 1% of the total incorporated radioactivity being found in RNA. Moreover Kufe et al (1980) found a highly significant relationship ($P < 0.0001$) between incorporation of cytosine arabinoside into DNA and loss of clonogenic survival. It is therefore suggested that the

incorporation of cytosine arabinoside into DNA is an important factor leading to cell death.

Mechanisms of Resistance to Cytosine Arabinoside

In order to function as an inhibitor of DNA synthesis cytosine arabinoside has to be converted to its triphosphate, and this can be decreased by decreased phosphorylation or increased deamination. Steuart et al (1971b) showed an inverse relationship between cytidine deaminase activity in cell free extracts of leukaemic blasts and therapeutic responsiveness to cytosine arabinoside. The development of resistance to cytosine arabinoside therefore correlated with increased cytidine deaminase activity. This aroused much interest, for if this proposal was correct, agents which inhibited cytidine deaminase could potentially overcome resistance to cytosine arabinoside. However, Tattersall et al (1974) studied acute myeloblastic leukaemia cells both sensitive and resistant to cytosine arabinoside and found no correlation between cytidine deaminase levels and sensitivity to this drug. Similarly Chou et al (1977), Smythe et al (1976) and Chang et al (1977) found no correlation between levels of cytidine deaminase and the degree of sensitivity or resistance to cytosine arabinoside.

Several studies with cell lines and animal tumours (Kessel 1967, Schrecker et al 1967, Bach 1969, Uchida et al 1968 and Kreis et al 1972), have demonstrated reduced levels of the

phosphorylating enzyme deoxycytidine kinase in mutant cell lines resistant to cytosine arabinoside. Tattersall et al (1974) demonstrated the same phenomemon in resistant human acute leukaemic myeloblasts. Decreased deoxycytidine kinase activity therefore appears to be a major mechanism of resistance to cytosine arabinoside in both experimental cell lines and human acute leukaemia.

Phosphorylated cytosine arabinoside (araCTP) competes with dCTP, one of the natural substrates for DNA polymerase, and increased levels of dCTP might therefore protect cells from cytosine arabinoside toxicity. Some leukaemic cell lines resistant to cytosine arabinoside have been shown to have expanded pools of dCTP (Bach 1969 and Momparler et al 1968). Tattersall et al (1974) showed relatively increased levels of dCTP in the myeloblasts of patients with resistant acute leukaemia.Chou et al (1977) examined the relationship between in vitro araCTP formation in human acute leukaemic myeloblasts and sensitivity to cytosine arabinoside in vivo.It was shown that in previously untreated patients with sensitive leukaemia the average amount of araCTP formed was significantly higher than in cells from patients who failed to achieve complete remission. However, patients who were initially sensitive to cytosine arabinoside but who subsequently became resistant when they relapsed, produced more araCTP than any other patient category. It is suggested that this could be due to high levels of dCTP in resistant cells, but they did not measure this parameter.

Rustum et al (1979b) found a significant correlation between the ability of acute myelogenous leukaemia blasts to form and retain araCTP and the duration of complete remission. Seven out of eleven "high retainers" remained in complete remission for greater than 55 weeks, while only 1/9 of the "low retainers" stayed in complete remission for a similar period. The median remission duration in the "high retainers" was greater than 63 weeks while that of the "low retainers" was 28 weeks. This study differs from the other clinical studies described in that it correlates the in vitro araCTP formation with duration of remission rather than complete remission rate. This may be very important, as complete remission rate can be affected by many factors,such as infection and haemorrhage, other than biochemical resistance. Another major difficulty in correlating in vitro araCTP formation with patient outcome is that it does not take into account the wide interpatient variability in cytosine arabinoside pharmacokinetics (Ho et al 1971) and araCTP production in vitro has been shown to be linearly related to extracellular cytosine arabinoside concentration (Harris et al 1980). Inter-patient variation in cytosine arabinoside pharmacokinetics could therefore significantly affect the in vivo formation of araCTP,and this may explain some of the difficulty correlating in vitro araCTP formation and patient outcome.

CHAPTER 2

MATERIALS AND METHODS.

MATERIALS AND METHODS.

In order to avoid repetition the materials and methods common to the majority of the studies are described in this chapter.Those specific to individual studies will be discussed in the relevant chapters.

Patients

Adults with acute myelogenous and acute lymphoblastic leukaemia, and adults and children with high grade non hodgkin's lymphoma, treated in the Imperial Cancer Research Fund Department of Medical Oncology at St. Bartholomew's Hospital, have been studied. Clinical details of the patients and the treatment they received are described in the relevant sections of the thesis. Where appropriate, approval was obtained from the St Bartholomew's Hospital Ethical Committee. An example of the consent forms used is shown in Figure 12.

Pathological Diagnosis.

Haematology.

Peripheral blood and bone marrow preparations were stained by May-Grunwald-Giemsa, Sudan black, periodic acid Schiff, and either combined esterase or non-specific esterase with and without sodium fluoride, and examined by Dr. John Amess, in the Department of Haematology at St. Bartholomew's Hospital.

PATIENTS CONSENT FORM

Consultant.....T A LISTER..... **Investigator**.....M L SLEVIN.....

Brief description of study to be carried out.	As you know, you are now in complete remission of your acute leukaemia (there is no evidence of leukaemia in your blood or bone marrow). It is normal practice at this stage to perform a lumbar puncture. This is to exclude the possibility that there are leukaemia cells in the fluid surrounding the brain and spinal cord (cerebro-spinal fluid). Cerebro-spinal fluid is a site which the drugs you have received do not normally reach. We are undertaking a study to see if we can get adequate levels of these drugs into the cerebro-spinal fluid by giving them in a different manner. If this is possible, it would be a great advantage in the treatment of patients with leukaemia cells in the cerebro-spinal fluid. If you agree to participate in this study, we would give you an infusion of one of the drugs you have already received (Cytosine Arabinoside) via a drip for 3 hours. This would be given in such a way that it would not affect your bone marrow or your blood count, and would not cause any other side effect apart from possible nausea. At the end of the infusion we would perform your lumbar puncture and could measure the levels of Cytosine Arabinoside in the cerebro-spinal fluid at the same time as we do the normal tests. A second lumbar puncture would be of great value to determine how long the drug stays in the cerebro-spinal fluid, and if the first lumbar puncture does not cause you any discomfort, you may be asked if you would consent to another lumbar puncture a few hours later.
Purpose of study.	The purpose of this study is to determine how much Cytosine Arabinoside crosses from the blood into the cerebro-spinal fluid during a short infusion. This information will help us to design better treatment for patients with leukaemia cells in the cerebro-spinal fluid.

I UNDERSTAND WHAT THIS STUDY INVOLVES AND I AGREE TO TAKE PART IN IT ON THE UNDERSTANDING THAT REFUSAL TO BE INCLUDED WILL NOT AFFECT MY TREATMENT IN ANY WAY.

Signature of Patient..... **Date**.....

I HAVE BEEN PRESENT WHILE THE PROCEDURE HAS BEEN EXPLAINED TO THE PATIENT, AND I HAVE WITNESSED HIS/HER CONSENT TO TAKE PART.

Signature of Witness..... **Date**.....
(The Witness should be a person not connected with the study)

Full name and address of patient: .....

.....

.....

.....

.....

Figure 12
Patient Consent Form

Histopathology.

Biopsy specimens were stained with haematoxylin and eosin, and examined by Dr. A. Stansfeld and Dr D. Levison in the Department of Histopathology at St. Bartholomew's Hospital.

Sampling

Peripheral Blood

Blood samples were taken via a peripheral intravenous cannula or, if available,via an indwelling subcutaneously tunnelled subclavian line (Hickman et al 1979,Figures 13 and 14).These lines are used for long term venous access in most patients with acute leukaemia treated at St Bartholomew's Hospital(Dhaliwal et al 1981).

Each blood sample was taken into a precooled tube ($4^{o}C$) containing tetrahydrouridine ($1x\ 10^{-5}$ molar), and 50 units of heparin. The tubes were chilled immediately ($4^{o}C$) and centrifuged at 1000 revs per minute for 5 minutes (0.7g). The plasma was then decanted, frozen and stored at $-20^{o}C$.

Cerebrospinal Fluid.

The lumbar punctures were all performed by myself using a 20 gauge disposable lumbar puncture needle. Cerebrospinal fluid samples (1 ml) were collected into tubes containing tetrahydrouridine and stored frozen at -20^{o} C. Sampling from the Ommaya reservoir (Ommaya 1963) (Figure 15) was performed as described by Shapiro et al (1975). To obtain samples, the skin was sterilized with iodine and the reservoir was then entered with a 23 gauge butterfly needle. Three millilitres of

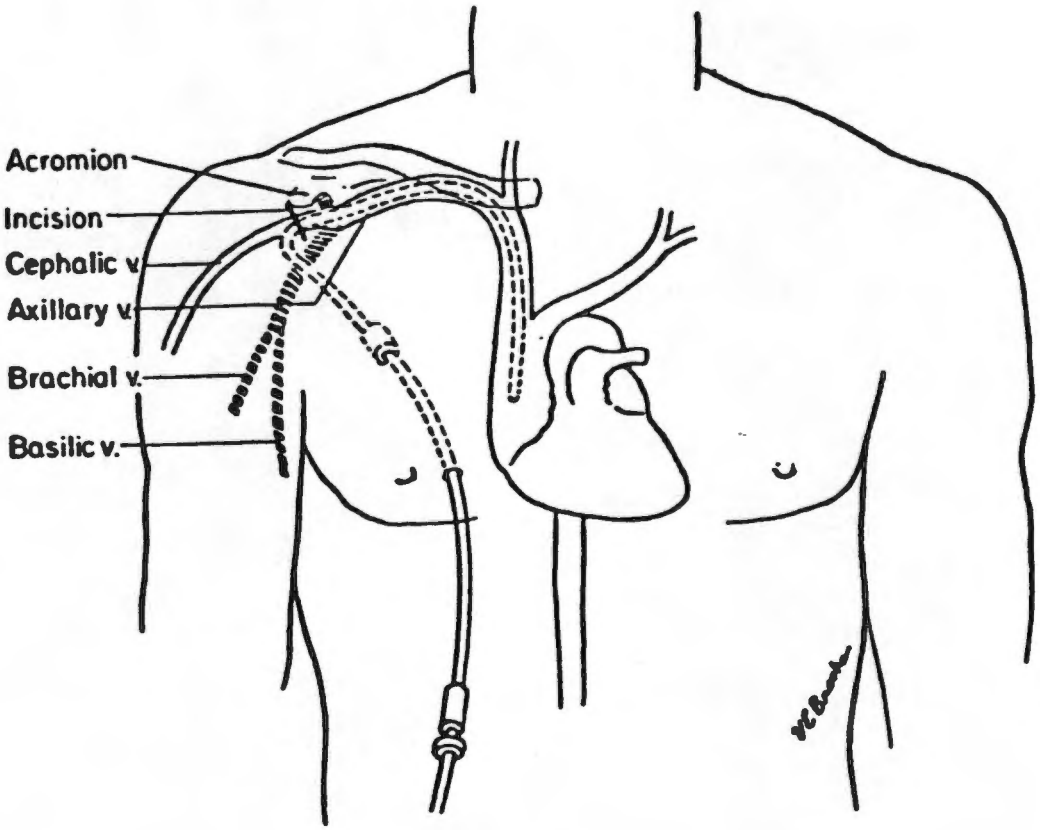


Figure 13
Hickman catheter
(Hickman et al 1979)

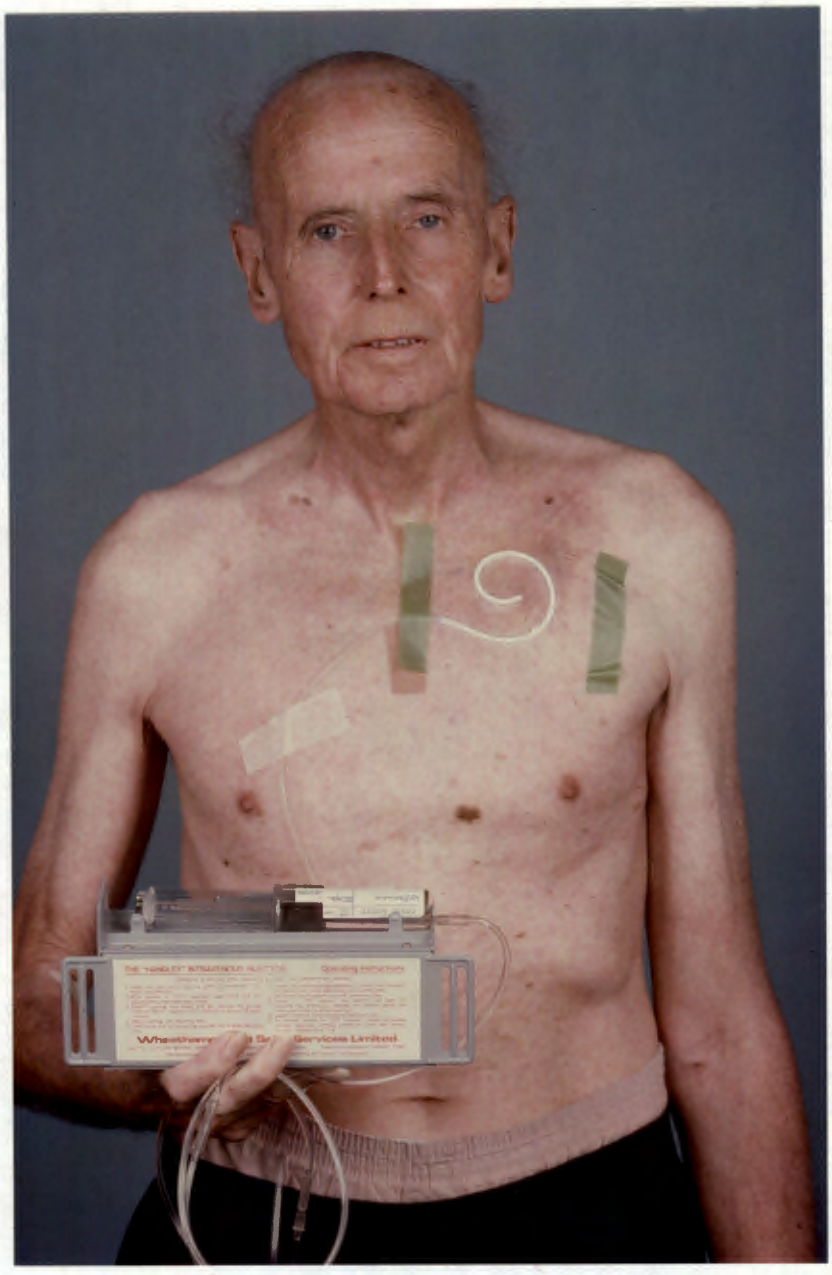


Figure 14
Hickman catheter.
(Hickman et al 1979)

cerebrospinal fluid were withdrawn and discarded (representing cerebrospinal fluid in the reservoir and tubing): the fourth millilitre was then removed for assay.

Saliva

Saliva samples (2 ml) were collected after the patients had rinsed their mouths with water. Parafilm, an inert tasteless plastic substance, was used to stimulate saliva production. The saliva samples were collected into pre-cooled tubes containing tetrahydrouridine (1×10^{-5} molar) and 50 units of heparin. They were chilled immediately and centrifuged at 1000 revs per minute for 5 minutes (0.7g) and the saliva was then separated from any particulate material at the bottom of the tube and stored frozen at $-20^{\circ}C$.

Radioimmunoassay.

The cytosine arabinoside concentrations in plasma, saliva and cerebrospinal fluid were measured using a radioimmunoassay developed at the University of Surrey (Piall et al 1979). I evaluated the assay myself and obtained the interassay and intra-assay variation shown in Tables 1 and 2. A sample standard curve is shown in Figure 16. These results are similar to those obtained by Mrs. E. Piall who assayed the samples.

Immunization

The antibody used was produced at the University of Surrey by immunizing a sheep with a cytosine arabinoside monophosphate

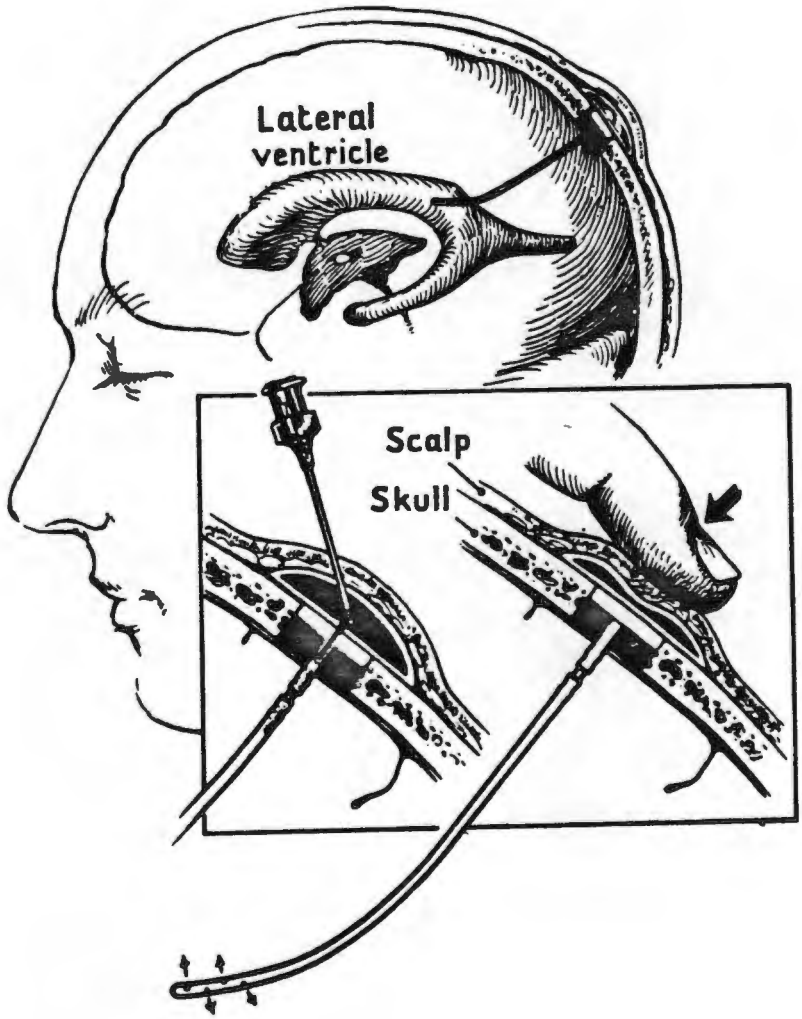


Figure 15
Ommaya reservoir
(Ommaya 1963)

CYTOSINE ARABINOSIDE RADIOIMMUNOASSAY

STANDARD CURVE AND QUALITY CONTROL

Interassay Variation

(5 assays performed on consecutive days)

cytosine arabinoside ng added	Percentage Bound of Zero Bound					Mean ±SD	Coefficient of variation
	Day1	Day2	Day3	Day4	Day5		
0.05	73.6	74.5	75.4	72.6	75.8	74.4 1.3	1.7%
0.10	61.1	62.8	64.5	58.1	59.1	61.1 2.6	4.2%
0.15	48.3	54.5	56.1	52.9	48.2	52.2 3.6	6.9%
0.20	38.7	43.9	46.5	45.2	41.1	43.1 3.2	7.4%
0.30	30.3	32.9	35.5	36.5	32.0	33.4 2.5	7.5%
0.40	23.8	28.9	25.2	28.9	27.4	26.8 2.3	8.6%
0.50	21.3	24.6	23.0	25.5	24.9	23.9 1.7	7.1%
0.70	15.7	19.3	20.1	19.4	19.3	18.8 1.7	9.0%
1.00	12.2	11.2	13.4	14.1	12.3	12.6 1.1	8.7%
1.50	7.5	8.9	7.3	10.1	9.2	8.6 1.2	13.9%
Low Quality Control (ng/ml)	19.8	20.5	21.5	18.5	22.0	20.5 1.4	6.8%
High Quality Control (ng/ml)	365.1	370.5	386.3	341.7	362.7	365.3 16.1	4.4%

Table 1.

CYTOSINE ARABINOSIDE RADIOIMMUNOASSAY

STANDARD CURVE AND QUALITY CONTROL

Intra-assay Variation

(5 assays performed simultaneously)

cytosine arabinoside ng added	Pecentage Bound of Zero Bound Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	MEAN ± SD	Coefficient of variation
0.05	72.9	73.8	71.8	74.1	75.8	73.1 1.5	2%
0.10	62.2	57.6	56.9	60.1	59.1	59.2 2.1	3.5%
0.15	49.4	50.4	49.1	46.7	48.2	48.8 1.4	2.8%
0.20	40.8	41.8	41.7	41.2	41.5	41.4 0.4	0.9%
0.30	34.5	31.8	33.7	35.5	32.3	33.6 1.5	4.5%
0.40	24.1	24.1	28.4	24.5	27.1	25.6 1.8	7%
0.50	22.6	19.2	23.4	20.5	23.2	21.8 1.8	8.2%
0.70	15.5	16.4	18.7	16.6	19.5	17.3 1.7	9.8%
1.00	10.6	11.8	13.2	11.5	12.1	11.8 0.9	7.6%
1.50	7.4	8.8	9.2	8.3	9.5	8.6 0.8	9.3%
Low Quality Control (ng/ml)	21.8	22.3	19.8	23.0	18.5	21.0 1.9	9%
High Quality Control (ng/ml)	384.6	346.4	359.5	368.1	349.8	361.7 15	4.1%

Table 2.

ovalalbumin conjugate. This animal produced antibody for use at an initial dilution of 1/80.

Assay

Materials

The assay buffer used for all dilutions was 0.05 molar phosphate buffer, pH 7.4, containing 0.6 g% (w/v) NaCl and 0.1 g% (w/v) gelatin. A freeze dried cytosine arabinoside standard was used . The antiserum was kept at 4°C, the amount to be used for standard curves being established by the antiserum dilution curves as that dilution which bound 40% of the added label. The radiolabel used was tritiated cytosine arabinoside (15 Ci/mmol) from the Radiochemical Centre ,Amersham. This was diluted in buffer for use in each assay, so that 0.1 pmol (24.3 pg) cytosine arabinoside was added to each assay tube. Dextran-coated charcoal was used to separate antibody bound from free cytosine arabinoside.

As a quality assurance check high and low quality control samples were prepared from pooled preassayed patient plasma. These pooled samples were then assayed, aliquotted and stored frozen at -20° C. To ensure batch to batch consistency these quality controls were subsequently included in each sample run and thus provided an external control separate from the calibration standards.

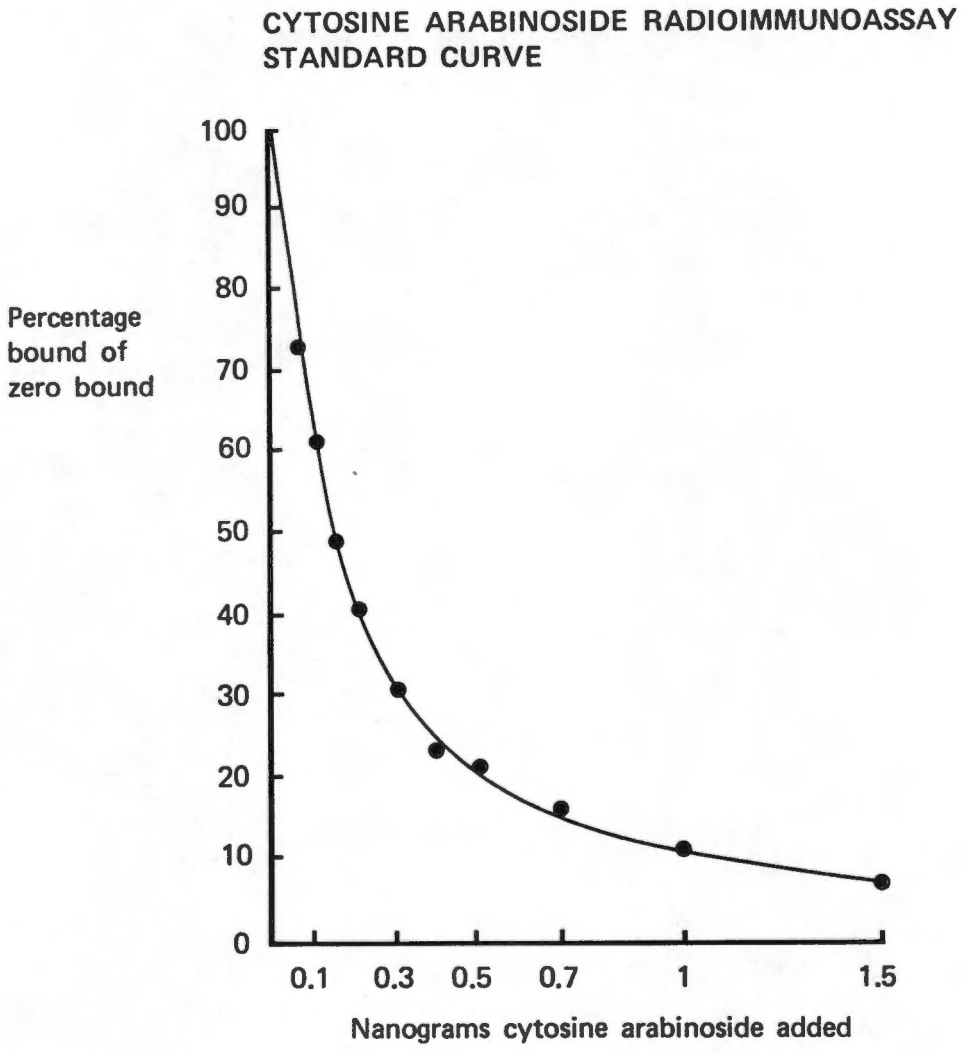

CYTOSINE ARABINOSIDE RADIOIMMUNOASSAY
STANDARD CURVE
Percentage bound of zero bound
100
90
80
70
60
50
40
30
20
10
0
0.1
0.3
0.5
0.7
1
1.5
Nanograms cytosine arabinoside added

Figure 16

Procedure

The freeze dried standard was diluted to give the standard curve points, each point being set up in duplicate. Samples were assayed at 3 to 4 dilutions, each dilution set up in duplicate. To set up the assay, the reagents were added in the order indicated in Table 3 to plastic tubes, and the contents mixed and left at room temperature for 50 minutes. The tubes were then placed on ice for 10 minutes. Dextran-coated charcoal was resuspended over a magnetic stirrer, and added rapidly to the tubes during stirring. The assay tubes were mixed again and placed in ice for 15 minutes, when they were centrifuged at 4°C for 10 minutes. Five hundred microlitre aliquots of supernatant were taken for liquid-scintillation counting in order to quantitate the bound antigen.The amount bound in the samples was compared with the standard curve set up in the same batch.

Assessment

The assay had a sensitivity of 1ng/ml in unextracted plasma and cerebrospinal fluid and recovery of cytosine arabinoside was complete over a 100 fold range from 10ng/ml to 1ug/ml. The interassay and intra-assay variation was less than 10%. The antibody did not cross-react with related nucleotides and drugs which are commonly co-administered to patients receiving cytosine arabinoside, such as other cytotoxics, antibiotics, tranquilisers and analgesics. There was also no cross reaction with tetrahydrouridine which is added to each blood sample to prevent deamination of cytosine arabinoside. The assay did however cross react greater than 100% with cytosine arabinoside monophosphate and cytosine arabinoside triphosphate. These

Reagent	Total counts tube	Non-specific binding tube	Maximum binding tube	Zero Tube or antiserum dilution curve	Standard or sample tube
Diluent buffer	600	500	100	400	300
Standard or sample	-	-	-	-	100
Antiserum	-	-	400	100	100
(^{3}H) AraC (0.1 pmol)	100	100	100	100	100
Incubate 50 min at room temperature followed by 10 min at 4°C					
DCC (2.5%)	-	100	100	100	100
Leave for 15 min in ice, centrifuge and count 500 ul supernatant.					

Table 3.
Procedure for radioimmunoassay of cytosine arabinoside
[Cytosine Arabinoside (araC),Dextran Coated Charcoal (DCC)]

nucleotides are found only intracellularly, are unable to cross the cell membrane and should not normally interfere with the measurement of plasma and cerebrospinal fluid cytosine arabinoside.

Pharmacokinetics

The pharmacokinetic parameters were calculated using"Stripact" (Leferink et al 1979). This is an interactive computer programme which uses least squares regression analysis of the log concentration versus time data to derive the best exponential fit by "back stripping" of the curves starting with the terminal exponential. This programme calculates the area under the curve by the trapezoidal method for the area from time To-Tn (Tn = last time point) and the area Tn - T infinity is estimated by Cn over B (Cn = the concentration at the last time point and B = the elimination rate constant).

Volume of distribution was calculated by dividing the administered dose by the area under the curve and the elimination rate constant for the terminal exponential. Total body clearance was derived by dividing the administered dose by the area under the curve.

CHAPTER 3

A COMPARISON OF TWO SCHEDULES OF CYTOSINE ARABINOSIDE USED IN COMBINATION WITH DOXORUBICIN AND 6 THIOGUANINE IN THE TREATMENT OF ACUTE MYELOGENOUS LEUKAEMIA.

Introduction

Cytosine arabinoside is conventionally administered by continuous intravenous infusion (Weinstein et al 1980 and McCredie et al 1981). This schedule is based on the S phase specificity of the drug and the experience derived from single agent cytosine arabinoside studies (Bodey et al 1969 and Frei et al 1969) which demonstrated greater efficacy and toxicity when cytosine arabinoside was administered as a prolonged infusion rather than repeated intravenous injection. However, these studies are difficult to assess because of problems with study design and small numbers of patients; the data are at times conflicting. (Frei et al 1969,Burke et al 1968,and Rai et al 1981 a and b).

There are no studies comparing intravenous bolus cytosine arabinoside with the same total dose given by continuous intravenous infusion when used in conjunction with other cytotoxic drugs. This confusion has led many investigators to use repeated intravenous or subcutaneous injections of cytosine arabinoside rather than continuous infusions (Gale et al 1977 , Wiernik et al 1979,Armitage et al 1976,Bell et al 1982,Rees et al 1977 and Glucksberg et al 1981).

The efficacy and toxicity of cytosine arabinoside given by two different schedules together with doxorubicin and 6 thioguanine have therefore been compared in patients with acute myelogenous leukaemia.

Patients and Methods

Patients

Seventy two previously untreated patients with acute myelogenous leukaemia received remission induction chemotherapy comprising doxorubicin, cytosine arabinoside and 6 thioguanine.

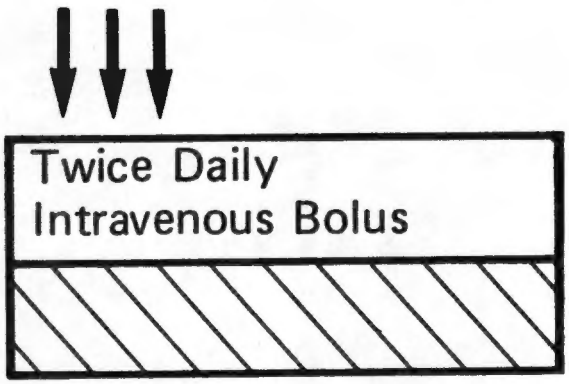
Chemotherapy Regimens

Two consecutive studies using different cytosine arabinoside schedules have been compared .

a. Forty one patients received doxorubicin 75 mg/m^2 over 3 days (25 mg/m^2 per day), 6 thioguanine 200 mg/m^2 for 7 days and cytosine arabinoside 200 mg/m^2 per day given as 100 mg/m^2 administered by twice daily intravenous injection for 7 days (Figure 17).

b. Thirty one patients received the same therapy but with cytosine arabinoside given by continuous intravenous infusion. Seven of these patients received doxorubicin over 3 days (25 mg/m^2 per day), the remainder receiving 40 mg/m^2 on day 1, and 35 mg/m^2 on day 2 (Figure 18).

INTRAVENOUS BOLUS CYTOSINE ARABINOSIDE



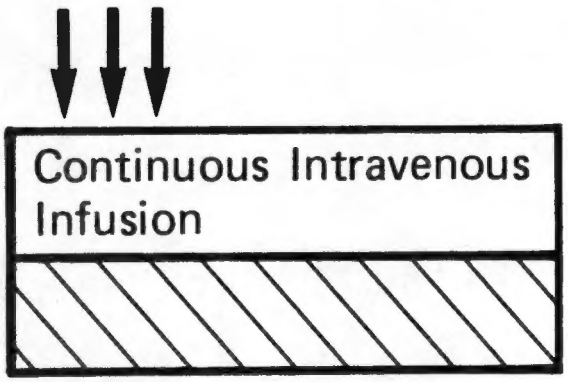
Doxorubicin 25mg/m^2/Day
for 3 Days

Cytosine Arabinoside 200mg/m^2/Day
for 7 Days

6 Thioguanine 200mg/m^2/Day
for 7 Days

Figure 17

CONTINUOUS INFUSION CYTOSINE ARABINOSIDE



Doxorubicin 40mg/m² Day 1, and 35mg/m² Day 2

Cytosine Arabinoside 200mg/m²/Day for 7 Days

6 Thioguanine 200mg/m²/Day for 7 Days

Figure 18

Neutrophil and Platelet Counts

Neutrophil and platelet counts were monitored from the first day of chemotherapy until the date of complete remission or the day of death, being obtained three times weekly in in-patients, and twice weekly in out-patients. Absolute neutrophil counts were grouped as follows: less than 0.1 x 10^9/l, 0.1 - 0.5 x 10^9/l and 0.5 - 1.0 x 10^9/l.

Peripheral Blast Counts

The peripheral blast counts on day 1 were regarded as 100% and subsequent counts were calculated as a percentage of the day 1 count. Blast counts were plotted against time in days, and the best exponential fit determined by least squares regression analysis. The rate at which the blasts cleared from the peripheral blood was determined for each patient from the exponential. The "T 1/2 blasts" was the time taken for the circulating blasts to decrease by 50%.

Results

Effect on Peripheral Blast Count.

Leukaemic blasts cleared from the peripheral blood more rapidly in patients receiving cytosine arabinoside by continuous intravenous infusion ($P < 0.001$). There was no

correlation between T 1/2 blasts and age, sex, FAB classification or absolute blast count on day 1. Furthermore, absolute blast count on day 1 did not correlate with cycles to less than 5% blasts in the bone marrow. The mean "half life" of blasts in the peripheral blood was shorter and the standard deviation smaller in patients receiving cytosine arabinoside infusions (Table 4, Figures 20 and 21).

Number of Cycles to less than 5% Blasts (Table 4)

Complete remission requires the patient to be in a normal state of health with a normal bone marrow and peripheral blood count. Patients were re-treated without necessarily fulfilling all the criteria for complete remission, although the bone marrow showed less than 5% blasts. Complete remission was therefore occasionally documented later than the time at which the bone marrow showed less than 5% blasts. The time to less than 5% blasts in the bone marrow therefore represents a more realistic estimate of the rate at which leukaemic blasts were cleared from the bone marrow.

Significantly fewer cycles of therapy were required to achieve less than 5% blasts in the bone marrow in patients treated with infusions of cytosine arabinoside (mean number of cycles to less than 5% blasts = 1.57 and 3.4 for cytosine arabinoside by infusion and twice daily intravenous injection respectively) ($P < 0.001$, Table 4, Figure 19). All the patients who achieved less than 5% blasts in the bone marrow

Regimen	Half life of Circulating Blasts	No. of Cycles to <5% blasts
IV Bolus	1.4 Days (0.3-5.5)	3.4 (1-6)
Continuous Infusion Cytosine Arabinoside	0.7 Days (0.2-1.2)	1.57 (1-2)

Table 4.

Effect of Cytosine Arabinoside Schedule on Rate of Decline of Peripheral Blood Blasts and Number of Cycles to Less Than 5% Bone Marrow Blasts.

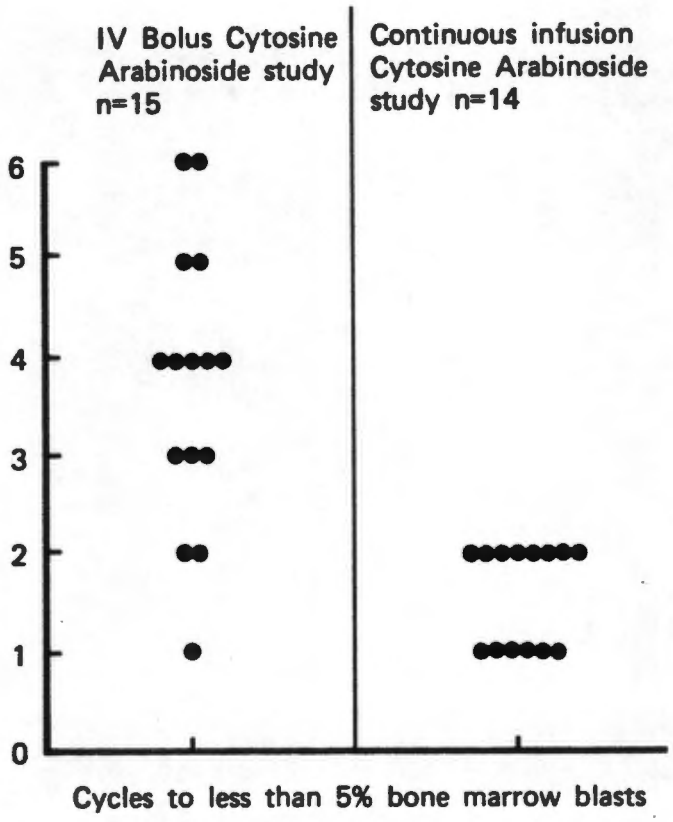


Figure 19

The effect of cytosine arabinoside schedule on number of cycles to less than 5% blasts.

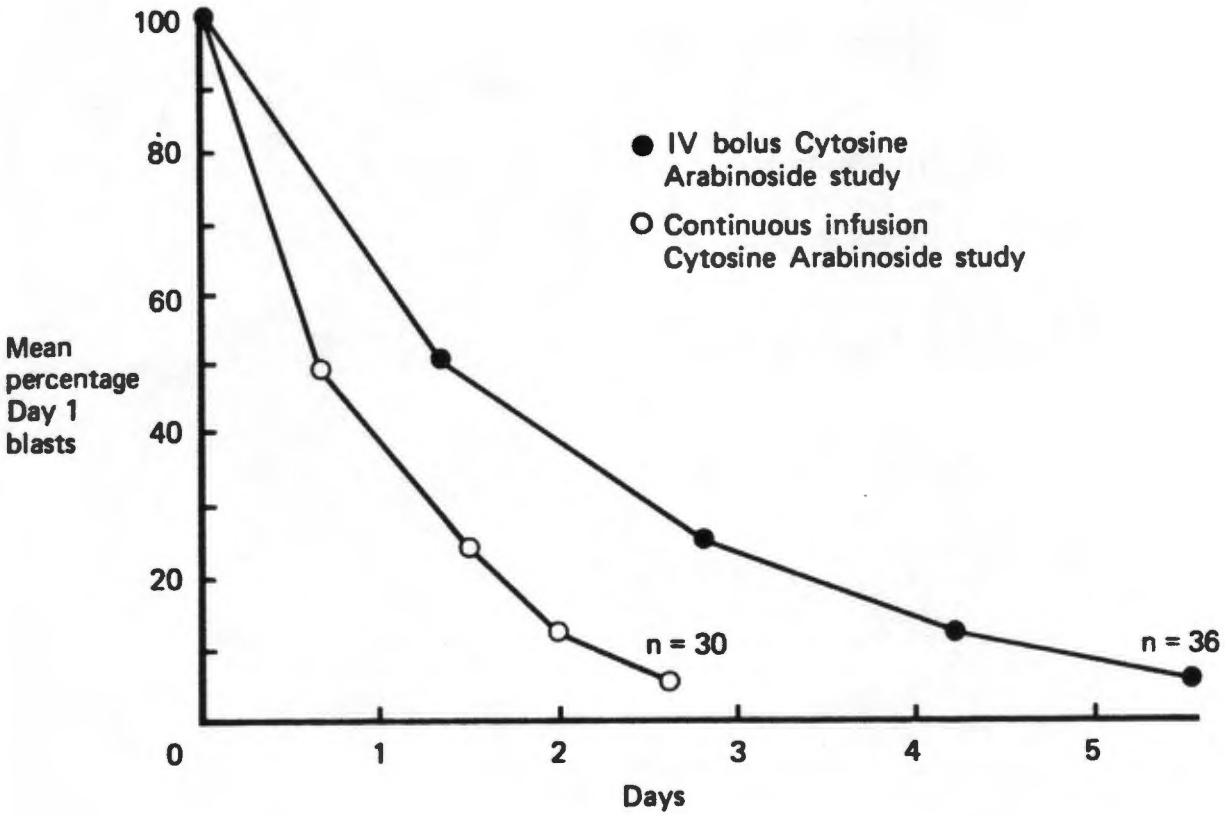


Figure 20

The effect of cytosine arabinoside schedule on the mean peripheral blast count.
(Day 1 regarded as 100%)

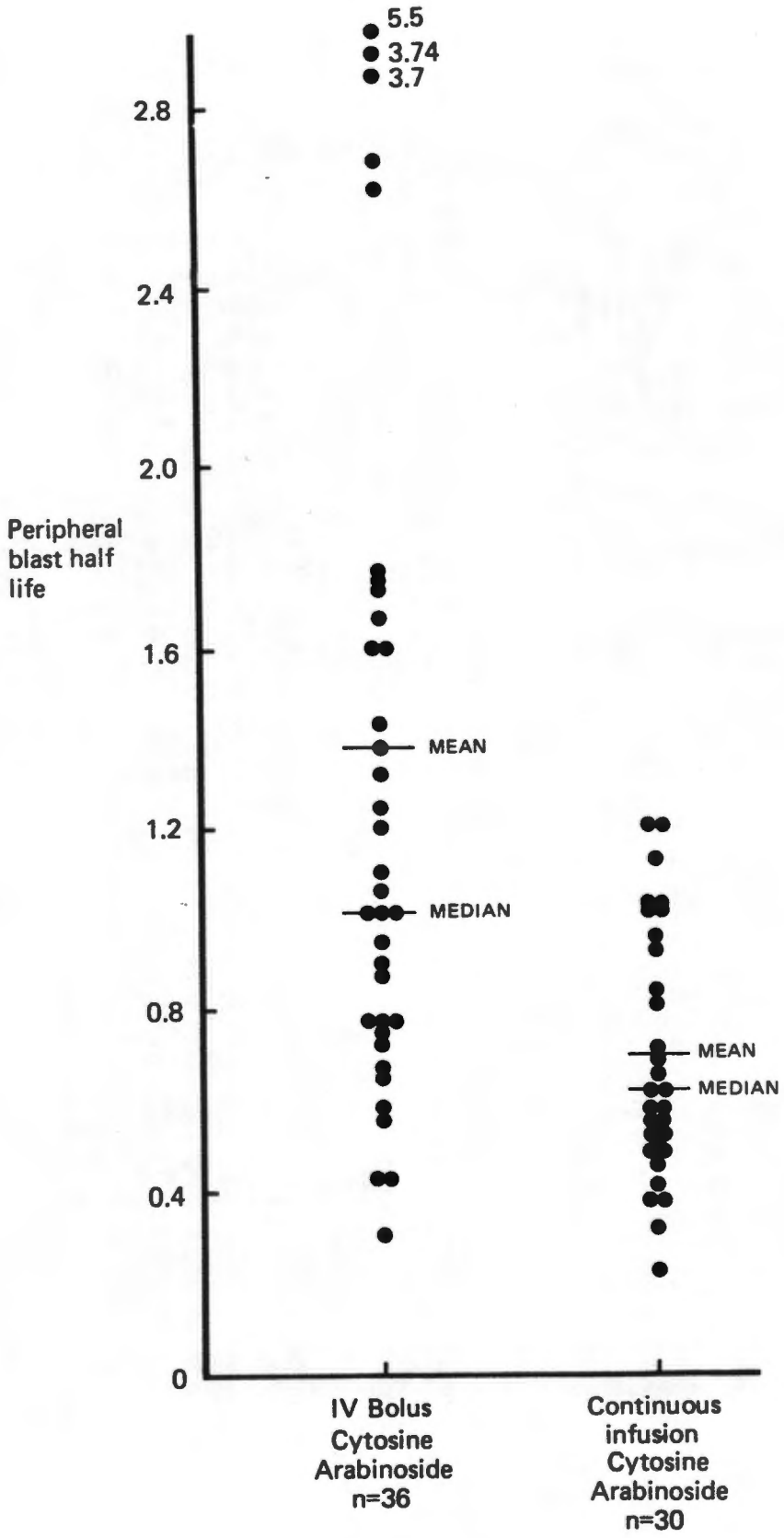


Figure 21

Effect of cytosine arabinoside schedule on half-life of peripheral blasts in individual patients.

with the continuous infusion schedule did so within 2 cycles of treatment (Figure 19).

Response to Initial Therapy

Overall, 15/41 patients receiving cytosine arabinoside by twice daily intravenous injection achieved complete remission, as compared with 14/31 patients treated with cytosine arabinoside by continuous intravenous infusion. The two populations are not strictly comparable as there was a greater proportion of older patients (greater than 60 years) in the former study (14/41 versus 8/31).

The complete remission rate in patients under 60 years is 11/27 (41%) for the intravenous bolus study and 12/23 (52%) for the infusion study.

Toxicity

a) Myelosuppression

Comparison of the degree and duration of neutropenia and thrombocytopenia associated with the two regimens revealed no significant difference following cycle 1 (Table 5). However, following cycle 2 (Table 6) the number of days spent at a neutrophil count of less than 0.1×10^9 /l was greater in patients treated with cytosine arabinoside infusions ($P < 0.05$). There was no difference in the time to recovery of the

Regimen	No. of Patients completing Cycle 1	Mean No of Days Neutrophils x 10^9/l			Mean number of days <1.0 x 10^9/l	Mean No of Days Platelets x 10^9/l <100
		<0.1	0.1-0.5	0.5-1.0		
IV Bolus Cytosine Arabinoside	28	12.3 (0-31)	6.1 (0-24)	4 (0-28)	22.4	24 (11-40)
Continuous Infusion	20	16.1 (9-25)	4.8 (1-15)	2.3 (0-7)	23.2	22.6 (6-33)

Table 5.

Effect of Cytosine Arabinoside Schedule on the Degree and Duration of Neutropenia and Thrombocytopenia During Cycle 1

Regimen	No. of Patients completing Cycle 2	Mean No of Days Neutrophils x 10^9/l			Mean number of days <1.0 x 10^9/l	Mean No of Days Platelets x10^9/l <100
		<0.1*	0.1-0.5	0.5-1.0		
IV Bolus	19	10.1 (0-35)	3.4 (0-12)	3.6 (0-16)	17.1	17.6 (13-38)
Continuous Infusion	15	15.9 (6-36)	3.0 (0-8)	1.6 (0-7)	21	20.4 (6-38)

* P <0.05

Table 6.

Effect of Cytosine Arabinoside Schedule on the Degree and Duration of Neutropenia and Thrombocytopenia During Cycle 2.

Regimen	Mean No of Days between Cycles I and II	Mean No of Days between Cycles II and III*
IV Bolus	28.1	28.3
Continuous Infusion.	32.3	35.8

*P <0.05

Table 7.

Effect of Cytosine Arabinoside Schedule on Intercycle Time.

platelet count. The intercycle time was also significantly longer between cycles 2 and 3 in patients treated by infusion ($P < 0.05$) (Table 7).

b) Gastrointestinal Tract Toxicity

Gastrointestinal tract toxicity was apparent in 5 patients receiving cytosine arabinoside by infusion, who developed a syndrome characterised by severe diarrhoea, abdominal distention and absent bowel sounds. This was not seen in patients treated by intravenous bolus. A further measure of toxicity is the total number of cycles patients were able to tolerate. Of the complete remitters 9 out of 15 (60%) patients completed 5 or 6 cycles of therapy in the group treated by twice daily intravenous injection, while only 4 out of 14 patients (28%) treated by continuous infusion were able to tolerate more than 4 cycles.

Discussion

Two intensive remission induction regimens comprising doxorubicin, cytosine arabinoside and 6 thioguanine have been compared in terms of efficacy and toxicity in patients with acute myelogenous leukaemia. Leukaemic blasts cleared more rapidly from the peripheral blood and bone marrow when cytosine arabinoside was administered by continuous intravenous infusion than when the same dose was given by twice daily injection, suggesting a greater antileukaemic

effect with the continuous infusions. Complete remission was achieved in 15 out of 41 patients treated by intravenous bolus cytosine arabinoside and 14 out of 31 patients who received cytosine arabinoside by infusion. In the intravenous bolus study, 67% of patients achieving complete remission and 80% of patients under the age of 60 remain in continuous first remission with a minimum follow up of more than 2 years. It is too early to assess the results of the infusion study in terms of remission duration .

The remission rate for the intravenous bolus schedule is lower than that reported by others using similar chemotherapy (Gale et al 1977). Although remission duration is longer than that reported in the latter study, the number of patients is small.

The duration of neutropenia and thrombocytopenia during the first cycle of therapy was similar with both regimens. In the second cycle, the duration of severe neutropenia was greater in patients receiving cytosine arabinoside by infusion. The toxicity associated with the continuous infusion schedule precluded giving the full therapy to the majority of patients. The Cancer and Leukaemia group B data (Rai et al 1979, 1981 a and b) showed that continuous infusions of cytosine arabinoside were more effective than twice daily intravenous bolus cytosine arabinoside given at twice the dose. This suggests that the dose of cytosine arabinoside used for

continuous intravenous infusion need not be the same as that used in the twice daily intravenous bolus schedule, but should rather be that dose which is equitoxic.

Recent data from St. Bartholomew's Hospital suggest that rapid achievement of complete remission may confer an advantage in terms of remission duration (Bell et al 1982).However, in this study patients were given a fixed number of treatment cycles, and it is therefore difficult to distinguish the time to remission from the effect of consolidation, as the patients who entered remission earlier also received more consolidation.

These data show that when used in combination with doxorubicin and 6 thioguanine, continuous infusions of cytosine arabinoside result in more rapid eradication of leukaemia than the same dose given by twice daily intravenous bolus. The toxicity associated with this regimen would be acceptable provided it results in a greater proportion of patients achieving long term remission.

CHAPTER 4

A PHASE I AND II STUDY OF THE USE OF HIGH DOSE CYTOSINE ARABINOSIDE IN PATIENTS WITH ACUTE LEUKAEMIA AND NON HODGKIN'S LYMPHOMA

Introduction.

The majority of patients with acute leukaemia will achieve complete remission when treated with combination chemotherapy. However, although a minority will go on to long term remission and probable cure, most of the remaining patients will relapse within a year. Re-treatment with the same drugs reveals that in many of the patients the leukaemia is now resistant to the therapy to which it previously responded. It is likely that the majority of patients who achieve complete remission still have a significant residual leukaemic burden which is not detectable by conventional methods and that this disease proliferates and manifests a few months or years later as recurrent leukaemia.

The demonstration that much higher doses of cytosine arabinoside given by a short infusion twice daily for several days could overcome resistance to conventional doses , has therefore aroused considerable interest.

Momparler (1974) first demonstrated the potential for high dose cytosine arabinoside in a single patient with acute leukaemia and Rudnick et al (1979) reported a Phase I study in which he demonstrated that this therapy was tolerable. A number of small studies have subsequently been conducted in patients with acute leukaemia and non hodgkin's lymphoma.(Karanes et al 1979 , Capizzi et al 1982 , Early et al 1982 a & b and Herzig et al 1982). Although these studies involved small numbers of patients, they all demonstrate that approximately 50% of

patients with acute myelogenous leukaemia resistant to conventional doses of cytosine arabinoside will achieve complete remission when treated with these high doses. A small number of patients with acute lymphoblastic leukaemia and non hodgkin's lymphoma have also been treated (Early et al 1982a and b).

High dose cytosine arabinoside has been associated with significant toxicity. The median duration of neutropenia and thrombocytopenia has ranged from 3 to 4 weeks. Conjunctivitis and photophobia has been found in all studies but has generally been mild and controlled with topical steroids. A major problem has been the incidence of central nervous system toxicity with up to 20% of patients (Herzig et al 1982 and Early et al 1982a) developing cerebellar ataxia. Several patients have also become somnolent and confused and seizures have been reported in a few patients. Other toxicity such as nausea and vomiting, diarrhoea, skin rashes and abnormal liver function tests are usually mild and transient.

All reported studies of high dose cytosine arabinoside have used 3 g/m^2 twice daily over 4-6 days. This dose was arrived at following the Phase I experience of Rudnick et al (1979) where 3 g/m^2 twice daily was found to be the maximum tolerated dose. In this study the doses ranged from 3 to 7.5 g/m^2. It is thus apparent the dose of cytosine arabinoside has been increased from conventional doses of 100 to 200 mg/m^2 per 24 hours to 6 g/m^2 per 24 hours in a single step without any evaluation being

made of the therapeutic efficacy of intermediate doses. The following Phase I and II study was therefore conducted to evaluate the efficacy and toxicity of $2g/m^2$ cytosine arabinoside twice daily.

MATERIALS AND METHODS

Patients

Thirty seven adults and 3 children were studied.

A. Ten patients with acute myelogenous leukaemia were treated. Seven patients had failed to respond or had relapsed after combination chemotherapy comprising adriamycin, cytosine arabinoside and 6-thioguanine. Three patients who had achieved complete remission on this chemotherapy received high dose cytosine arabinoside as consolidation therapy.

B. Nine patients with acute lymphoblastic leukaemia (6 adults and 3 children) and 1 patient with acute undifferentiated leukaemia who had relapsed after conventional therapy (vincristine, prednisolone, doxorubicin ,L asparaginase and cyclophosphamide) were reinduced with high dose cytosine arabinoside.

C. Five patients with previously untreated blast crisis of chronic myeloid leukaemia (Three lymphoid and 2 myeloid).

D. Fifteen patients with non hodgkin's lymphoma who had relapsed after conventional therapy were also treated; 5 of these

patients had centrocytic lymphoma, 5 immunoblastic lymphoma, 2 centroblastic lymphoma, 2 lymphoblastic lymphoma and 1 Burkitt's lymphoma.

Treatment Schedule

Cytosine arabinoside was administered in normal saline at a dose of $2g/m^2$ as an intravenous infusion over 3 hours. This was repeated every 12 hours for 6 days. A second course was administered to 4 patients. All patients received prophylactic non absorbable antibiotics and prednisolone eye drops were given 2 hourly for 10 days.

Results

Complete remission was achieved in 6 out of 7 patients with acute myelogenous leukaemia, 2 out of 5 patients with blast crisis of chronic myeloid leukaemia (both lymphoid), 3 out of 9 patients with acute lymphoblastic leukaemia and 4 out of 15 patients with non hodgkin's lymphoma (1 lymphoblastic,2 immunoblastic and 1 centroblastic).A short lived response was also seen in a patient with acute undifferentiated leukaemia.

In addition to the complete responses, partial responses were seen in 3 patients with acute lymphoblastic leukaemia and 5 patients with non hodgkin's lymphoma.

Duration of Response

Four of the 6 patients with acute myeloblastic leukaemia who achieved complete remission relapsed within 9 months, and 2 remain disease free. Two of the 3 patients with acute lymphoblastic leukaemia have also relapsed at 2 and 10 months respectively. Only 1 of the 4 patients with lymphoma who achieved complete remission remains disease free, the others having relapsed within 5 months.

Toxicity

Total alopecia occurred in all patients and nausea and vomiting were experienced by most. This was most distressing on the first 2 days of therapy and many patients found the nausea and vomiting less severe than that experienced with their previous therapy. Conjunctivitis was noted in 7 patients despite the use of 2-hourly prednisolone eye drops. In 2 patients vision was diminished to the extent that they could only detect gross movement and could not count the fingers on a hand held in front of them. Slit lamp examination showed clouding of the anterior chamber of the eye. Both patients rapidly recovered full visual acuity after receiving systemic steroids.

Central nervous system abnormalities were noted in 2 patients. A patient with acute lymphoblastic leukaemia who had intrathecal chemotherapy and cranial irradiation developed cerebellar ataxia

after 1 course of high dose cytosine arabinoside. She was treated with oral dexamethasone and recovered completely over the next few weeks. A patient with non hodgkin's lymphoma had a grand mal seizure 6 hours prior to death from septicaemia. A post mortem was not obtained and it is therefore difficult to be certain that this could be attributed to the high dose cytosine arabinoside. An extensive exfoliative dermatitis developed in 1 patient.

The bone marrow toxicity is shown in Table 8. The duration of neutropenia and thrombocytopenia was assessable only in those patients who survived long enough for the bone marrow to recover. It is interesting that the duration of neutropenia and thrombocytopenia was very similar to that found previously using combination chemotherapy comprising cytosine arabinoside, 6-thioguanine and doxorubicin (Chapter 3).

Diagnosis	No of Patients	Neutrophils x 10^9/l 0-0.1	0.1-0.5	0.5-1.0	Platelets <100 x10^9/l
Remission induction of Acute Leukaemia (AML + ALL)	9	14	3	1	25
Remission consolidation (AML)	3	13	2	2	22
Lymphoma	4	14	2	3	27

Table 8

Mean number of days neutropenic and thrombocytopenic after high dose cytosine arabinoside 2g/m^2/day for 6 days.

DISCUSSION

This study has evaluated high dose cytosine arabinoside at a dose of 2 g/m^2 administered as a 3 hour infusion twice daily for two days, rather than the conventional dose of 3 g/m^2. The activity of this therapy has been confirmed, particularly in patients with acute myelogenous leukaemia, in whom a complete remission was achieved in 6 out of 7 patients treated. Patients with high grade non hodgkin's lymphoma were also responsive to this therapy. This data is comparable to that previously reported, and although the patient numbers remain small, there would appear to be no loss of activity with the use of this lower dose.

The nausea and vomiting and the duration of neutropenia and thrombocytopenia are similar to that previously reported at 3 g/m^2. However, the incidence of central nervous system toxicity is much less than that found in other studies, with only 1 out of 40 patients developing cerebellar abnormalities. This patient had previous cranial irradiation and intrathecal chemotherapy. The Roswell Park Memorial Institute noted cerebellar toxicity in 27% of patients in the pilot study, and 13% of patients in the subsequent studies (Early et al, 1982a & b). Similarly, Herzig et al (1982) found that 22% of patients developed cerebellar ataxia. The patients treated in the above studies were given the cytosine arabinoside as a 1 hour infusion compared with the 3 hour infusion used here. It is, however,

unlikely that this difference in schedule would account for the lower incidence in cerebellar toxicity, as it has been shown that cerebrospinal fluid concentrations of cytosine arabinoside are similar irrespective of whether the infusion is given over 1 or 3 hours (Chapter 6). It was demonstrated in the same study that cerebrospinal fluid cytosine arabinoside concentrations have a linear relationship to the dose administered over a range of 1-3 g/m^2. This probably accounts for the lower incidence of central nervous system toxicity found in the patients described here.

Further studies of high dose cytosine arabinoside at intermediate doses such as 1 and 2 g/m^2 need to be conducted. In addition the duration of therapy needs to be further evaluated in order to achieve the optimal balance between efficacy and toxicity.

CHAPTER 5

THE PHARMACOKINETICS OF SUBCUTANEOUS CYTOSINE ARABINOSIDE IN PATIENTS WITH ACUTE MYELOGENOUS LEUKAEMIA.

THE PHARMACOKINETICS OF SUBCUTANEOUS BOLUS CYTOSINE ARABINOSIDE

Introduction

The S phase specificity of cytosine arabinoside has led most workers to administer it by continuous intravenous infusion (Weinstein et al 1980 and McCreadie et al 1981), despite the fact that some of the clinical evidence supporting this method of administration is contradictory (Frei et al 1969 and Burke et al 1968). The data presented in chapter 3 demonstrates that even when used as part of combination chemotherapy, continuous intravenous infusions of cytosine arabinoside result in more rapid eradication of leukaemia than the same dose given by twice daily intravenous bolus. This suggests that the maintenance of sustained plasma levels of cytosine arabinoside is a worthwhile goal.

When administered by intravenous bolus injection, cytosine arabinoside is rapidly deaminated, and consequently single intravenous doses have little effect on the bone marrow. Recently, subcutaneous bolus administration of cytosine arabinoside has been used as a practical alternative to intravenous infusion(Rees et al 1977 and Bell et al 1982). The rationale for this method of administration was based on the convenience to the patient, and the assumption that absorption from the subcutaneous tissues would be slow and would thus approximate a continuous infusion.

Little is known of the pharmacokinetics of subcutaneous cytosine arabinoside in man. The only study that has been

reported (Finklestein et al 1970) is unassessable because of the inability to separate cytosine arabinoside from the inactive metabolite uracil arabinoside.

This study was therefore undertaken to determine the kinetics of cytosine arabinoside when administered by subcutaneous bolus injection to patients with acute myelogenous leukaemia.

Materials and Methods

Patients

Five adult patients with acute myelogenous leukaemia were studied. All the patients received cytosine arabinoside 200 mg/m^2 per day for 7 days. In addition they received doxorubicin 25 mg/m^2 on days 1, 2 and 3 and 6 thioguanine 200 mg/m^2 per day for 7 days. The patients were studied during the first 3 days of treatment. On the first day they received cytosine arabinoside 100mg/m^2 12 hourly by intravenous bolus injection. On the second day cytosine arabinoside was given at a dose of 100mg/m^2 12 hourly by subcutaneous bolus and on the third day it was administered by continuous intravenous infusion over 12 hours at the same dose. In order to achieve a steady state rapidly in the infusion studies, a loading dose of cytosine arabinoside was administered at the start of the infusion. This was given at a dose of three times the hourly rate of infusion as suggested by Wan et al (1974). Samples were taken following cytosine arabinoside injection for 12 hours on each of the 3 successive days of the study. Blood (4 ml) was taken at the

following intervals during the first hour: 3, 6, 10, 15, 21, 30, 45 and 60 minutes, and then at 1.5, 2, 2.5, 3, 4, 6, 8 , 10 and 12 hours. Blood was taken into tubes containing tetrahydrouridine ($1x10^{-5}$ M) and 50 units of heparin. Details of the plasma separation and storage,and the radioimmunoassay have been described in chapter 2.

Results

The mean plasma levels of cytosine arabinoside versus time for the intravenous bolus, subcutaneous bolus and continuous intravenous infusion are displayed in Figure 22. After an intravenous bolus injection, plasma cytosine arabinoside levels declined triexponentially. The mean half life for the initial phase was 1.9 $\pm$ 0.6 minutes,for the intermediate phase 14.2 $\pm$ 2.9 minutes and for the terminal phase 8.4 $\pm$ 8.5 hours. Following subcutaneous administration there was a rapid absorption phase (mean half life of absorption was 3.4 $\pm$ 2.1 minutes), and then the decline in cytosine arabinoside was biexponential with a mean initial half life of 15.7$\pm$ 10.7 minutes, and a mean terminal half life of 1.4 $\pm$ 0.2 hours. The individual patients' pharmacokinetic parameters are shown in Tables 9 and 10. In patients E.M. and G.W. erratic values were obtained during the elimination phase of the intravenous bolus and these gave rise to the extended terminal half lives. Levels were above steady state infusion levels for only 40 minutes following an intravenous bolus and for 100 minutes following the subcutaneous bolus. The decline in

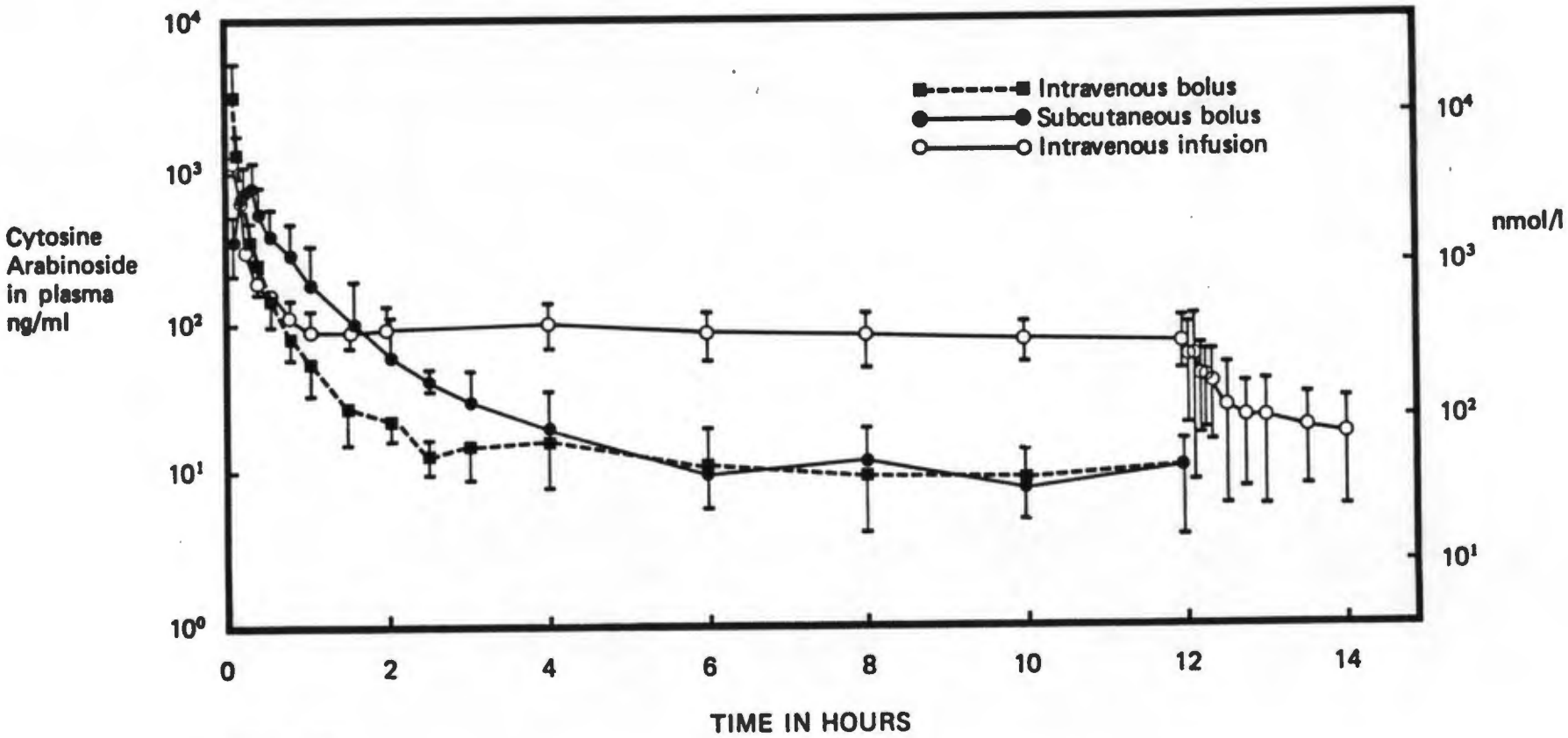


Figure 22

Mean plasma concentration (± SD) after intravenous and subcutaneous bolus and during intravenous infusion of cytosine arabinoside (100mg/m^2) in 5 patients with myelogenous leukaemia.

PATIENT	IV HALF LIVES			Area Under the Curve ng/ml h	Volume of Distribution (area) l	Clearance l/min	Post Infusion Half Life B (hr.)
	∝ 1 min	∝ 2 min	B hour				
J.A.	2.8	18.4	3.1	533	1430	5.3	1.6
E.M.	1.1	12.3	16.1	574	7404	4.9	1.2
P.W.	2.0	12.3	2.2	463	995	6.1	1.2
J.S.	1.6	16.0	1.5	472	764	6.0	1.6
G.W.	2.1	11.8	19.0	1011	4203	2.8	2.4
Mean	1.9	14.2	8.4	611	2959	5.0	1.6
S.D.	0.6	2.9	8.5	228	2842	1.3	0.5

Table 9.

Pharmacokinetics of Cytosine Arabinoside after Intravenous Bolus (100 mg/m^2) and following Completion of Intravenous Infusion in patients with Acute Myeloblastic Leukaemia

PATIENT	Subcutaneous Bolus Half Lives			Area Under the Curve ng/ml h	Volume of Distribution (area) l	Clearance l/min
	Ka min	α min	B hr			
J.A.	1.4	11.4	1.3	562	567	5.0
E.M.	1.9	7.3	1.2	473	622	5.9
P.W.	5.3	8.7	1.4	483	711	5.9
J.S.	6.0	33.4	1.6	1085	361	2.6
G.W.	2.2	17.5	1.3	833	382	3.4
Mean	3.4	15.7	1.4	687	528	4.6
S.D.	2.1	10.7	0.2	265	152	1.5

Table 10.

Pharmacokinetics of Cytosine Arabinoside after Subcutaneous Bolus (100mg/m^2) in Patients with Acute Myeloblastic Leukaemia.

plasma cytosine arabinoside concentrations continued rapidly following both these routes of administration and after 5 hours were approximately 10% of the steady state infusion levels.

Discussion

The pharmacokinetics of intravenous bolus injection of cytosine arabinoside reported here are similar to those described by others using different methods of assay including tritiated drug (Ho et al 1971), high performance liquid chromatography (Kreis et al 1977) and gas chromatography-mass spectrometry (Harris et al 1979). The majority of published reports describe biphasic elimination rather than the triphasic elimination reported here. However Harris et al (1979), using frequent early sampling, found triphasic elimination in two patients, and van Prooijen et al (1977a) found that the initial phase could be divided into two exponentials.

The terminal half lives following the intravenous bolus, subcutaneous bolus, and discontinuation of the continuous infusion are all similar with the exception of the prolonged half lives following the intravenous bolus in patients E.M and G.W. The prolonged half lives were associated with erratic cytosine arabinoside concentrations during the terminal phase. These erratic points were reassayed twice and found to represent real values and not analytical errors.

A number of possible explanations for the erratic levels and prolonged half lives in these two patients can be considered. Enterohepatic recycling is a possibility. However this is unlikely for a number of reasons. Cytosine arabinoside has a low molecular weight, and enterohepatic recycling is uncommon for compounds with a molecular weight of less than three hundred (Creasey 1979). In addition it is poorly absorbed via the oral route (Goldenberg et al 1968, Finklestein et al 1970 and Ho et al 1971) and has a high hepatic extraction ratio with deamination to the inactive metabolite. This metabolite, uracil arabinoside, is not detected by the radioimmunoassay used in this study.

The data was further examined by calculating the residuals from the fitted regression line for the terminal phase in these two patients. This showed that there was random scatter above and below the line and there was no evidence of a post prandial peak such as might have occurred with enterohepatic recycling.

Another possible explanation for the erratic values and prolonged half lives is that they may be due to the release from leukaemic blasts of phosphorylated cytosine arabinoside nucleotides which cross react with the radioimmunoassay.It is interesting that a similar plateau was found by Harris et al (1979) when plasma cytosine arabinoside levels were measured with the bioassay but not with gas chromatography - mass spectrometry.

It was hoped that cytosine arabinoside would be released slowly from subcutaneous tissues, and plasma levels might thus approximate a continuous infusion. However, the data demonstrate that the pharmacokinetics of subcutaneous cytosine arabinoside bolus more closely approximate that of an intravenous bolus rather than a continuous intravenous infusion. The plasma cytosine arabinoside concentrations following subcutaneous bolus are higher than those following an intravenous bolus only during the first few hours. However, recent data from the Cancer and Leukaemia Group B (Rai et al 1979, 1981 a and b) have shown that the duration of remission of patients with acute myelogenous leukaemia is much greater when maintenance therapy is administered by subcutaneous bolus rather than intravenous bolus cytosine arabinoside. This suggests that relatively small differences in cytosine arabinoside pharmacokinetics may significantly affect patient outcome.

THE PHARMACOKINETICS OF SUBCUTANEOUS CYTOSINE ARABINOSIDE IN AN ARACHIS OIL PLUS ALUMINIUM DISTEARATE SUSPENSION

Introduction

It has been demonstrated that cytosine arabinoside is rapidly absorbed following subcutaneous bolus administration and then declines with a half life similar to that of intravenous bolus cytosine arabinoside, and that prolonged plasma levels can only be obtained by continuous intravenous infusions. However, subcutaneous bolus injection is considerably more convenient for the patient than continuous intravenous infusion and it would clearly be advantageous to have a slow release formulation of cytosine arabinoside that could be administered by subcutaneous injection.

Several formulation approaches could be used to develop a parenteral slow release preparation of cytosine arabinoside. Ho et al (1977) studied water insoluble 5` esters of cytosine arabinoside and these were not successful. Cyclocytidine, a cytosine arabinoside analogue that is resistant to deamination but is slowly hydrolysed to cytosine arabinoside, successfully prolongs the terminal half life of cytosine arabinoside. However, the induction of postural hypotension which was not dose related is likely to limit the usefulness of this drug (Sakai et al 1972, Lokich et al 1975 and Finklestein et al 1979). Further attempts to delay release of cytosine arabinoside have involved dispersion in encapsulated polymer complexes and the use of liposomes. These approaches are limited by the practical difficulties discussed earlier.

The use of vegetable oils together with an aluminium stearate results in a thixotropic preparation which is viscous on standing but becomes liquid when shaken. This vehicle has been used to create delayed release suspensions of lipid insoluble drugs such as procaine penicillin (Buchi et al 1948) and the cytotoxic drug bleomycin (Ikeda et al 1976 and Kimura et al 1976). These preparations delay the release of drugs over a period of hours rather than weeks or months which may occur when insoluble esters are formed. This formulation has the advantage of simplicity of preparation, and the viscosity can be controlled by adjusting the concentration of the aluminium stearate. Furthermore procaine penicillin in arachis oil and aluminium monostearate has been extensively used in patients without adverse affect. This approach was therefore adopted in an attempt to create a delayed release formulation of cytosine arabinoside.

Materials and Methods.

Formulation.

The freeze dried cytosine arabinoside powder was produced by Upjohn. Aluminium mono and distearate were supplied by Glaxo Pharmaceuticals. The vegetable oil used was arachis oil B.P. Aluminium mono and distearates were dissolved in arachis oil by heating to 160 ^{o}C for 1 hour. This was then filtered using a Watson-Marlow peristaltic pump and then autoclaved to

sterilise. Suspensions of cytosine arabinoside (200 mg/ml) in arachis oil, arachis oil plus aluminium monostearate, and arachis oil plus aluminium distearate were prepared with a mortar and pestle in a vertical laminar flow cabinet, and were aliquotted into 2 ml sterile vials.

One hundred microlitre samples of the suspension were extracted into 10 millilitres of water by shaking for 60 seconds in an automated shaker, and then centrifuged at 2000 revs per minute for 15 minutes. Evenness of distribution within the suspension was assessed by assaying several samples from individual aliquots and samples were also assayed to assess the within batch and between batch variation. The stability of cytosine arabinoside in the suspension was determined by assaying aliquots from samples that had been kept at room temperature on 6 occasions over 2 weeks. Sterility testing was carried out by the Pharmacy Department at St. Bartholomew's Hospital. Preliminary acceptability testing was performed by administering the arachis oil plus aluminium distearate solution, without cytosine arabinoside, by subcutaneous injection to 10 normal volunteers.

In Vivo Investigations

Rabbit Studies

Nine New Zealand White female rabbits weighing between 3 and

3.5 kg were studied on 2 consecutive days. The dose of cytosine arabinoside received was 30 mg/kg on each occasion. Six rabbits received cytosine arabinoside in saline on day 1 and cytosine arabinoside in arachis oil plus 1.5% aluminium distearate on day 2. The remaining 3 rabbits received cytosine arabinoside in saline on day 1 and cytosine arabinoside in arachis oil with 4% aluminium distearate on day 2. The cytosine arabinoside was injected subcutaneously 3cm lateral to the midspine. Blood samples (1ml) were obtained from the marginal vein of the ear at the following times on each day of the study: 0, 5,10,15,20,30 and 45 minutes and 1,1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 hours. Three of the 6 rabbits receiving 1.5% aluminium distearate were sacrificed on day 5 and 3 on day 10. The 3 rabbits receiving the 4% aluminium distearate were all sacrificed on day 5. The site of the injection was excised and fixed in formalin for 48 hours and a paraffin section was stained with haematoxylin and eosin and examined histologically, by Dr.D Levison of the Department of Histopathology at St Bartholomew's Hospital, for signs of tissue necrosis.

Patient Studies

Six patients receiving remission induction or consolidation therapy for acute myelogenous leukaemia who had platelet counts greater than 100 x 10^9/l were studied on the first 3 days of treatment. Cytosine arabinoside was administered in 3 different formulations: cytosine arabinoside in saline,

cytosine arabinoside in arachis oil plus 1.5% aluminium distearate and cytosine arabinoside in arachis oil plus 2.5% aluminium distearate. Patients were randomised as to the order in which they received the different formulations of cytosine arabinoside. Cytosine arabinoside (100 mg/m^2) was injected subcutaneously over the deltoid muscle. The site of injection was examined regularly over the next few days and weekly over the next month. Blood samples (4ml) were taken at the following times: 0, 5, 10, 15, 20, 30 and 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 hours. Blood samples were collected into tubes containing tetrahydrouridine ($1x10^{-5}$ M) and 50 units of heparin. Separation and storage of plasma and the radioimmunoassay of cytosine arabinoside have been described in chapter 2.

Results

Formulation

The arachis oil plus aluminium monostearate preparations (1-3%) were not sufficiently thixotropic and the cytosine arabinoside settled into a firm precipitate that was difficult to resuspend. Cytosine arabinoside was however maintained in suspension when aluminium distearate in concentrations greater than 1%(w/v) was used.The efficiency of the extraction of cytosine arabinoside from the arachis oil plus aluminium distearate preparation into water was 70%, and cytosine arabinoside was evenly distributed throughout

the suspension. Furthermore the cytosine arabinoside was stable for at least 2 weeks at room temperature. Sterility testing carried out in the Pharmacy Department at St. Bartholomew's Hospital showed no evidence of bacterial contamination. In 10 normal volunteers who received the arachis oil plus aluminium distearate preparation without cytosine arabinoside given subcutaneously over the deltoid muscle, there was minimal discomfort for approximately 5 minutes after the injection and no inflammatory response or induration.

Rabbit Studies

The New Zealand White rabbits treated with the cytosine arabinoside in arachis oil plus aluminium distearate did not show any clinical evidence of inflammation or induration at the site of injection. The mean plasma cytosine arabinoside concentrations obtained in the rabbits after subcutaneous injection of cytosine arabinoside in saline, cytosine arabinoside in arachis oil with 1.5% aluminium distearate and cytosine arabinoside in arachis oil with 4% aluminium distearate are shown in Figures 23 and 24. The results obtained from the individual rabbits are shown in Tables 11,12,13 and 14.The pharmacokinetics of cytosine arabinoside were not influenced by the arachis oil plus aluminium distearate preparation at either the 1.5 or 4% concentration. The area under the curve was similar for all 3 methods of administration showing that the bioavailability of cytosine

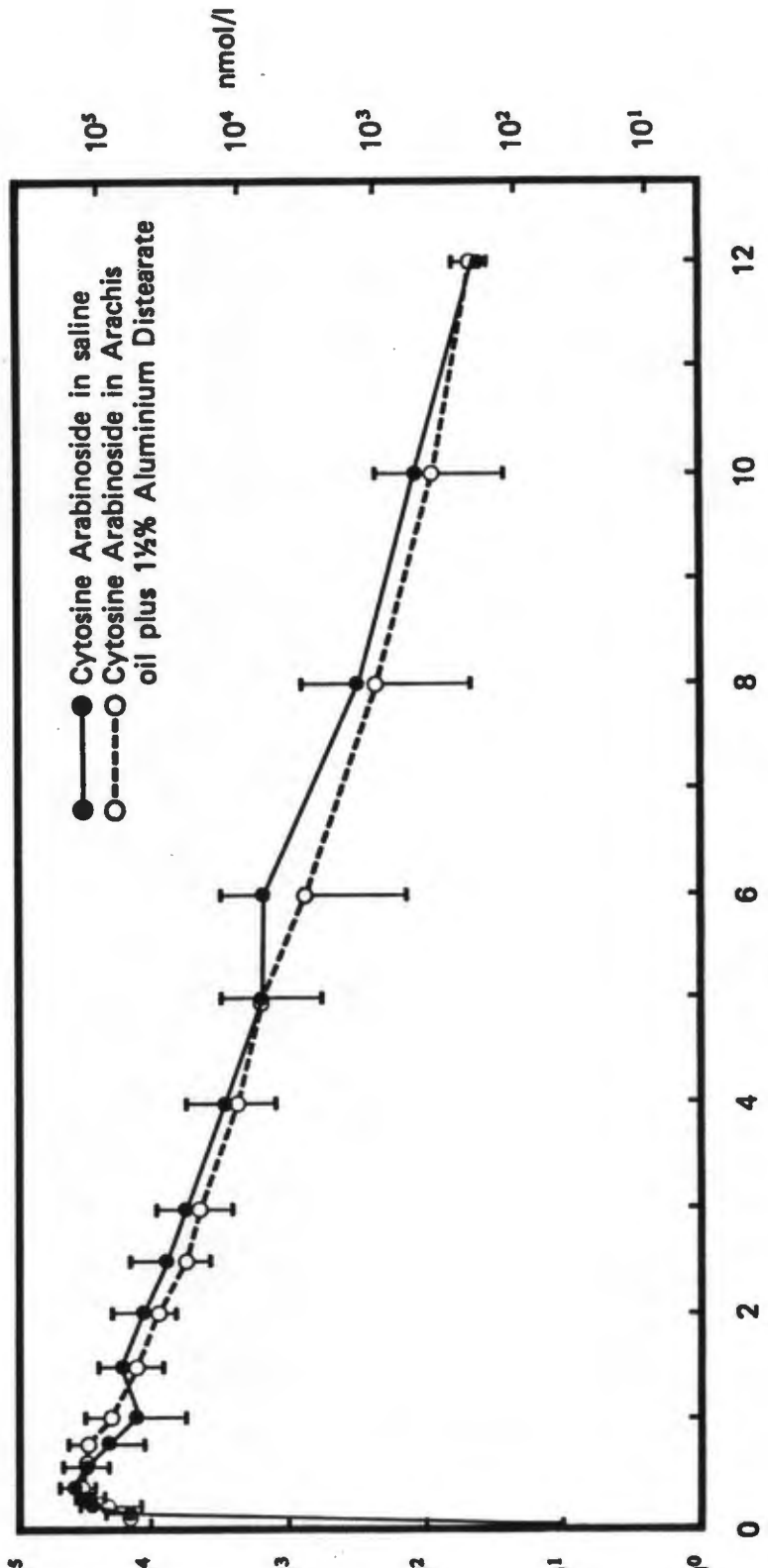


Figure 23

The pharmacokinetics of cytosine arabinoside (30mg/Kg in saline and arachis oil plus 1.5% aluminium distearate in New Zealand white rabbits.

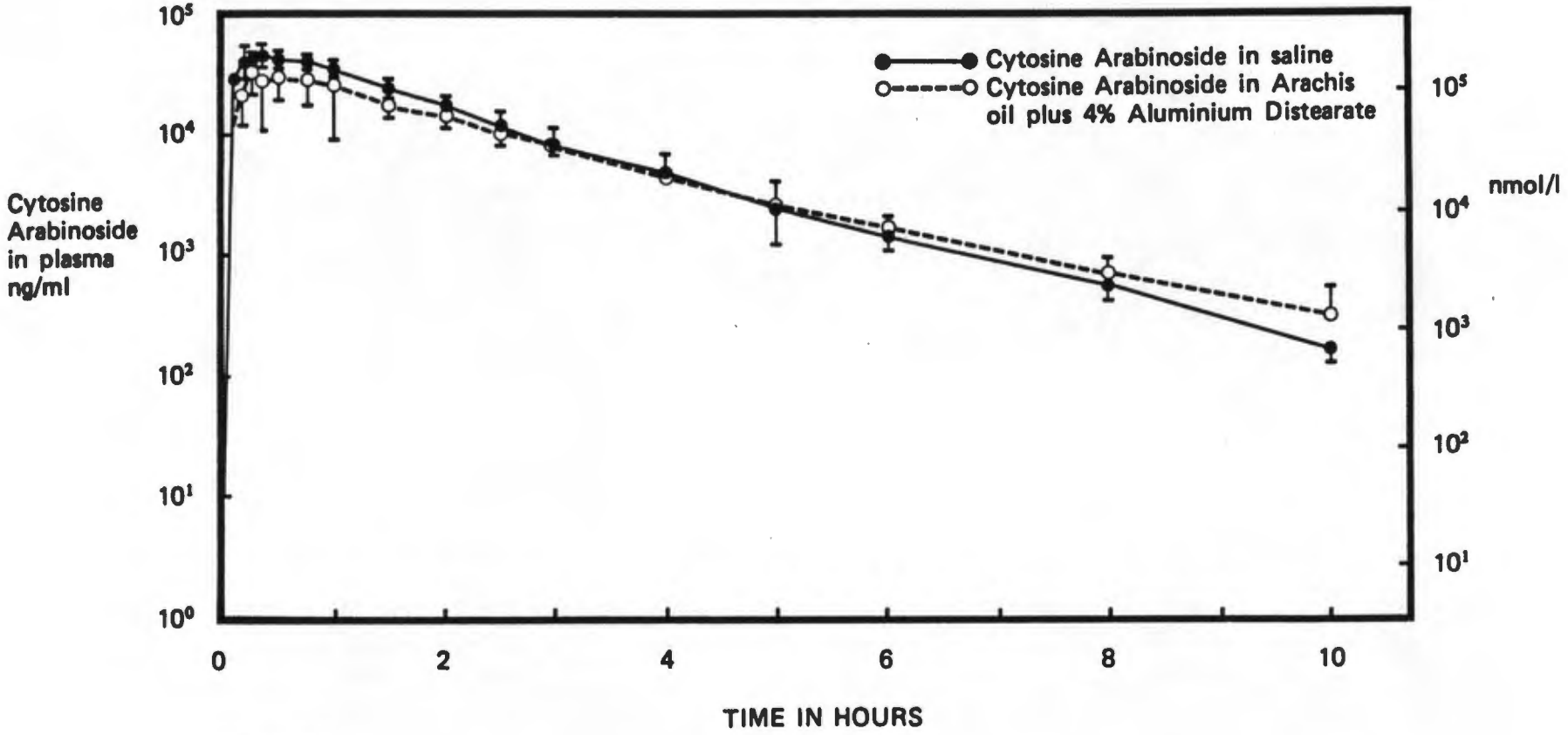


Figure 24

The pharmacokinetics of cytosine arabinoside (30mg/Kg) in saline and arachis oil plus 4% aluminium distearate in New Zealand white rabbits.

RABBIT	HALF LIVES		Area Under the Curve ng/ml h	Volume of Distribution (area) l	Clearance ml/min
	Ka min	B hours			
A	2.4	0.8	39048	3.0	42.7
B	3.4	1.2	86651	2.1	19.2
C	4.2	1.0	47060	3.1	35.4
Mean	3.3	1.0	57586	2.7	32.4
S.D.	0.9	0.2	25487	0.5	12.0

Table 11.

Pharmacokinetics of Cytosine Arabinoside (30 mg/kg) in Saline in New Zealand White Rabbits.

RABBIT	HALF LIVES		Area Under the Curve ng/ml h	Volume of Distribution (area) l	Clearance ml/min
	Ka min	B hours			
A	6.0	0.8	39008	2.9	42.5
B	5.4	1.1	72745	2.2	22.9
C	3.0	1.1	48830	3.3	34.1
Mean	4.8	1.0	53528	2.8	33.1
S.D.	1.6	0.2	17352	0.5	9.8

Table 12.

Pharmacokinetics of Cytosine Arabinoside (30 mg/kg) in Arachis Oil Plus 1.5% Aluminium Distearate in New Zealand White Rabbits.

RABBIT	HALF LIVES		Area Under the Curve ng/ml h	Volume of Distribution (area) l	Clearance ml/min
	Ka min	B hours			
D	7.8	2.1	92561	3.3	18.0
E	15.6	1.2	101256	1.6	16.0
F	6.6	1.4	79985	2.6	20.8
Mean	10.0	1.6	91267	2.5	18.3
S.D.	4.9	4.7	10694	0.8	2.4

Table 13.

Pharmacokinetics of Cytosine Arabinoside (30 mg/kg) in Saline in New Zealand White Rabbits.

RABBIT	HALF LIVES		Area Under the Curve ng/ml h	Volume of Distribution (area) l	Clearance ml/min
	Ka min	B hours			
D	7.2	1.3	73416	2.5	22.7
E	10.8	1.4	62107	3.3	26.8
F	3.0	1.5	62002	3.5	26.9
Mean	7.0	1.4	65841	3.1	25.5
S.D.	3.9	0.1	6559	0.5	2.4

Table 14.

Pharmacokinetics of Cytosine Arabinoside (30 mg/kg) in Arachis Oil Plus 4% Aluminium Distearate in New Zealand White Rabbits.

arabinoside for the arachis oil plus aluminium distearate preparation was equivalent to cytosine arabinoside in saline. Histological examination of the sites of injection showed signs of a moderate inflammatory response in some specimens after the 4% aluminium distearate preparation but not with the other two formulations. There was no evidence of fat necrosis with the 1.5% formulation but small areas of fat necrosis were seen with the 4% suspension (Figures 25 and 26).

Patient Studies

The patients experienced less discomfort with the oil based cytosine arabinoside preparation than conventional cytosine arabinoside in saline. In 2 patients a palpable subcutaneous induration that was slightly tender was present for 2 to 3 weeks after the injection, but there was no sign of an acute inflammatory response in any patient. The mean plasma levels of cytosine arabinoside for these patients following the 3 different cytosine arabinoside formulations are shown in Figure 27 and the individual patients' results in Tables 15, 16 and 17. As with the rabbit studies, the plasma levels of cytosine arabinoside were not influenced by the arachis oil plus aluminium distearate formulation. The bioavailability as measured by the area under the curve was similar for both methods of administration.

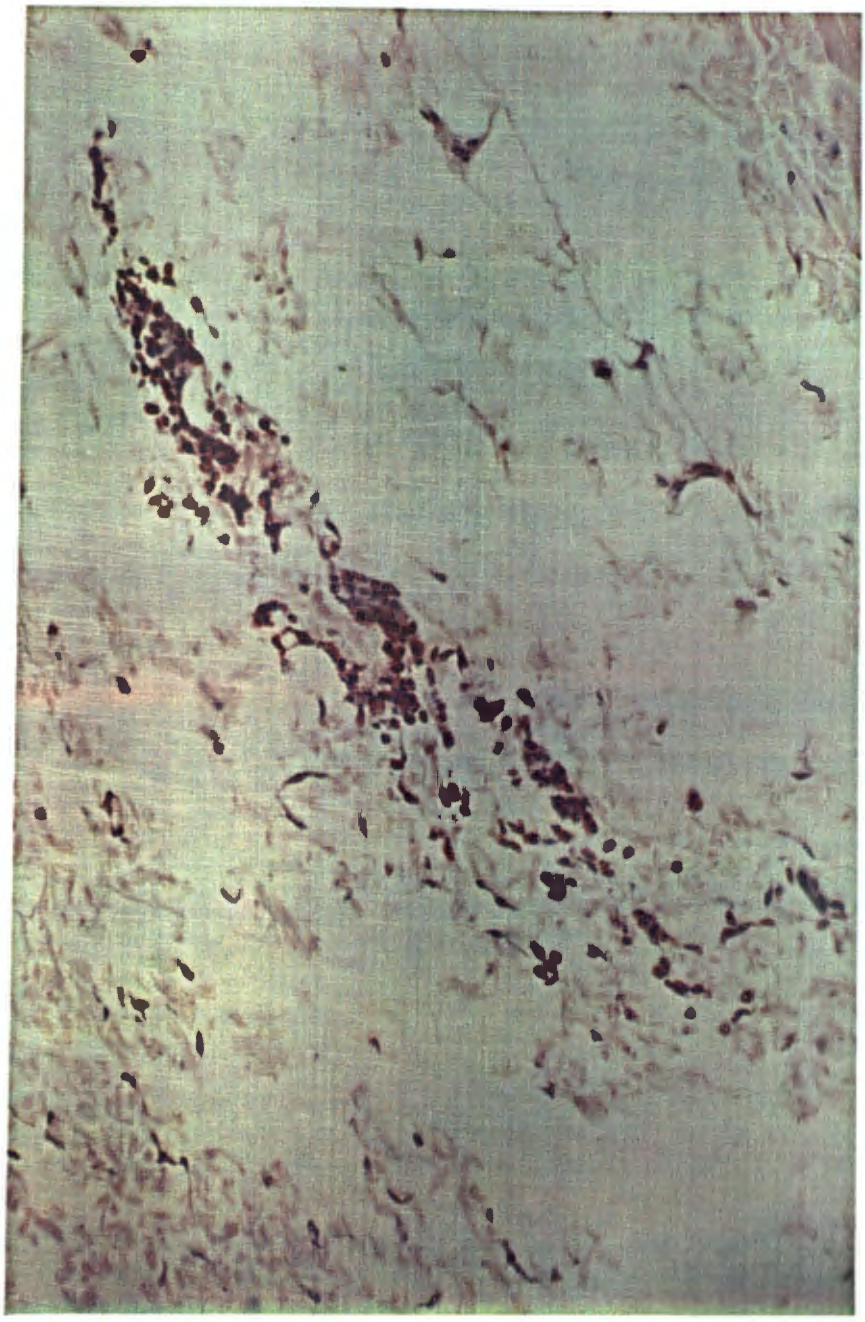


Figure 25

Injection site following subcutaneous administration of cytosine arabinoside in saline to New Zealand white rabbits.

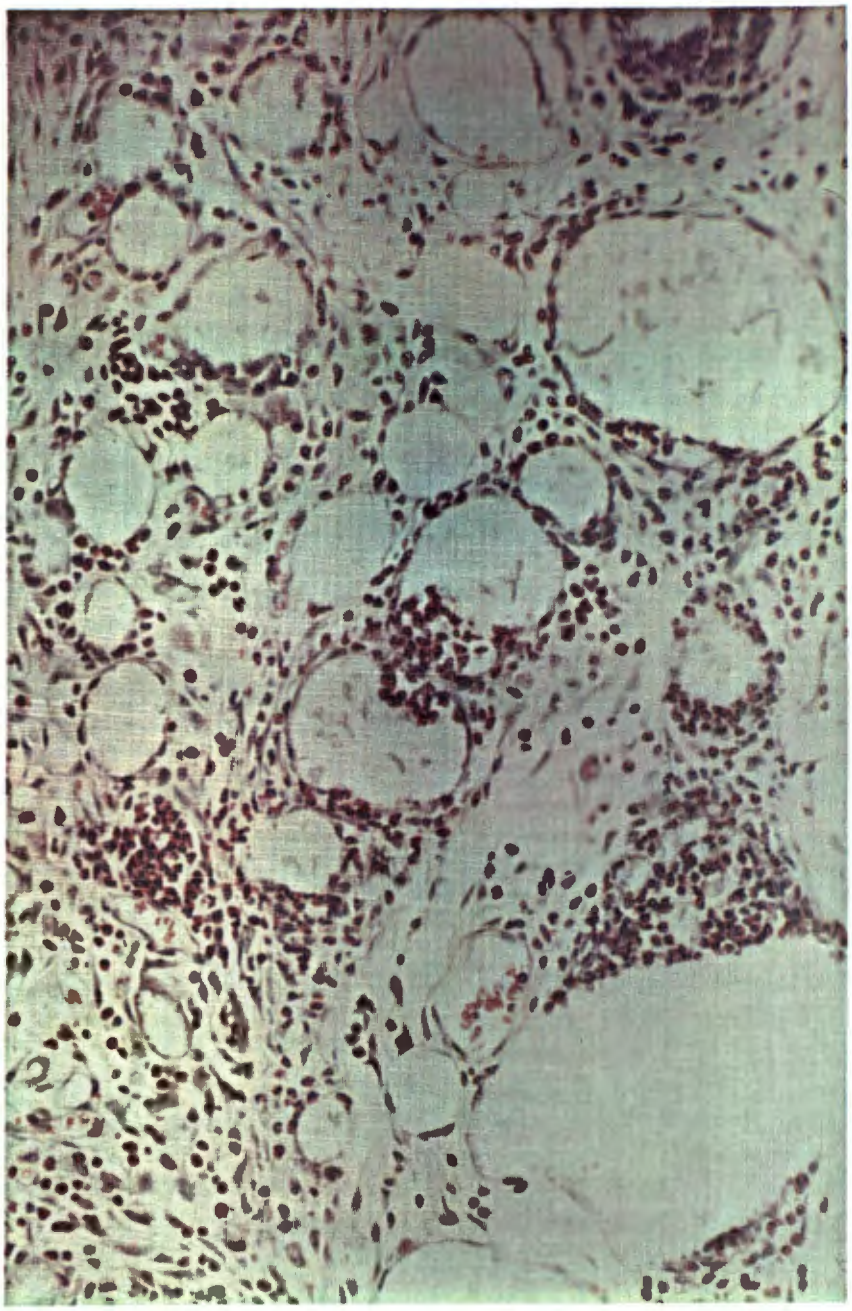


Figure 26

Injection site following subcutaneous administration of cytosine arabinoside in arachis oil plus 4% aluminium distearate to New Zealand white rabbits.

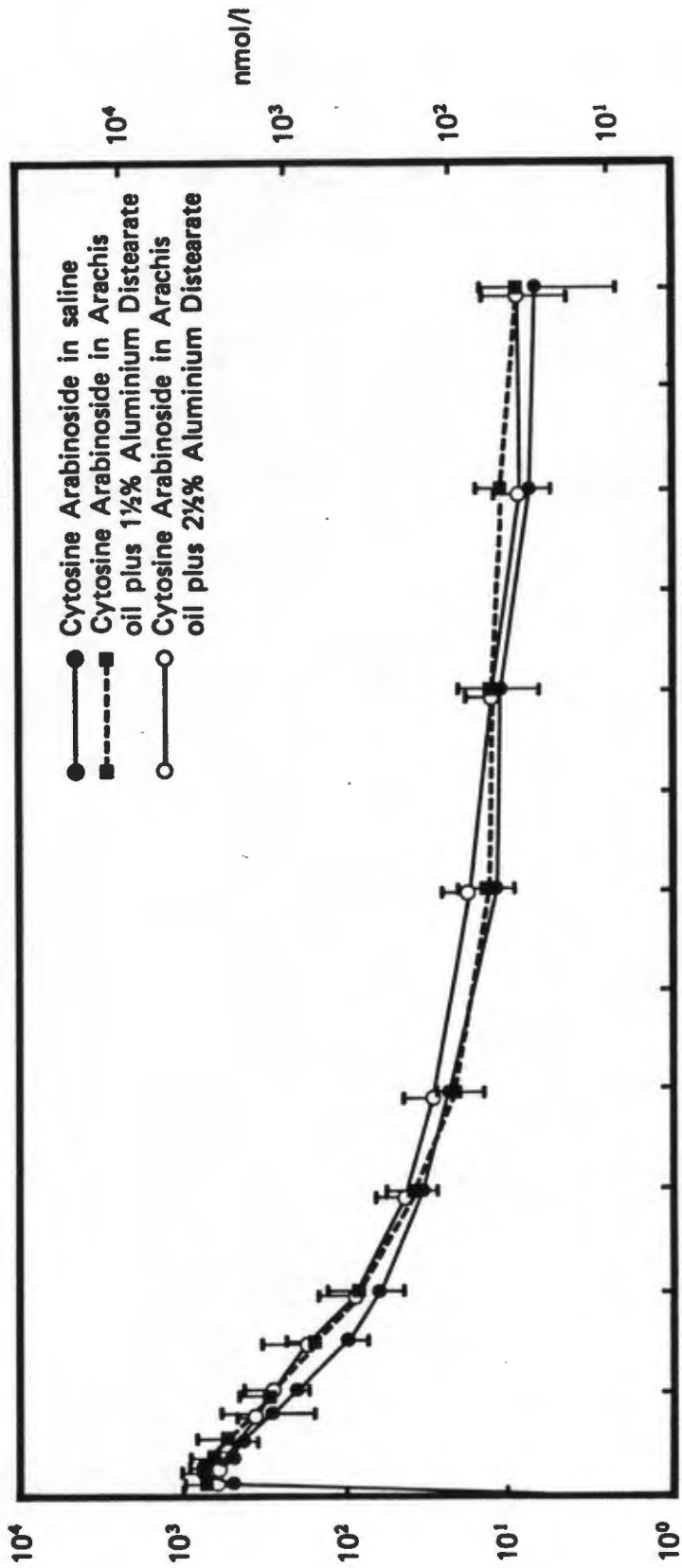


Figure 27

The pharmacokinetics of cytosine arabinoside (100mg/m^2) in saline and arachis oil plus 1.5% and 2.5% aluminium distearate in patients with acute myelogenous leukaemia.

PATIENT	Half Lives			Area Under the Curve ng/ml h	Volume of Distribution area) l	Clearance l/min
	Ka min	α min	B hours			
S.J.	-	19.8	2.2	518	871	4.5
J.C.	2.4	13.8	1.5	653	482	3.7
J.R.	7.8	13.8	2.0	577	713	4.0
G.B.	-	16.2	1.2	702	344	3.3
Mean	5.1	15.9	1.7	612	602	3.9
S.D.	3.8	2.8	0.4	81	235	0.5

Table 15.

Pharmacokinetics of Subcutaneous Cytosine Arabinoside (100mg/m^2) in Saline

PATIENT	Half Lives			Area Under the Curve ng/ml h	Volume of Distribution (area) l	Clearance l/min
	Ka min	∝ min	B hours			
S.J.	-	37.8	2.8	694	807	3.4
J.C.	-	29.4	1.5	823	383	2.9
J.R.	4.8	21.6	1.4	1183	241	2.0
G.B.	-	21	2.8	438	1643	6.8
Mean	4.8	27.4	2.1	784	768	3.8
S.D.	-	7.9	0.8	310	630	2.1

Table 16.

Pharmacokinetics of Subcutaneous Cytosine Arabinoside (100mg/m^2) in Arachis Oil Plus 1.5% Aluminium Distearate in Patients with Acute Myeloblastic Leukaemia.

PATIENT	Half Lives			Area Under the Curve ng/ml h	Volume of Distribution (area)	Clearance l/min
	Ka min	∝ min	B hours			
S.J.	9	23.4	4.8	568	1711	4.1
J.C.	-	56.4	1.5	730	429	3.3
J.R.	-	58.8	1.7	1222	281	1.9
G.B.	3	11.4	1.3	595	567	5.0
Mean	6	37.5	2.3	779	747	3.6
S.D.	4.2	23.7	1.6	304	653	1.3

Table 17.

Pharmacokinetics of Subcutaneous Cytosine Arabinoside (100 mg/m^2) in Arachis Oil Plus 2.5% Aluminium Distearate in Patients with Acute Myeloblastic Leukaemia.

Discussion

The use of an arachis oil plus aluminium distearate vehicle for administering cytosine arabinoside parenterally does not delay its release from the subcutaneous tissues. It had been expected that the dissolution time would be delayed by this oily thixotropic preparation. However, the data suggest that cytosine arabinoside goes into solution in the interstitial fluid almost immediately and is then rapidly absorbed . Although results of the the rabbit experiments were negative this formulation was given to patients. The skin and subcutaneous tissue in rabbits is very loosely fixed to the underlying muscular tissue allowing rapid dispersion of the suspension. It was felt the resulting increase in surface area might increase the rate of dissolution of the cytosine arabinoside and thus not be representative of the human situation.

It has been reported that this formulation is an effective method of delaying the absorption of procaine penicillin (Buchi et al 1948) and bleomycin (Ikeda et al 1976 and Kimura et al 1976) after intramuscular injection. Procaine penicillin is however poorly water soluble in contrast to cytosine arabinoside which has a very low oil:water partition coefficient. The comparative pharmacokinetics of bleomycin in saline and bleomycin oil suspension are reported in 2 studies. While the data shown demonstrate a similar rate of

absorption and elimination for both formulations, the area under the curve is much greater with the Bleomycin oil suspension. This suggests that the dose of bleomycin administered may not have been the same in both instances.

In conclusion this data demonstrates that a vegetable oil based thixotropic preparation of cytosine arabinoside does not delay the rate of absorption from subcutaneous tissues and casts some doubt as to the efficacy of this formulation as an effective method of delaying release of water soluble drugs.

THE PHARMACOKINETICS OF SUBCUTANEOUS INFUSIONS OF CYTOSINE ARABINOSIDE

Introduction

The potential therapeutic advantages of continuous intravenous infusion of cytosine arabinoside have to be balanced against the disadvantages of hospitalisation and the medical and nursing supervision required to establish and maintain intravenous therapy . Furthermore, prolonged intravenous cannulation frequently results in thrombophlebitis, which compromises the patient's future venous access. For many patients, the discomfort associated with repeated re-siting of intravenous cannulae is one of the most unpleasant aspects of remission induction chemotherapy for acute leukaemia.

Having failed to achieve sustained release of cytosine arabinoside with an arachis oil and aluminium distearate formulation other alternatives to continuous intravenous infusion were examined. The rapid absorption of cytosine arabinoside from the subcutaneous tissues, the lack of local excoriation and the high aqueous solubility made it likely that subcutaneous infusion would provide a rational alternative to intravenous infusion. This method of administration has been used for continuous infusions of insulin (Pickup et al 1980) and desferrioxamine (Jefferys et al 1979). Furthermore Weinstein et al (1978) recently compared steady state levels of cytosine arabinoside in 3 patients who received both intravenous and subcutaneous infusions for greater than 24 hours and similar plasma concentrations of cytosine arabinoside were demonstrated.

The time taken to achieve these steady state levels by both methods of administration was not, however, examined.

This study was therefore undertaken to determine whether continuous subcutaneous infusion of cytosine arabinoside would be tolerated by the patients, and whether the time to reach a plateau and steady state levels achieved would be comparable to continuous intravenous infusions.

Materials and Methods

Patients

Six patients with acute myelogenous leukaemia receiving remission induction or consolidation therapy with cytosine arabinoside (200 mg/m^2 per day x 7), doxorubicin (40 mg/m^2 on day 1 and 35 mg/m^2 on day 2) and 6 thioguanine (200 mg/m^2 per day for 7 days) were studied. All patients had a platelet count of greater than 100 $x10^9$ /l at the time of the study. They were investigated during the first two days of treatment. On one day they received cytosine arabinoside 100 mg/m^2 by continuous intravenous infusion and on the other day 100 mg/m^2 by continuous subcutaneous infusion, both over 12 hours. At least 3 hours were allowed between the end of one infusion and the start of the second in order to allow cytosine arabinoside plasma levels to decline. Three of the patients received the intravenous infusion first and the other three started with the subcutaneous infusion. Infusions

were administered via a Handley Intravenous Injector. The pump is designed to take a 20 ml syringe but for the purpose of the subcutaneous infusion it was modified to take a 1 ml insulin syringe. The 12 hour infusions of cytosine arabinoside were delivered in 22 ml of saline for the intravenous infusions, and 1.1 ml of saline for the subcutaneous infusions (Figure 28).

Blood Sampling

Blood samples were taken during the cytosine arabinoside infusions for 12 hours on each of the study days. Blood (4 millilitres) was taken at 0, 15, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 5, 6, 8, 1 0 and 12 hours. The samples were taken into precooled tubes containing tetrahydrouridine (10^{-5} M) and 50 units of heparin. The separation and storage of plasma and the radioimmunoassay have been described fully in the Materials and Methods section of this thesis (Chapter 2).

Results

The mean plasma levels of cytosine arabinoside versus time $\pm$ standard deviation, in the six patients are shown in Figure 29. The mean plasma concentrations of cytosine arabinoside reached a plateau within 2 hours, and the plasma concentrations and the area under the curve were similar for both methods of administration (The mean area under the curve

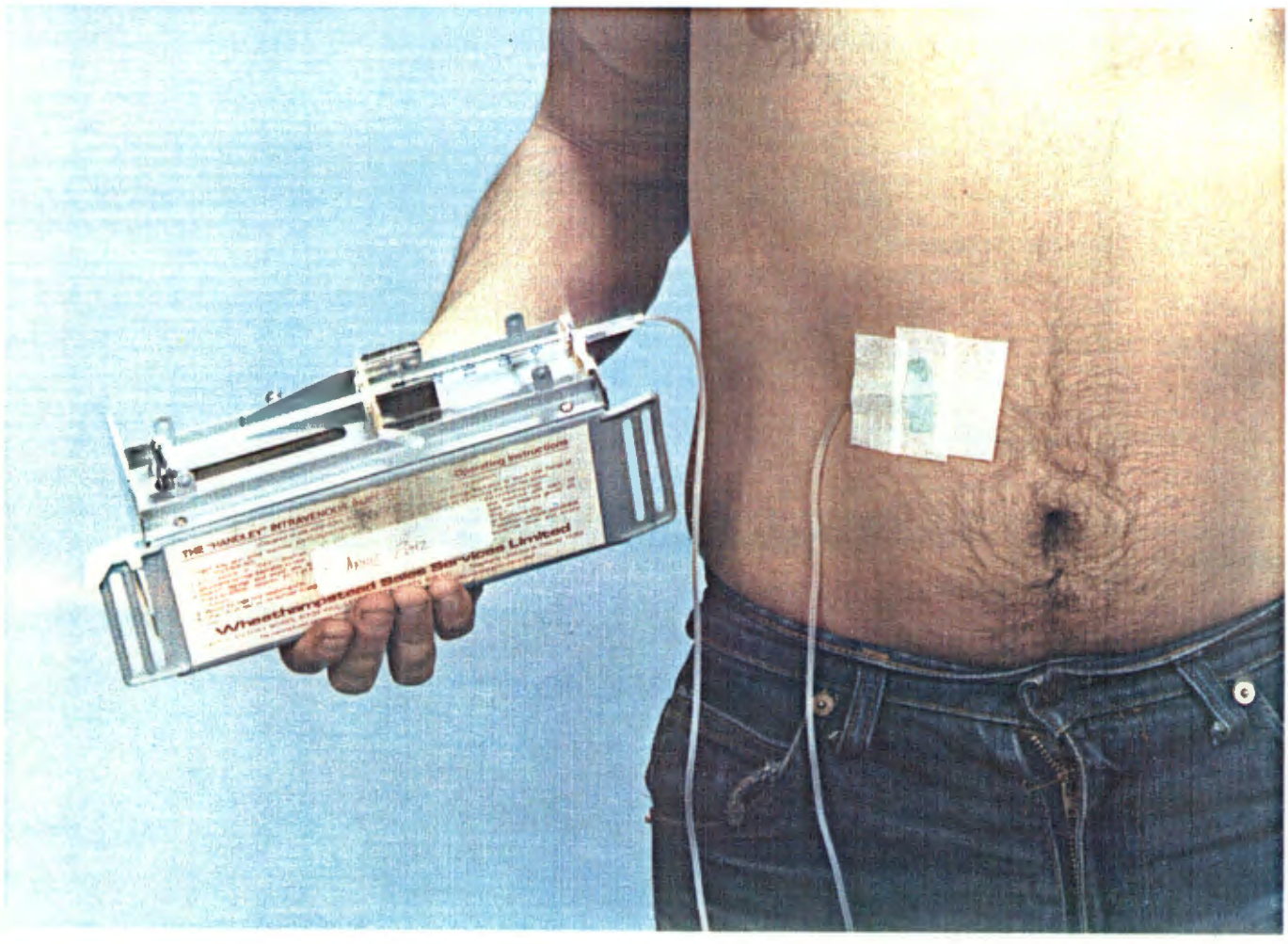


Figure 28
Subcutaneous infusion of cytosine arabinoside.

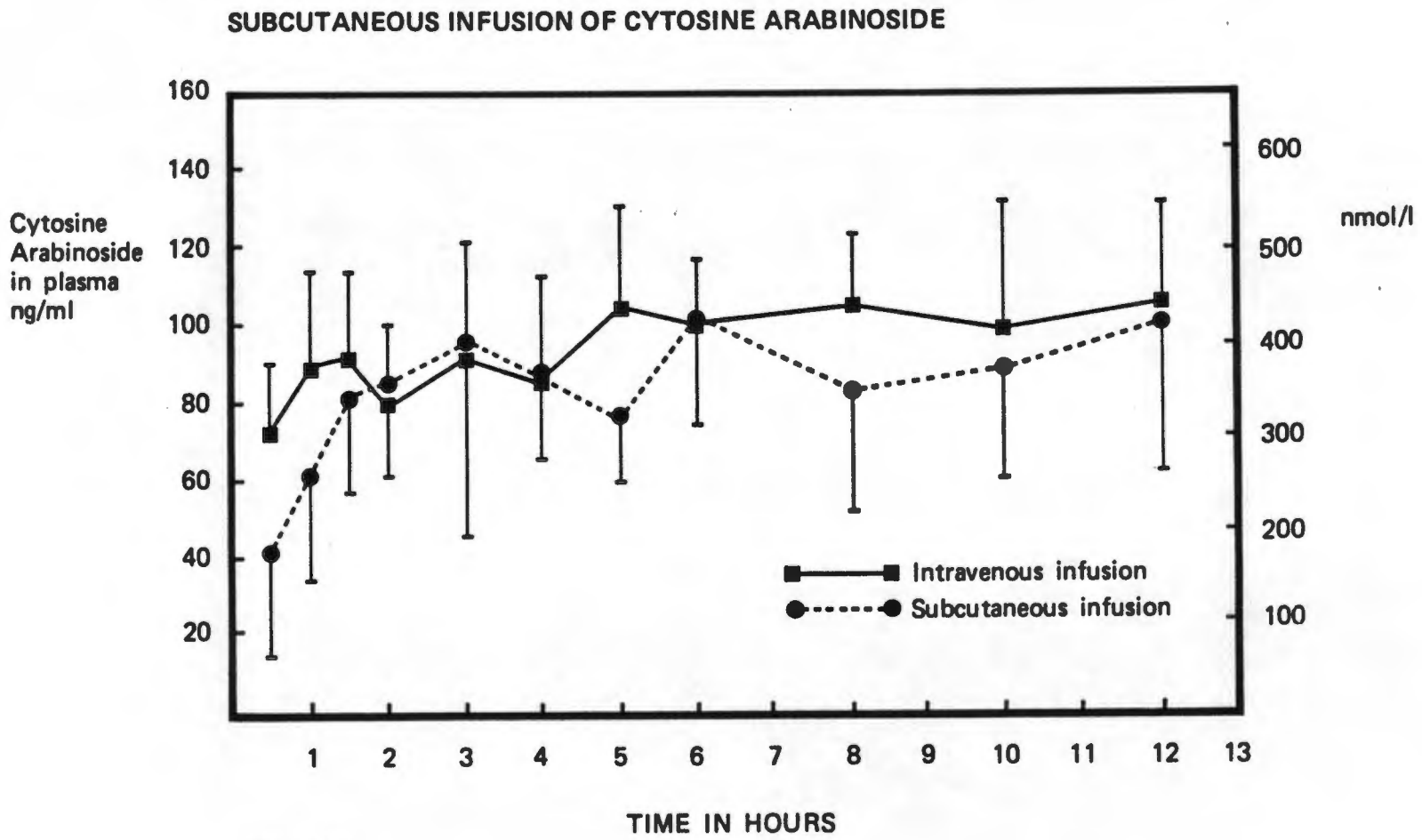


Figure 29

The mean plasma concentrations (± SD) of cytosine arabinoside (100mg/m^2) during intravenous and subcutaneous infusions in 6 patients with acute myelogenous leukaemia.

was 1147 ± 230 for intravenous infusions and 1017 ± 238 for subcutaneous infusions). A comparison of the area under the curve during the intravenous and subcutaneous infusions for the individual patients is shown in Table 18. The standard deviations shown in Figure 29 demonstrate the wide interpatient variation, but there was also a large variability in the plasma concentrations of cytosine arabinoside in the individual patients after a plateau had apparently been reached. A greater than twofold within patient difference between the minimum and maximum plasma cytosine arabinoside concentrations during the "plateau" phase of the infusion was demonstrated in some patients. The range of cytosine arabinoside concentrations in individual patients during intravenous and subcutaneous infusions is shown in Figure 30.

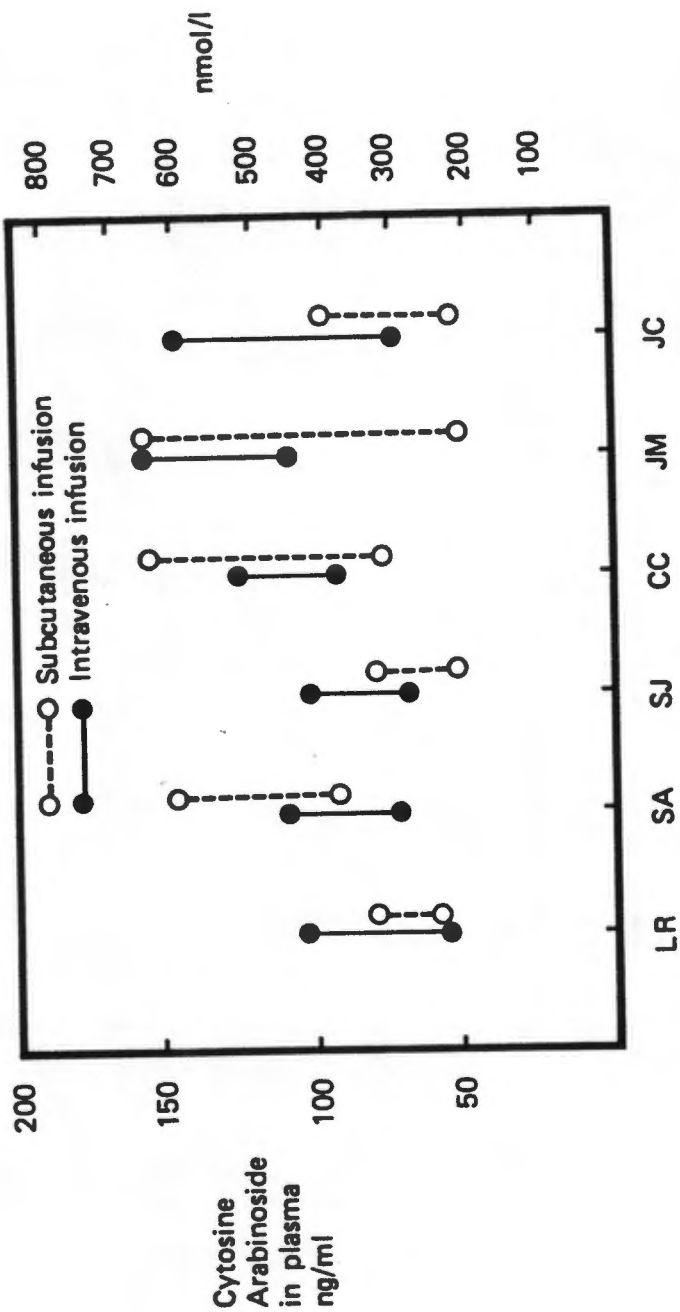


Figure 30
The range of cytosine arabinoside concentrations in individual patients during the "plateau" phase of intravenous and subcutaneous infusions.

PATIENT	AREA UNDER THE CURVE	
	IV INFUSION	SC INFUSION
L.R.	898	806
S.A.	1016	1215
S.J.	976	799
C.C.	1252	1370
J.M.	1521	1033
J.C.	1221	872
Mean	1147	1017
S.D.	230	238

Table 18.

A Comparison of the Area Under the Curve during Intravenous and Subcutaneous Cytosine Arabinoside Infusions (100mg/m^2) in Patients with Acute Myeloblastic Leukaemia.

Discussion

The data presented here shows that the time to achieve a "plateau" and the steady state plasma concentrations of cytosine arabinoside during subcutaneous infusions are similar to those achieved during intravenous infusions at the same dose. This is not surprising in view of the data presented earlier in this chapter showing the rapid absorption of cytosine arabinoside following subcutaneous bolus injection.

There was a relatively large interpatient difference in plasma levels during the infusion. Furthermore there was a wide variation in the plasma concentrations in the same patients after the "plateau" had been achieved. Similar observations were made by Harris et al (1979), who showed up to twofold within patient variation in the plasma cytosine arabinoside concentrations during intravenous infusions that had continued for at least 48 hours. These authors suggest that this wide interpatient variation during infusions could be explained by variations in patient posture resulting in alterations of hepatic blood flow . The hepatic extraction ratio for cytosine arabinoside has been calculated at 0.76. Wilkinson et al (1975) demonstrated that hepatic clearance of drugs with high hepatic extraction ratios is related to hepatic blood flow. This may be markedly dependent on posture (Culbertson et al 1951) and may double on lying down. The patients reported here were receiving their second or

subsequent courses of chemotherapy, and were all active and mobile, and this may explain the failure to achieve a true steady state. The mean values for the intravenous and subcutaneous infusions are, however, similar, and within each patient the area under the curve is similar for both methods of administration. The variable plasma concentrations during the so called "steady state" would need to be taken into account in any attempt to correlate steady state cytosine arabinoside levels during continuous infusion with therapeutic outcome.

This study confirms that subcutaneous infusion of cytosine arabinoside is a feasible alternative, and is comparable to intravenous infusion. It allows the patient the physical, psychological, and financial benefits of being at home, and prevents unnecessary thrombophlebitis.

CHAPTER 6

THE PHARMACOKINETICS OF HIGH DOSE CYTOSINE ARABINOSIDE

Introduction

High dose cytosine arabinoside has now been evaluated in several institutions. The dose and schedule of high dose cytosine arabinoside has varied in these different centres. Early et al(1982a and b) administered high dose cytosine arabinoside as a 1 hour infusion, Karanes et al (1979) as a 2 hour infusion and Rudnick et al (1979) as a 3 hour infusion. These studies have used a dose of $3g/m^2$ 12 hourly. Chapter 4 describes the use of $2g/m^2$ in an attempt to diminish central nervous system toxicity. It has been suggested by Dedrick et al (1972) and Capizzi et al (1982) that cytidine deaminase might be saturated at high doses and that the pharmacokinetics at these doses might therefore be non linear and dose dependent. This suggestion is, however, based upon in vitro data and there is no data to support it in man. If confirmed this would make it difficult to predict the plasma concentrations likely to be achieved by different methods of high dose cytosine arabinoside administration.

Central nervous system leukaemia is conventionally treated with intrathecal chemotherapy, and radiotherapy. Intrathecal cytosine arabinoside is effective therapy for some patients with overt central nervous system leukaemia (Band et al 1973 and Wang et al 1970a), and is used prophylactically together with methotrexate to prevent central nervous system relapse of acute lymphoblastic leukaemia (Simone et al 1979). Repeated

lumbar punctures are often most unpleasant, and it would clearly be to the advantage of the patient were it possible to produce prolonged cytotoxic levels of cytosine arabinoside in the cerebrospinal fluid by systemic therapy, thereby preventing the necessity for multiple lumbar punctures. Following intralumbar injections of methotrexate, ventricular levels are much lower than those achieved in the lumbar cerebrospinal fluid. In contrast, with high dose intravenous infusions of this drug, a uniform level is achieved throughout the cerebrospinal fluid axis. (Bleyer et al 1978). Furthermore, patients with neoplastic meningitis show significant abnormalities of cerebrospinal fluid flow, which can prevent even distribution of cytotoxic drugs administered by either the lumbar or ventricular route, and may also contribute towards neurotoxicity following intrathecal or intraventricular chemotherapy (Grossman et al 1981).

The phase 1 and 2 studies of high dose cytosine arabinoside described by Rudnik et al (1979), Karanes et al (1979) and Early et al (1982 a and b) show that these large doses are tolerable, and may be effective therapy for patients with relapsed and resistant acute leukaemia. It seemed likely that the use of these doses would result in greater cerebrospinal fluid cytosine arabinoside concentrations than during conventional dose infusions, and that these might be useful therapeutically in the treatment of central nervous system

leukaemia. The following studies were therefore undertaken to investigate the effect of the dose and schedule of high dose cytosine arabinoside on the plasma and cerebrospinal fluid pharmacokinetics.

Materials and Methods

Patients

A. The Effect of Schedule of High Dose Cytosine Arabinoside on Plasma Concentrations.

Twenty four patients with acute myelogenous and lymphoblastic leukaemia or high grade non hodgkin's lymphoma were studied.

Eight patients were investigated on 3 occasions within a single course of treatment. All received cytosine arabinoside at $2g/m^2$ twice daily for 6 days. In 6 patients cytosine arabinoside levels in plasma were studied during 1 and 3 hour infusions without a loading dose and during a 3 hour infusion preceded by a loading dose (one third of the total dose given as a bolus, and two thirds given as an infusion). Patients were randomised as to the order in which the different cytosine arabinoside schedules were given. In 2 patients cytosine arabinoside levels in plasma were studied during a 3 hour infusion on days 1, 3 and 6 of the course of therapy.

B. The Relationship between Cerebrospinal Fluid and Plasma Levels during High Dose Cytosine Arabinoside Infusions.

In 15 patients in whom a lumbar puncture was required to exclude central nervous system leukaemia, the levels of cytosine arabinoside in the plasma and cerebrospinal fluid were examined during high dose intravenous infusion. Each patient was studied on a single occasion during the first dose of high dose cytosine arabinoside therapy. Five patients received cytosine arabinoside at a dose of $1g/m^2$ as a 1 hour infusion. Five patients received $1g/m^2$ as a 3 hour infusion preceded by a loading dose and 5 patients received $3g/m^2$ given as 3 hour infusion preceded by a loading dose. In those patients who received a loading dose, one third was given as a bolus and the remainder as an infusion over 3 hours. During the 3 hour infusion, blood samples were taken at: 0,5,10,15,20,30 and 45 minutes, 1,1.5,2,2.5,and 3 hours and during the 1 hour infusion at 0,15,30 and 45 minutes and 1 hour. Following the completion of the infusions, blood samples were taken at 5, 10,15,20,30 and 45 minutes, 1,1.5,2,2.5, and 3 hours. A cerebrospinal fluid sample was obtained by lumbar puncture just before the infusion stopped. If this lumbar puncture was well tolerated the patients were asked if they would agree to a further lumbar puncture 3 hours later. A second sample of cerebrospinal fluid was obtained in 9 patients.

C. Time to Reach Equilibrium Between Plasma and Cerebrospinal Fluid

A patient with acute undifferentiated leukaemia had relapsed in both the bone marrow and central nervous system. An Ommaya reservoir was inserted. She received an infusion of cytosine arabinoside on 2 consecutive days. On day 1 she received 100mg/m^2 as a infusion over 6 hours and on day 2 she was given 1g/m^2 as an infusion over 6 hours. In each case one third was given as a loading dose and the remainder as an infusion. Blood samples were taken at 0, 5, 15, 20, 30 and 45 minutes, 1, 1.5, 2, 3, 4, 5 and 6 hours during the infusion and following the infusion at 5, 10, 15, 20, 30 and 45 minutes, 1, 2 and 3 hours. Cerebrospinal fluid samples were obtained by the Ommaya resevoir at 0, 30 minutes, 1, 2, 3, 4, 5 and 6 hours during the infusion and at 1 and 3 hours after completion of the infusion.

D. Intraventricular Cytosine Arabinoside

On a separate occasion the disappearance of cytosine arabinoside from the cerebrospinal fluid following an injection of 50 mg/m^2 into the Ommaya reservoir was followed in the same patient. Cerebrospinal fluid samples were taken at 1, 2, 4, 6, 10,, 23.5, 26.5 and 30 hours.

Sampling and Assay

Blood and cerebrospinal fluid samples were taken into precooled tubes containing tetrahydrouridine (10^{-5} M) and 50 units of heparin . The separation and storage of these samples and the radioimmunoassay have been described in Materials and Methods section of this thesis (Chapter 2).

Results

A. The Effect of The Schedule of High Dose Cytosine Arabinoside on Plasma Concentrations

The mean plasma cytosine arabinoside concentrations and the standard deviation measured in 6 patients during the 1 hour infusions and the 3 hour infusions with and without a loading dose are shown in Figure 31, and the individual patients pharmacokinetic parameters in Tables 19, 20 and 21. A plateau was rapidly achieved with all 3 methods of administration. Plasma cytosine arabinoside concentrations varied considerably between patients. The intra-patient variation seen during the plateau phase of the infusion was similar to that seen with conventional dose cytosine arabinoside (Harris et al 1979, Chapter 5). As expected the plateau cytosine arabinoside concentrations were much higher during the 1 hour infusions than during the 3 hour infusions, but the mean area under the

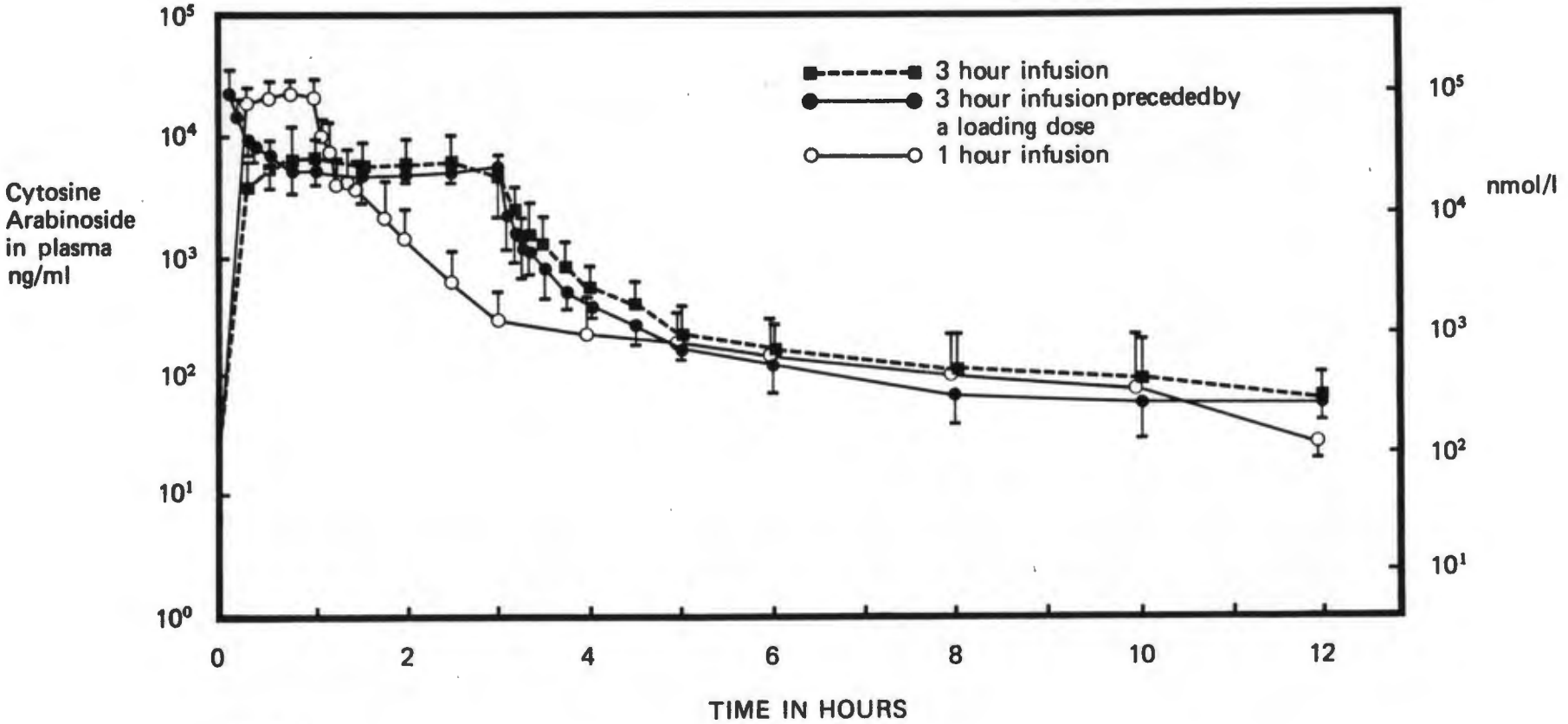


Figure 31

Mean plasma concentrations (± SD) in 6 patients treated with high dose cytosine arabinoside (2g/m^2) as a 3 hour infusion, a 3 hour infusion preceded by a loading dose, and a 1 hour infusion.

PATIENT	POST INFUSION HALF LIVES		Cytosine Arabinoside Plateau (end of infusion level) ng/ml	Area Under the Curve ng/ml
	α min	B hours		
R.W.	9.0	1.7	1100	14234
G.Mo.	10.8	1.5	5300	14603
A.B.	19.8	3.7	6100	17970
G.S.	7.8	1.4	3150	17563
G.M.	8.4	2.2	6700	21727
H.W.	17.4	0.9	9400	38614
Mean	12.2	1.9	5291	20785
S.D.	5.1	1.0	2886	9145

Table 19.

Plasma Pharmacokinetics of High Dose Cytosine Arabinoside (2 g/m^2) given as a 3 hour Intravenous Infusion .

PATIENTS	POST INFUSION HALF LIVES		Cytosine Arabinoside Plateau (end infusion level) ng/ml	Area Under the Curve ng/ml h
	α min	B hours		
R.W.	13.8	3.2	6300	24516
G.Mo.	12	1.9	7000	22275
A.B.	15.6	2.5	5800	22894
G.S.	4.2	1.6	7300	26130
G.M.	12.0	3.7	4400	15611
H.W.	8.4	2.8	6200	19498
Mean	11.0	2.6	6166	21820
S.D.	4.0	0.8	1025	3772

Table 20.

Plasma Pharmacokinetics of High Dose Cytosine Arabinoside ($2g/m^2$) given as a 3 hour Intravenous Infusion Preceded by a Loading Dose.

PATIENT	POST INFUSION HALF LIVES		Cytosine Arabinoside Plateau (end infusion level) ng/ml	Area Under the Curve ng/ml h
	α min	B hours		
R.W.	13.2	1.9	11,100	14002
G.Mo	13.8	1.8	27,200	24049
A.B.	18.0	2.4	20,100	20271
G.S.	4.8	1.8	21,500	21738
G.M.	7.8	1.5	26,100	26066
H.W.	17.6	2.3	36,300	42663
Mean	12.5	1.9	23716	24798
S.D.	5.3	0.3	8406	9672

Table 21.

Plasma Pharmacokinetics of High Dose Cytosine Arabinoside (2 g/m^2) Given as a 1 hour Intravenous Infusion.

curve was similar for all 3 methods of administration (24798 ± 9672 for the 1 hour infusion, 20785 ± 9145 for the 3 hour infusion and 21820 ± 3772 ng/ml hours for the 3 hour infusion preceded by a loading dose). The initial and terminal half lives of cytosine arabinoside following completion of the infusion were not influenced by the schedule of administration (Tables 19, 20, 21).

A comparison of the mean levels of cytosine arabinoside in the plasma during infusions on days 1, 3 and 6 (doses 1, 5 and 11) is shown in Figure 32. The area under the curve, the plateau concentrations and pharmacokinetics were not influenced by the preceding therapy (Table 22). There was no evidence of accumulation of cytosine arabinoside with successive doses.

The mean plasma cytosine arabinoside concentrations from patients in the above studies who received infusions of 1 g/m^2, $2g/m^2$ and $3g/m^2$ are shown in Figure 33, and the individual patients' pharmacokinetic parameters in Tables 23,20 and 25 respectively. The plateau concentrations and the area under the curve rose proportionately to the dose of cytosine arabinoside administered, and the elimination half lives were similar at all 3 doses. Furthermore the half lives were similar to those following intravenous bolus of 100 mg/m^2 (Chapter 5).

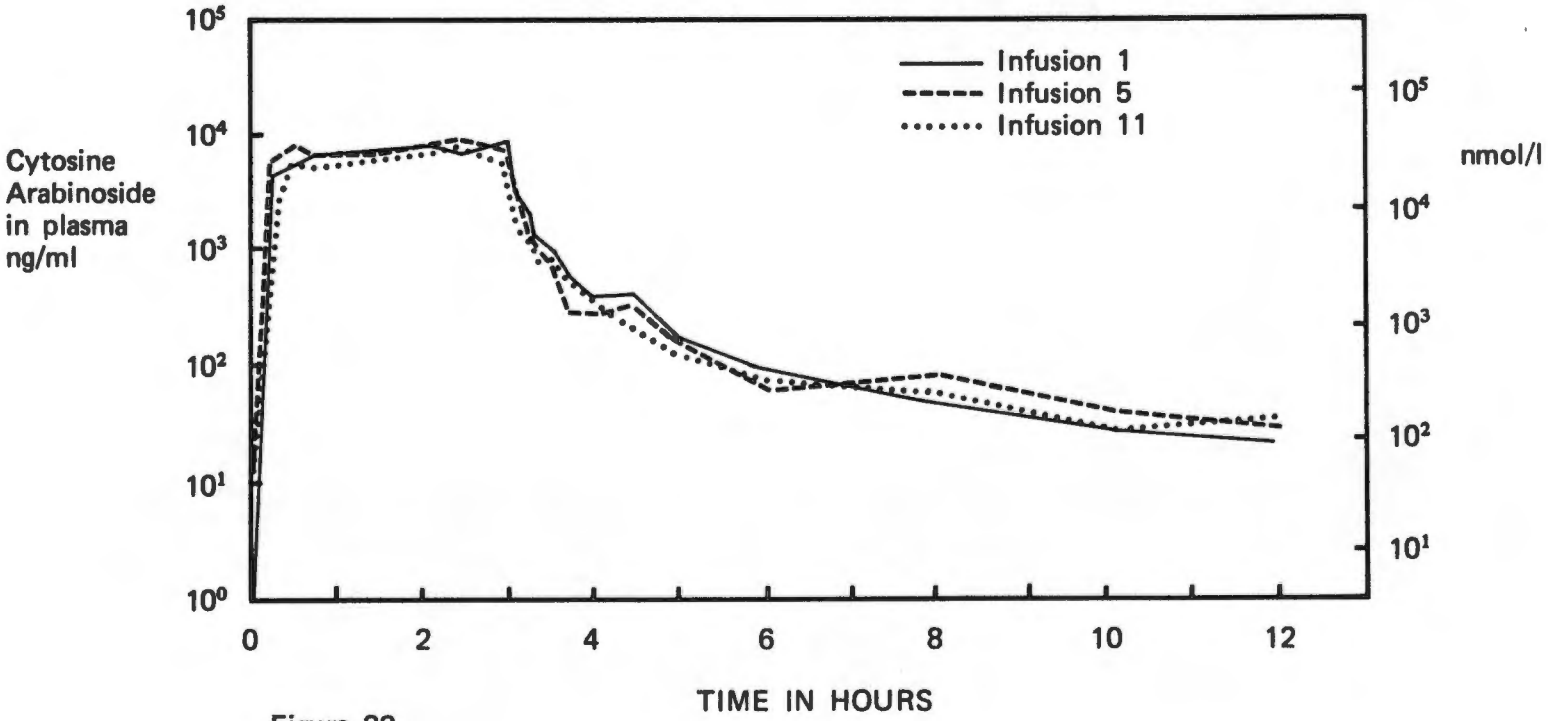


Figure 32

Mean plasma concentrations of cytosine arabinoside during doses 1, 5 and 11 of high dose cytosine arabinoside ($2g/m^2$) therapy in 2 patients.

Patient	Post Infusion Half Lives						Cytosine Arabinoside End Infusion Level ng/ml			Cytosine Arabinoside Area Under the Curve ng/ml h		
	α Minutes			B Hour			DOSE			DOSE		
Dose	1	5	11	1	5	11	1	5	11	1	5	11
J.Sp.	13.2	10.2	11.4	2.5	4.5	3.7	6,100	6,200	6,000	14844	18042	12767
J.Sa	18.0	12.0	11.4	2.6	2.0	1.4	10,100	8,900	6,900	25465	25254	25817

Table 22.

Plasma Pharmacokinetics of High Dose Cytosine Arabinoside ($2g/m^2$) during doses 1, 5 and 11 (Days 1, 3 and 6).

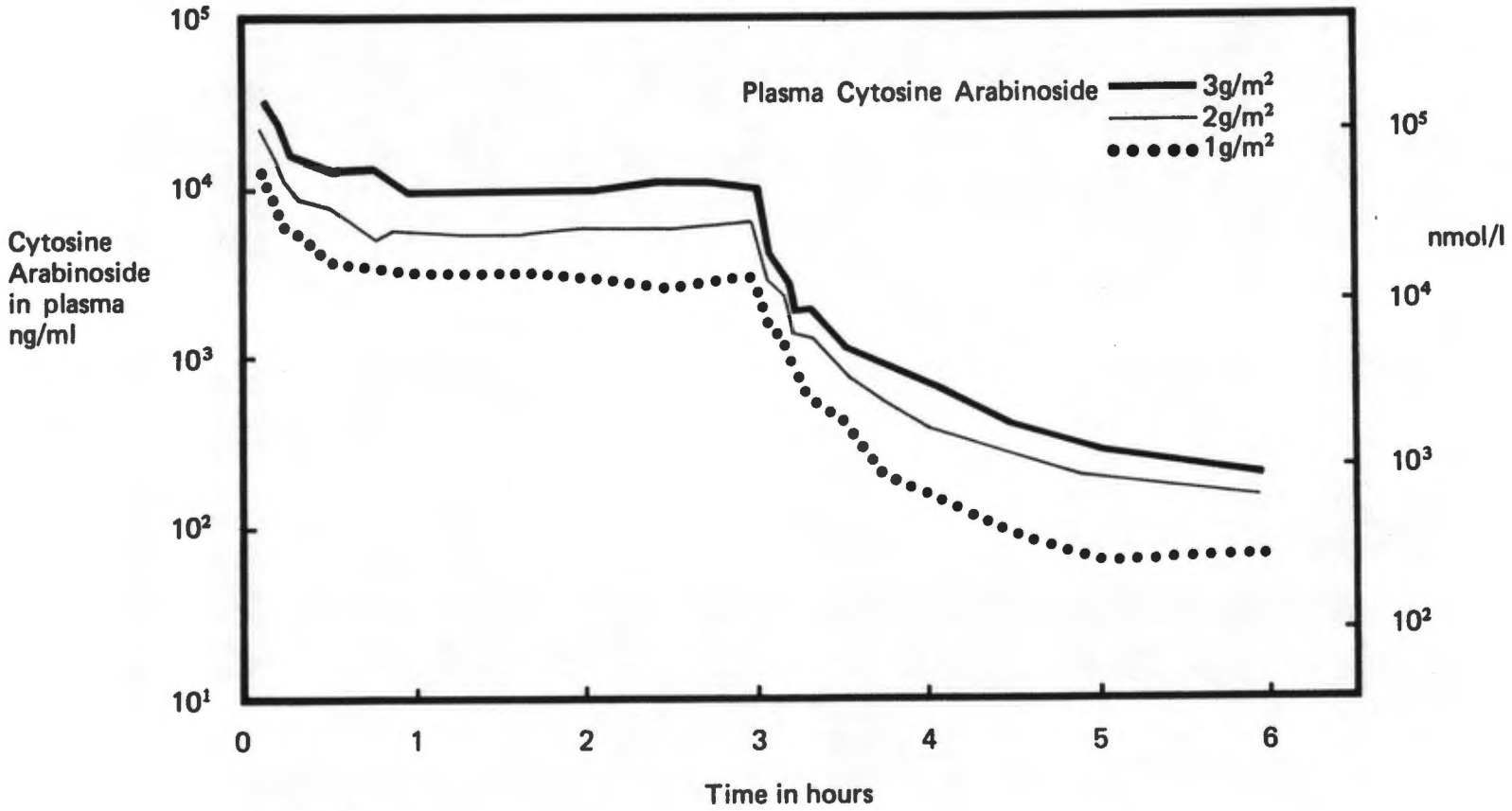


Figure 33

Mean plasma concentrations of cytosine arabinoside in 5 patients who were treated with 1g/m², 5 with 2g/m² and 5 with 3g/m² cytosine arabinoside over 3 hours (1/3 of total dose given as bolus).

PATIENT	POST INFUSION HALF LIVES		Cytosine Arabinoside Plateau (end infusion level) ng/ml	Area Under the Curve ng/ml h
	α min	B hours		
G.W.	10.2	0.6	3280	12659
M.C.	3.0	6.0	4350	17099
G.O.	5.4	1.9	2140	7945
M.M.	7.2	1.3	2600	11839
R.L.	12.0	1.7	2430	8521
Mean	7.6	2.3	2960	11612
S.D.	3.6	2.1	882	3682

Table 23.

Plasma Pharmacokinetics of High Dose Cytosine Arabinoside (1 g/m^2) given as a 3 Hour Intravenous Infusion Preceded by a Loading Dose.

PATIENT	CSF Cytosine Arabinoside ng/ml		CSF HALF LIVES hrs	CSF:PLASMA RATIO	
	3 hrs	6 hrs		3 hrs	6 hrs
G.W.	464	137	1.7	0.14	1.8
M.C.	425	-	-	0.1	-
G.O.	215	56	1.5	0.1	0.8
M.M.	253	139	3.5	0.1	1.5
R.L.	378	162	2.4	0.16	4.5
Mean	347	123	2.3	0.12	2.15
S.D.	108	46	0.9	0.03	1.62

Table 24.

Cerebrospinal Fluid (CSF) Pharmacokinetics of Cytosine Arabinoside during Intravenous Infusion of 1 g/m^2 given over 3 hours and Preceded by a Loading Dose.

Patient	Post Infusion Half Lives		Cytosine Arabinoside Plateau (end Infusion Level) ng/ml	Area Under the Curve ng/ml h
	α Minutes	B Hours		
S.J.	16.2	1.9	7,200	27547
R.B.	16.2	4.1	6,300	58376
J.C.	12.6	1.4	16,400	22747
J.S.	15.0	2.2	8,900	36429
R.W.	15.0	3.4	9,200	34445
Mean	15.0	2.6	9,600	35909
S.D.	1.4	1.2	3,986	13696

Table 25.

Plasma Pharmacokinetics of Cytosine Arabinoside during an Infusion of $3g/m^2$ over 3 hours with One Third of the Total Dose given as a Loading Dose.

Patient	CSF Cytosine Arabinoside (ng/ml) 3 hours	6 hours	CSF Half Lives (Hours)	CSF:Plasma Ratio 3 hours	6 hours
S.J.	921	798	14.4	0.13	7.9
R.B.	914	-	-	0.06	-
J.C.	758	395	3.2	0.12	4.2
J.S.	767	-	-	0.09	-
R.W.	1990	329	1.2	0.22	1.5
Mean	1070	507	6.3	0.12	4.5
S.D.	520	257	7.1	0.006	3.2

Table 26

Cerebrospinal Fluid (CSF) Pharmacokinetics of Cytosine Arabinoside ($3g/m^2$) as an Intravenous Infusion over 3 Hours with one-third of the Total Dose given as a Bolus.

PATIENT	POST INFUSION HALF LIVES		Cytosine Arabinoside Plateau (end infusion level) ng/ml	Area Under the Curve ng/ml h
	∝ min	B hours		
M.P.	13.8	2.5	10300	11228
R.W.	10.8	0.9	8400	11048
A.B.	12.8	1.5	8900	12430
J.P.	10.2	1.2	12200	10866
P.H.	15.6	1.8	8100	8023
Mean	12.6	1.6	9580	10719
S.D.	2.2	6.1	1690	1627

Table 27.

Plasma Pharmacokinetics of High Dose Cytosine Arabinoside (lg/m^2) given as a 1 hour Intravenous Infusion.

PATIENT	CSF Cytosine Arabinoside ng/ml		CSF:PLASMA RATIO	
	1 hr	4 hrs	1 hr	4 hrs
M.P.	198	-	0.02	-
R.W.	333	129	0.04	1.1
A.B.	180	195	0.02	1.7
J.P.	280	279	0.02	2.6
P.H.	295	-	0.04	-
Mean	257	201	0.03	1.8
S.D.	65	75	0.01	0.75

Table 28.

Cerebrospinal Fluid (CSF) Pharmacokinetics of High Dose Cytosine Arabinoside during Intravenous Infusion of 1 g/m^2 given over 1 hour.

B. The Relationship between Cerebrospinal Fluid and Plasma Levels of Cytosine Arabinoside During High Dose Cytosine Arabinoside Infusions

The mean plasma and cerebrospinal fluid concentrations in the patients treated with $1g/m^2$ and $3g/m^2$ cytosine arabinoside as a 3 hour infusion are shown in Figure 34, and in the patients treated with $1g/m^2$ as a 1 hour infusion in Figure 35. The individual patients' pharmacokinetic parameters are given in Tables 23 - 28. At the end of the 3 hour infusions, the ratio of the mean cerebrospinal fluid to plasma cytosine arabinoside levels was 0.12 for the $3g/m^2$ infusions and 0.12 for the $1g/m^2$ infusions. At the completion of the 1 hour infusion at $1g/m^2$, the mean cerebrospinal fluid:plasma ratio was 0.03. Three hours after stopping the infusion, the ratios were 4.5 ($3g/m^2$ over 3 hours), 2.15 ($1g/m^2$ over 3 hours) and 1.8 ($1g/m^2$ over 1 hour). The plateau cytosine arabinoside concentrations during the 1 hour infusion were as expected, much higher than when the same dose was given over 3 hours. Cerebrospinal fluid concentrations at the end of the infusion were, however, similar resulting in the lower cerebrospinal fluid:plasma cytosine arabinoside ratio.

C. Time to Reach Equilibrium Between Plasma and Cerebrospinal Fluid

The plasma and cerebrospinal fluid levels of cytosine

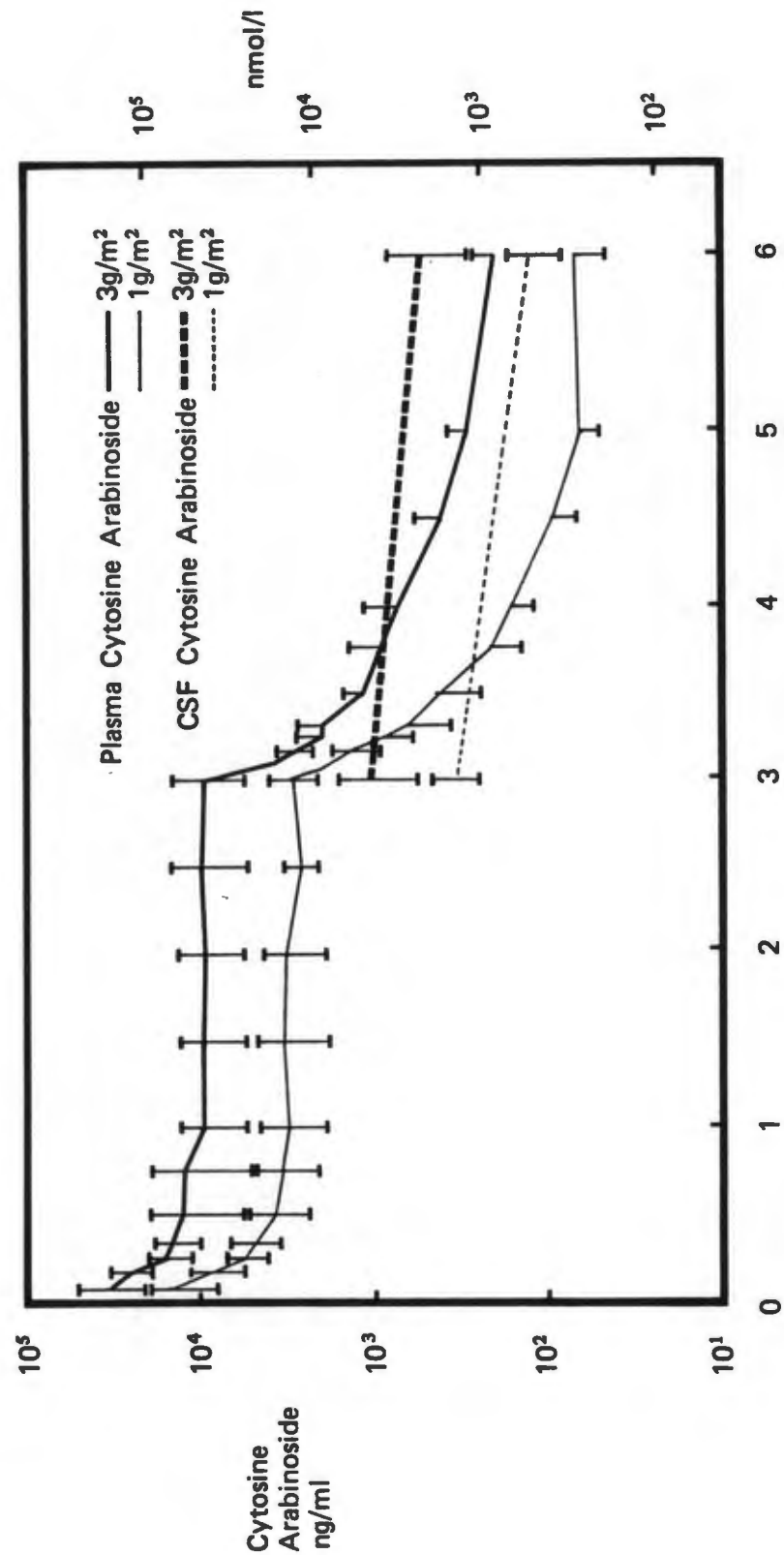


Figure 34

Mean plasma and cerebrospinal fluid concentrations of cytosine arabinoside (± SD) in 5 patients who received 1g/m² and 5 patients who received 3g/m² cytosine arabinoside over 3 hours. (1/3 total dose given as a bolus)

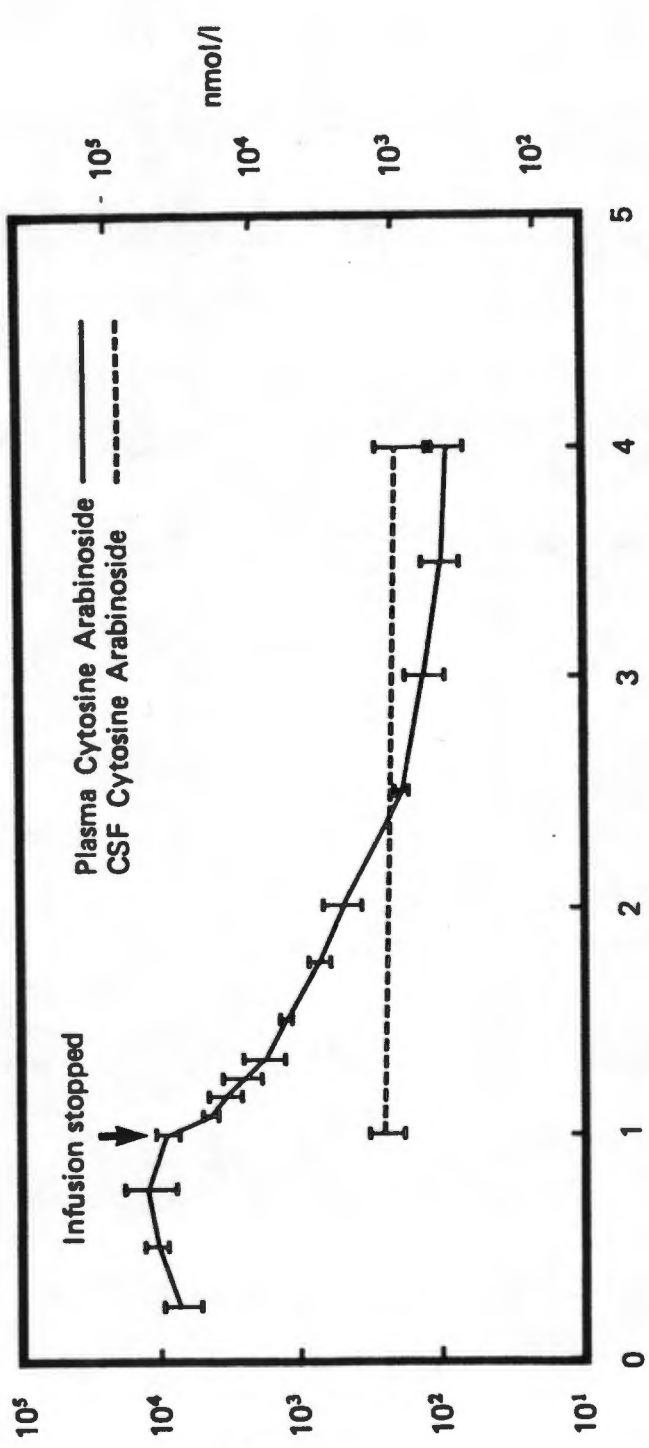


Figure 35

Mean plasma and cerebrospinal fluid levels of cytosine arabinoside (± SD) in 5 patients during an intravenous infusion of cytosine arabinoside over 1 hour.

arabinoside in the patient with the Ommaya reservoir are shown in Figure 36. A plateau was rapidly reached in the plasma and equilibration between the plasma and cerebrospinal fluid was reached within an hour at both the 100mg/m^2 and 1g/m^2 doses. The ratio of the mean cerebrospinal fluid and plasma levels during the 100 mg/m^2 infusion was 0.15 and during the 1g/m^2 infusion 0.24, suggesting that cytosine arabinoside crossed the blood brain barrier at least as effectively at 1g/m^2 as at 100 mg/m^2.

D. Intraventricular Cytosine Arabinoside

The disappearance of cytosine arabinoside from the cerebrospinal fluid over 30 hours following an injection of 50 mg/m^2 via an Ommaya reservoir is plotted in Figure 37. Cytosine arabinoside declined biexponentially with an initial half life of 0.5 hours and a terminal half life of 3.4 hours.

Discussion

The data presented here shows that cytosine arabinoside concentrations in plasma rise proportionately with the increase in dose of a range of 1-3g/m^2, and that the initial and terminal half lives are the same at 100 mg/m^2 as at 3g/m^2. A standard dose of cytosine arabinoside produces a constant area under the curve whether given over 1 or 3 hours and with or without a loading dose. If cytidine deaminase was saturated during the high dose cytosine arabinoside infusions,

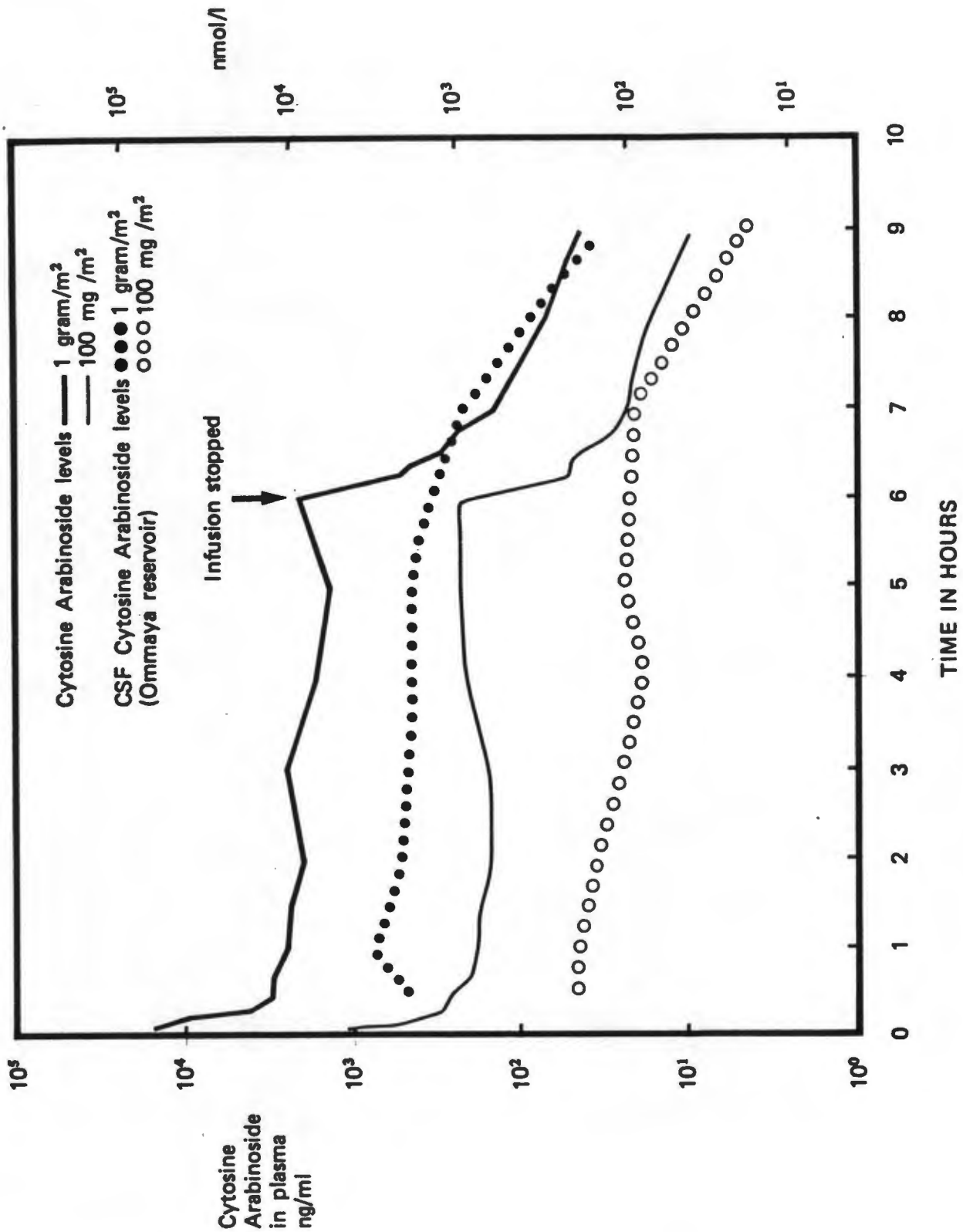


Figure 36

Plasma and cerebrospinal fluid concentrations of cytosine arabinoside in a patient with an Ommaya reservoir treated with an intravenous infusion of 100mg/m² and 1g/m² over 6 hours.

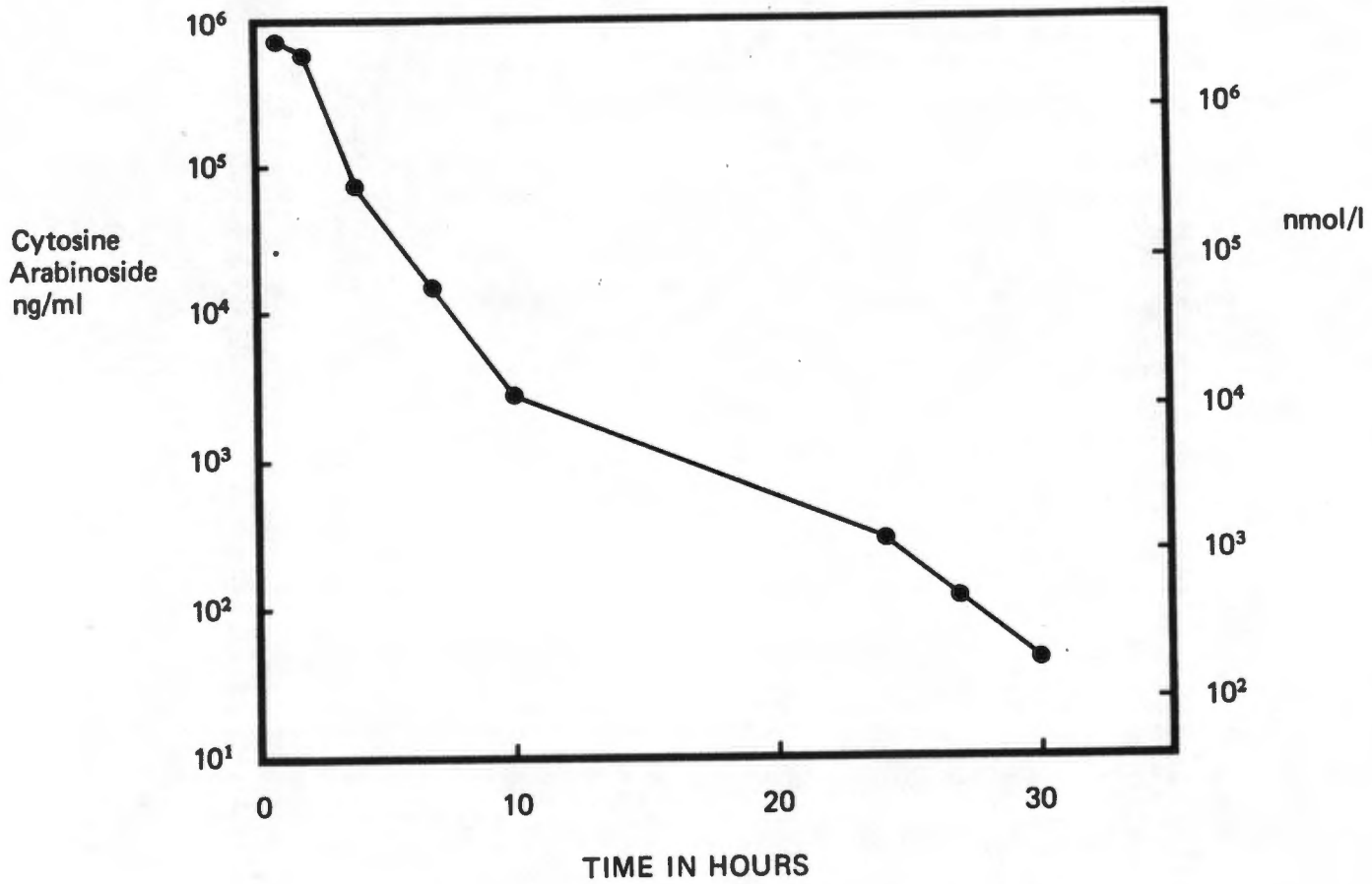


Figure 37

Cerebrospinal fluid levels of cytosine arabinoside following injection of cytosine arabinoside (50mg/m^2) into Ommaya reservoir.

the half life of cytosine arabinoside would be prolonged at the higher concentrations. In addition a disproportionate increase in plasma concentrations and in the area under the curve would occur. Furthermore successive doses of cytosine arabinoside given to the same patient resulted in no accumulation in the plasma. This suggests that cytidine deaminase does not become saturated over this dose range in man.

The studies of the relationship between plasma and cerebrospinal fluid cytosine arabinoside concentrations during therapy with high dose cytosine arabinoside showed that at the doses used cerebrospinal fluid concentrations increased in proportion to the doses administered. At the highest dose ($3g/m^2$ over 3 hours) cerebrospinal fluid concentrations were an order of magnitude higher than those obtained in the plasma during intravenous infusions of conventional doses (100-200 mg/m^2 over 24 hours). After the infusion cerebrospinal fluid cytosine arabinoside concentrations declined with a half life of greater than 2 hours. In view of the rapid equilibration between the plasma and cerebrospinal fluid, potentially therapeutic levels (above 100 ng/ml) will be maintained in the cerebrospinal fluid throughout the duration of high dose therapy, provided treatment is repeated every 12 hours.

The one hour infusions resulted in much higher plasma cytosine arabinoside concentrations, than the infusions over 3 hours. However, cerebrospinal fluid concentrations at the end of the

infusions were similar, resulting in the much lower cerebrospinal fluid:plasma cytosine arabinoside ratios during the shorter infusion. In two of three patients who had a second lumbar puncture 3 hours after completion of the 1 hour infusion, cerebrospinal fluid cytosine arabinoside concentrations had not decreased. The most likely explanation for this is that the cerebrospinal fluid cytosine arabinoside continued to rise after the completion of the infusion. This contrasts with the rapid equilibration between the plasma and cerebrospinal fluid in the patient with an Ommaya resevoir described here, and that previously described by Ho et al (1971). However, both these patients had an intravenous bolus preceding the infusion, and this may contribute to the rapid equilibration between the plasma and the cerebrospinal fluid. Multiple cerebrospinal fluid samples from the same patients would be necessary to answer these detailed questions, and would only be possible in patients with an Ommaya reservoir.

Central nervous system toxicity has been reported in patients receiving high dose cytosine arabinoside infusions (Rudnik et al 1979,Early et al 1982 a and b and Herzig et al 1982.). The data presented here demonstrate that the levels achieved in the cerebrospinal fluid during high dose intravenous infusions of cytosine arabinoside are several hundred times lower than those obtained following intraventricular injection of 50 mg/m^2. However, following intraventricular cytosine arabinoside the levels have fallen to below 100ng/ml shortly after 24 hours. The central nervous system toxicity during

high dose cytosine arabinoside infusions is unlikely therefore to relate directly to the peak cerebrospinal fluid concentration, and is probably due to the prolonged exposure to relatively high cerebrospinal fluid concentrations which occur during this method of administration.

Clinical studies to determine whether high dose cytosine arabinoside given as a short infusion twice a day over several days is effective in treating central nervous system leukaemia, are now needed to define the role of high dose cytosine arabinoside in the treatment of central nervous system leukaemia.

CHAPTER 7

PROTEIN BINDING OF CYTOSINE ARABINOSIDE

Introduction

The binding of a drug to plasma protein may have a marked effect on the distribution and pharmacological effect of the drug and on the rate at which it is eliminated from the body. The protein bound fraction of drug is not immediately available for distribution into the extravascular space or for elimination. It is the unbound drug which is pharmacologically and toxicologically active. Several in vitro methods such as equilibrium dialysis, ultrafiltration and ultracentrifugation are available for studying protein binding of drugs (Chignell 1977). However, the protein concentration in extravascular fluids such as cerebrospinal fluid, saliva and synovial fluid is considerably less than that in plasma and the total drug concentration in these fluids is often equal to the free concentration in the plasma. The concentration of drugs in these extravascular fluids is therefore considered to represent a physiological measure of protein binding (Bertilsson et al 1979 and Piafsky 1980).

The protein binding of basic drugs differs from that of acidic drugs. Basic drugs associate with a number of plasma constituents such as alpha 1 acid glycoprotein, lipoprotein, and albumin, whereas acidic drugs bind largely to albumin (Piafsky 1980). The variation in plasma albumin is relatively narrow and is almost always in the direction of decreased concentrations, whereas alpha 1 acid glycoprotein and lipoprotein show large fluctuations in disease (Snyder et al 1975 and Weeke et al 1971).Alpha 1 acid glycoprotein is regarded as an acute phase protein and is often

increased in patients who are acutely ill or infected.Furthermore alpha 1 acid glycoprotein may be increased in patients with malignant disease (Snyder et al 1971,Weiss et al 1979, Chio et al 1979). With many basic drugs such as dipyridamole, quinidine and imipramine there is a significant correlation between protein binding and the concentrations of alpha 1 acid glycoprotein, but no correlation with albumin (Kopitar et al 1971,Fremstad et al 1976 and Piafsky et al 1977).

The protein binding of cytosine arabinoside has been studied by van Prooijen et al (1977b). Protein binding was determined by ultrafiltration and ultracentrifugation using plasma from healthy volunteers. The results obtained by both methods did not differ significantly and cytosine arabinoside binding was found to be 13.3 $\pm$ 2.2%. The percentage bound was independent of drug concentration within the therapeutic range.

Cytosine arabinoside is a basic drug and might therefore bind significantly to alpha 1 acid glycoprotein. It is possible that patients with acute myelogenous leukaemia who are often acutely ill may have raised alpha 1 acid glycoprotein levels at presentation.

Equilibration between plasma and cerebrospinal fluid cytosine arabinoside in patients receiving intravenous infusions preceded by a loading dose has been shown to take place within an hour by Ho et al (1971) and in chapter 6 of this thesis. In the patient described by Ho et al (1971), 40% of the plasma cytosine

arabinoside levels were reached in the cerebrospinal fluid, and in the patients described in chapter 6, cerebrospinal fluid : plasma cytosine arabinoside ratios of 0.12 to 0.25 were demonstrated. It was therefore decided to investigate the protein binding of cytosine arabinoside further both in vivo by measuring saliva:plasma cytosine arabinoside ratios during steady state intravenous infusions and in vitro by equilibrium dialysis using serum from patients with acute myeloblastic leukaemia at presentation.

Materials and Methods

Patients

Six patients with acute myelogenous leukaemia undergoing remission induction or consolidation with chemotherapy including continuous infusions of cytosine arabinoside were studied during therapy. Simultaneous saliva (2 ml) and blood (4 ml) samples were obtained on 3 occasions on the second or subsequent days of the cytosine arabinoside infusion in 5 of these patients. In one patient simultaneous saliva and plasma samples were obtained at repeated intervals during the first 12 hours of the infusion to determine the time to equilibrium. Serum was obtained from 18 patients with acute myelogenous leukaemia at presentation and prior to therapy, and from 6 normal volunteers for the protein binding studies.

Sampling and Assay

Saliva was collected by asking the patients to rinse their mouths with water and then stimulating saliva production by chewing a bland inert plastic material (Parafilm). Blood and saliva samples for cytosine arabinoside assay were taken into pre-cooled tubes containing tetrahydrouridine (10^{-5} M) and 50 units of heparin. The samples were separated by centrifugation and stored at -20°C. Plasma and saliva cytosine arabinoside concentrations were measured by radioimmunoassay. Serum samples for the protein binding studies were also separated by centrifugation and stored at -20°C.

Plasma Protein Binding

Tritiated cytosine arabinoside (specific activity 1.54 GBq/mg) was supplied by the Radiochemical Centre , Amersham. Serum was placed in 1 ml acrylic cells and dialysed at 37° C against phosphate buffered saline at pH 7.3 (Dulbecco "A"with mineral salt solution: Oxoid Limited). The cells were separated by an 11.5 um Cuprophan membrane and rotated at 45 revs per minute to ensure constant mixing. Each experiment was performed in duplicate. The cytosine arabinoside concentrations in plasma and buffer dialysates were measured using a Packard Instrument PRIS PLD scintillation counter with automatic quench correction by external standard. The quench curve was established using a standard concentration of cytosine arabinoside (2 ul, 24 mg/ml;

4.44 x 10^6 Bq) in the scintillant mixture (4 ml Picofluor Packard Instrument) and varying concentrations (0-10% v/v) of picric acid to produce colour quenching. The equilibrium dialysis chambers are shown in Figure 38.

The time to equilibrium and the influence of concentration on protein binding were determined in the serum from the normal volunteers. Thirty six cells were used, 18 spiked with 200 ng cytosine arabinoside (containing 10ng H^3 cytosine arabinoside) on the buffer side and 18 on the serum side. Four cells were removed from the oven at the following times: 15, 35 and 45 minutes and 1, 2, 3, 4, 7 and 10 hours. The time to equilibrium was established by plotting buffer disintegrations per minute (DPM) over plasma DPM against time .

The influence of cytosine arabinoside concentration was studied using 9 concentrations: 20, 50, 100, 200 and 500 ng/ml and 1, 2, 5 and 10 ug/ml.

The intra-assay variability was determined by measuring the protein binding of cytosine arabinoside in the serum of one normal volunteer. Twenty four cells were used. Twelve contained the serum and 12 contained buffer spiked with 200ng cytosine arabinoside containing 10 ng H^3 cytosine arabinoside.
The serum obtained from patients with acute myelogenous leukaemia at presentation and prior to any drug therapy was dialysed

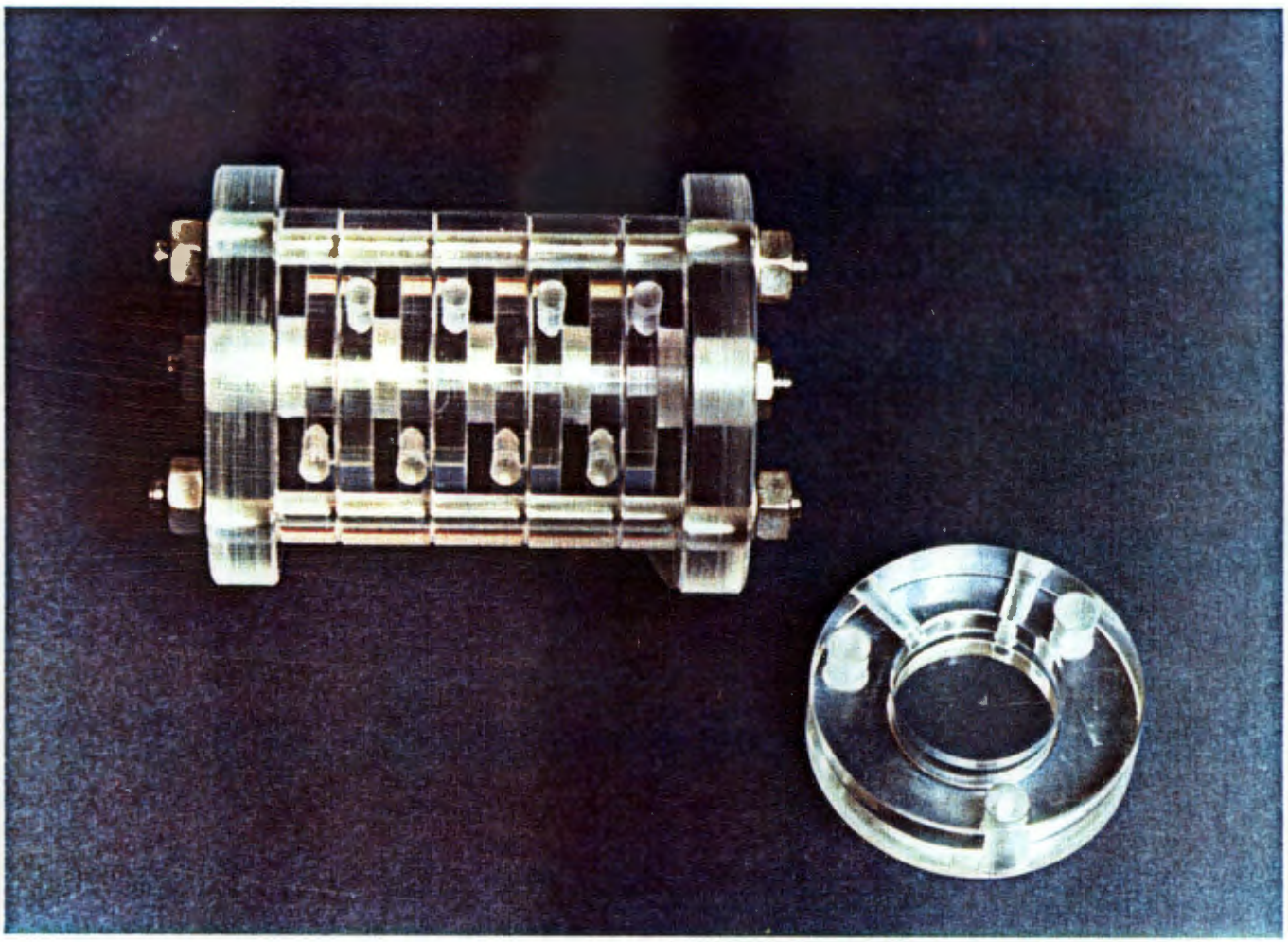


Figure 38
Equilibrium dialysis cells (x 1).

against 200 ng/ml of cytosine arabinoside so that at equilibrium the plasma cytosine arabinoside concentration would be in the conventional therapeutic range (100 ng/ml). Estimations were done in duplicate.

Total protein was estimated colorimetrically using Biuret reagent. Plasma albumin was measured by dyebinding (bromo-cresol green) and alpha 1 acid glycoprotein was measured using M-Partigen immunodiffusion plates. These plates contain monospecific antiserum to individual plasma proteins in an agar-gel layer. This antiserum is produced by immunising rabbits, sheep, goats or horses. The wells are filled with control and patient serum and are allowed to stand at room temperature for at least 2 days, following which the diameter of the precipitate is measured. This diameter is proportional to the concentration of the protein present in the serum.(Figure 39)

Results

The Relationship Between Plasma and Salivary Cytosine Arabinoside

The saliva:plasma cytosine arabinoside ratio in the 5 patients who had simultaneous samples taken during intravenous infusions of cytosine arabinoside ranged from 0.05 to 0.45 with a mean value of 0.22 ± 0.14. In the single patient who had simultaneous saliva and plasma samples taken at repeated intervals throughout the first 12 hours of the intravenous infusion, equilibrium

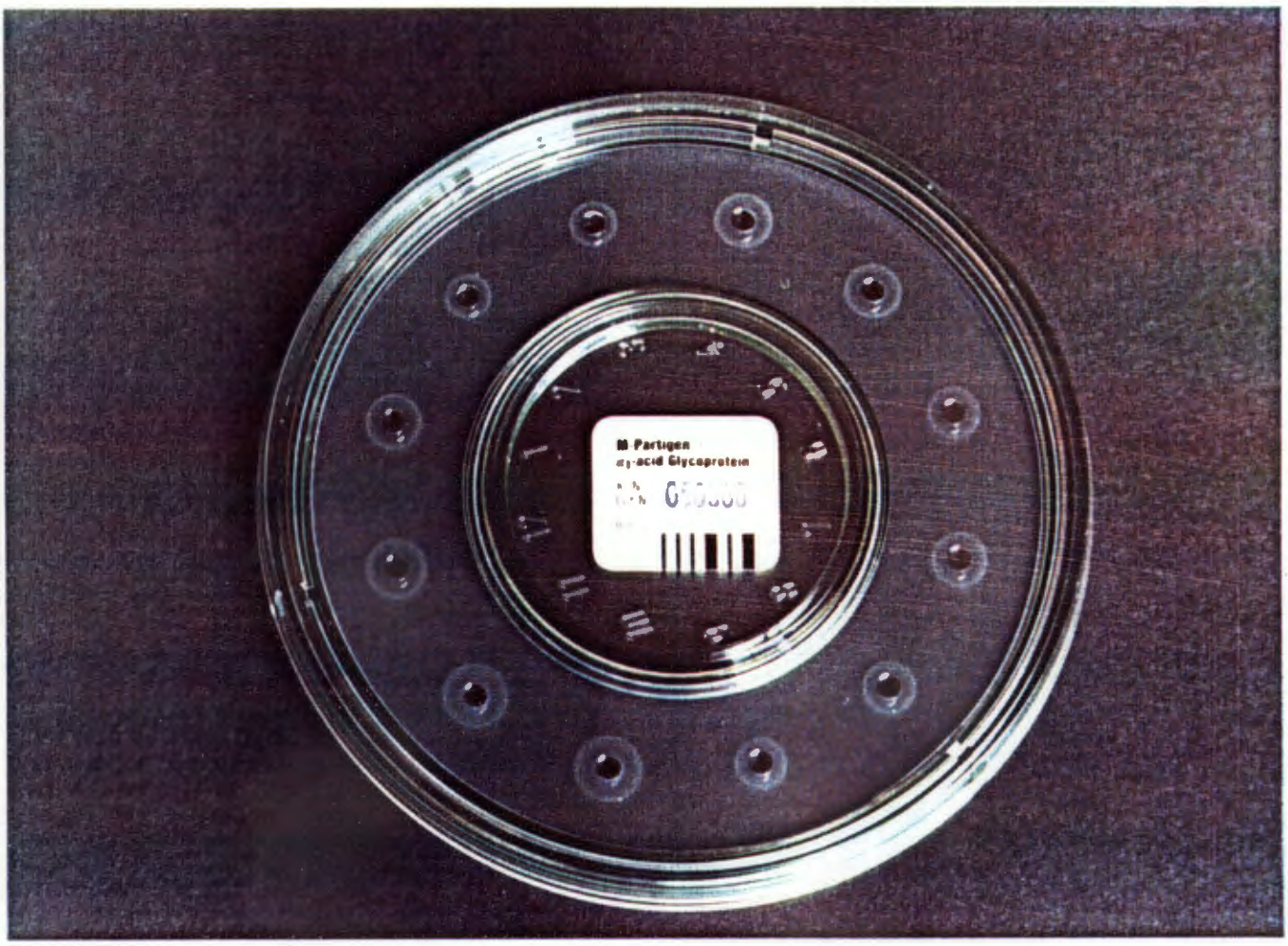


Figure 39
M-Partigen immunodiffusion plate (x 1).

between plasma and salivary cytosine arabinoside was established within 2.0 hours, and the mean saliva:plasma ratio during the infusion was 0.28.

Protein Binding Studies

The in vitro equilibrium dialysis studies in the serum taken from the 6 normal volunteers demonstrated rapid equilibration between serum and buffer cytosine arabinoside (2.5 hours),and no effect of concentration on protein binding was seen over a range of 20 ng to 10 ug/ml. In serum taken from 18 patients with acute myelogenous leukaemia at presentation protein binding ranged from -9.6 to 15.0 with a mean of 2.3 $\pm$ 6.8 , demonstrating that protein binding of cytosine arabinoside is negligible in patients with acute myelogenous leukaemia. This result was not significantly different from zero (95% confidence limits -1.1 to 5.7). The intra-assay coefficient of variation was 4.4%.

Serum Protein Concentrations in the Patients with Acute Myelogenous Leukaemia

In the majority of patients alpha 1 acid glycoprotein levels fell within the normal range (0.1-1.4 g/l) with a range of 0.43-1.92 g/l and a mean value of 1.05 $\pm$ 0.43 g/l. Five patients had values above 1.4 gm/l. Albumin and total protein were, however, below the normal range in many of the patients with a mean of 33.5 $\pm$ 6.8 and 61 $\pm$ 17.1 g/l respectively. There was no

correlation between cytosine arabinoside protein binding and the level of alpha 1 acid glycoprotein in individual patients.

Discussion

The protein binding of cytosine arabinoside in patients with acute leukaemia in this study was not significantly different from zero. This is in keeping with the data from van Prooijen et al (1977b) who found very low protein binding in serum from normal volunteers using ultrafiltration and ultracentrifugation. The apparently anomalous negative values for protein binding in some patients resulted from the variability in drug measurement.

It is regarded as a truism in clinical pharmacology that cerebrospinal fluid:plasma and saliva:plasma ratios of drug concentrations at equilibrium are a physiological measure of the free fraction (non protein bound) of a drug in plasma (Piafsky 1980, Bertilsson et al 1979 and Borga et al 1977). However, several factors influence the rate and extent to which drugs can cross from the plasma into extravascular fluid. Lipid solubility or oil:water partition coefficient play an important part and the more lipid soluble a drug the more easily it crosses the lipid membrane into extravascular fluid and tissue. Furthermore highly lipid soluble drugs tend to remain in tissues for longer than drugs with low oil:water partition coefficients. Since the lipid membrane is permeable only to the non-ionized form of a drug it is important to know the degree of ionization. The tendency to

ionize is expressed by the pKa value of a drug and for basic drugs such as cytosine arabinoside the higher the pKa relative to that of plasma the greater the tendency to ionize.

The movement of drugs from the blood into the brain is limited by end capillaries. These differ from peripheral capillaries in that their endothelium forms a continuous sheet of cells with no pores, and pinocytosis and fenestrae in the capillary wall are virtually absent (blood-brain barrier). Entry of drugs into the saliva from the plasma is affected by similar factors but not by the capillary endothelium.

Cytosine arabinoside is a weak base and has a pKa of 4.3. Thus at physiological pH it will be almost entirely un-ionized, which should aid its ability to cross lipid membranes. It is also a relatively small molecule with a molecular weight of 243. The theoretical equilibrium across lipid membranes for weak bases such as cytosine arabinoside is described by equation 1 (Taylor et al 1981).

$$R = \frac{1 + 10^{(pKa-pHCSF)}}{1 + 10^{(pKa-pHp)}} \times \frac{fp}{fCSF}$$

where: R = concentration ratio between cerebrospinal fluid or saliva and plasma

pKa = pKa of drug

pHp = pH of plasma (assumed to be 7.40) or saliva (assumed to be 6.5).

pHCSF = pH of cerebrospinal fluid (7.32 to 7.37)

fp = fraction of drug unbound to plasma proteins

fCSF = fraction of drug unbound to cerebrospinal fluid proteins (assumed to be 1.0)

Applying this equation to cytosine arabinoside the concentration ratio between extravascular fluids and plasma should approximate unity. There are two possible explanations for the low concentrations of cytosine arabinoside measured in the extravascular fluids compared to those predicted by this equation. Firstly, that equilibrium may not have been achieved. There is little data on the time taken to reach equilibrium between the cerebrospinal fluid and plasma during continuous infusions of cytosine arabinoside. However, the data from Ho et al(1971) and that in chapter 6 suggest that when a short infusion is preceded by a loading dose equilibrium is rapidly achieved. Furthermore, the simultaneous plasma and saliva samples were taken from patients who had received continuous infusions of cytosine arabinoside for at least 24 hours.

Secondly, cytosine arabinoside is a highly water-soluble drug with a very low oil-water partition coefficient, and this equation does not take lipid solubility into account. A study by Taylor et al(1981) examined the predicted and measured cerebrospinal fluid:plasma ratios for three beta-blockers. For propranolol,a highly lipid soluble beta-blocker, and pindolol, of intermediate lipid solubility , the measured cerebrospinal fluid:plasma ratios were very close to those predicted. However,

for atenolol, which has a very low oil-water partition coefficient, the measured values were only 20% of those predicted. It seems likely therefore that the poor lipid solubility of cytosine arabinoside is the major factor restricting its entry into extravascular fluids.

The extravascular concentrations of drugs may well represent a physiological measure of the non-protein bound fraction of lipid soluble drugs in plasma. However, the large discrepancy between the measured extravascular concentrations of water-soluble drugs and those predicted from a knowledge of the free fraction in plasma suggests that these ratios are not a reliable measure of the free fraction of lipid insoluble drugs.

CONCLUSIONS.

There is now little doubt that a proportion of patients with adult acute myelogenous leukaemia will achieve long term survival and possibly cure with combination chemotherapy. The first objective of chemotherapy for this disease is to achieve a complete remission, as patients failing to reach this state usually succumb rapidly. Initial attempts to treat this disease used relatively ineffective drugs such as 6 mercaptopurine, methotrexate and vincristine that had been useful in the treatment of acute lymphoblastic leukaemia. The introduction of cytosine arabinoside in 1963 represented a major advance in the treatment of acute myelogenous leukaemia. This drug is an analogue of cytidine that is phosphorylated to the triphosphate. Cytosine arabinoside triphosphate is a potent inhibiter of DNA synthesis by competitive inhibition of DNA polymerase. More recently it has been demonstrated that cytosine arabinoside is incorporated into DNA and this may prove to be the major mechanism of action

The use of combination chemotherapy in which cytosine arabinoside is combined with an anthracycline antibiotic such as doxorubicin or daunorubicin, with or without other drugs such as 6 thioguanine, vincristine and prednisolone, together with improvements in supportive care, has led to the majority of younger patients with acute myelogenous leukaemia achieving complete remission.

Cytosine arabinoside is a pyrimidine antagonist that has been shown to act predominantly in the S phase of the cell cycle. This property together with the rapid inactivation of the drug in plasma makes cytosine arabinoside the prime example of a phase specific drug.

The experiments of Skipper et al (1967) clearly showed that in mice with L1210 leukaemia continuous exposure to cytosine arabinoside was much more effective than single large doses. Following this lead, several groups investigated the effect of the schedule of cytosine arabinoside on its toxicity and efficacy in man. The majority (Frei et al 1969, Bodey et al 1969 and the South West Oncology Group 1974) suggested that continuous intravenous infusions of cytosine arabinoside over several days were more effective than repeated intravenous bolus injections or short intravenous infusions. However, in others (Burke et al 1968 and Wang et al 1970a) no effect of schedule could be demonstrated. More recently the use of combination chemotherapy and the high remission rate achieved have made it considerably more difficult to assess the place of the schedule of cytosine arabinoside in the treatment of these patients.

In view of this confusion many investigators now use repeated intravenous or subcutaneous bolus cytosine arabinoside rather than continuous intravenous infusion without compromising the complete remission rate. However, now that the attainment of complete remission is the expected rather than the exceptional

result of treatment programmes for acute myelogenous leukaemia, attention has been focused on the duration of these remissions. In the majority of reported studies most patients have relapsed within 12 months of achieving complete remission. The role of consolidation and maintenance therapy in prolonging remission duration remains controversial. Despite this a proportion of patients are alive and disease free 5 years after leukaemic blasts were cleared from the bone marrow and may well be cured. There are no consistent presentation features which help to identify these patients.

Recent data from the Cancer and Leukaemic Group B (CALGB) (Rai et al 1979, 1981a and b) have suggested that even when cytosine arabinoside is used in combination with other drugs such as daunorubicin,the schedule of administration might significantly influence the duration of response and survival of the patients. This remarkable result was achieved despite the fact that the cytosine arabinoside dose used in the continuous infusion schedules was much lower than that in the intravenous bolus schedules.

The study described in Chapter 3 is the only reported data comparing the same total dose of cytosine arabinoside given either by continuous intravenous infusion or twice daily intravenous bolus, used in combination with an anthracycline antibiotic and 6 thioguanine. Leukaemic blasts were cleared from the peripheral blood and bone marrow more rapidly when cytosine arabinoside was administered by continuous

intravenous infusion than when given by twice daily intravenous injection, demonstrating a greater anti-leukaemic effect for the intravenous infusion. This was, however, achieved at the cost of greater gastro-intestinal tract toxicity and more prolonged bone marrow suppression during the second cycle of chemotherapy. This study demonstrates that even when used with combination chemotherapy the schedule of cytosine arabinoside significantly affects its anti-leukaemic effect. The measurement of the exponential decline of the circulating blasts together with the time taken to achieve less than 5% blasts in the bone marrow is a simple and valuable means of evaluating the anti-leukaemic effect of cytotoxic drugs. Long term follow up on the duration of these remissions will be necessary to determine whether the increased toxicity resulting from the continuous infusion schedules is justified.

Despite the confusion about the best schedule of cytosine arabinoside administration, its S phase specificity has led many workers to administer it by continuous intravenous infusion. Recently, however, subcutaneous bolus injection has been used as an alternative to intravenous infusions.The rationale for this is based on the convenience to the patient and the suggestion from a study by Finklestein et al (1970) that following subcutaneous bolus injection, cytosine arabinoside declined with a half life of several hours. Methodological problems made this report difficult to assess and a study was therefore conducted to compare the

pharmacokinetics of subcutaneous bolus cytosine arabinoside to intravenous bolus and intravenous infusion. The results of this study showed that subcutaneous bolus cytosine arabinoside was rapidly absorbed and then declined with a half life similar to that of intravenous bolus injection. Plasma cytosine arabinoside concentrations following subcutaneous bolus were greater than those following intravenous bolus only during the first few hours and within 5 hours plasma cytosine arabinoside concentrations were only 10% of steady state infusion levels. However, the data from the CALGB (Rai et al 1979,1981 a and b) showed a significantly longer duration of remission in those patients who received subcutaneous bolus rather than intravenous bolus maintenance. This suggests that even relatively minor differences in cytosine arabinoside pharmacokinetics might significantly affect patient outcome.

These data suggested that the development of a delayed release formulation of cytosine arabinoside that could be subcutaneously administered would be a worthwhile objective. This was attempted using a suspension of cytosine arabinoside in arachis oil plus aluminium distearate. This formulation was chosen as it appeared to have the advantage of simplicity of preparation, and previous experience suggested that it delayed release of water soluble drugs for only a few hours which would be ideal for cytosine arabinoside. Initial quality control studies showed that the preparation was stable and evenly dispersed within the oily base. Studies in rabbits showed an acceptable degree of local tissue damage.

Investigation of the pharmacokinetics of this preparation in rabbits showed that neither the 1.5% nor the 4% aluminium distearate formulation delayed the release of cytosine arabinoside from the subcutaneous tissues. Similar studies in patients with 1.5 % and 2.5% aluminium distearate were also negative. The low oil/water partition coefficient of cytosine arabinoside presumably resulted in rapid dissolution of the suspended cytosine arabinoside in the interstitial fluid.

Having failed to create a delayed release formulation of cytosine arabinoside other alternatives to continuous intravenous infusion were examined. Continuous subcutaneous infusion has been successfully used for continuous administration of insulin and desferrioxamine. The high aqueous solubility of cytosine arabinoside and the rapid absorption from the subcutaneous tissues made this an attractive approach. It was subsequently demonstrated that subcutaneous infusions of cytosine arabinoside were equivalent to intravenous infusions and that both the time to achieve a plateau, the steady state levels, and the area under the curve were similar for both methods of administration. Subcutaneous infusion of cytosine arabinoside is thus a feasible alternative to intravenous infusion and offers the patients the benefit of outpatient therapy while preserving their venous access.

It was somewhat suprising that true steady state levels were difficult to achieve during continuous intravenous or

subcutaneous infusions of cytosine arabinoside at both conventional and very high doses. There was a greater than twofold within-patient variation in plasma cytosine arabinoside concentrations after the plateau had been achieved in some cases. It is possible that the high hepatic extraction ratio of this drug together with variations in hepatic blood flow resulting from alterations in posture might account for this variability. It is most important that future studies which attempt to correlate the plateau cytosine arabinoside concentrations with patient outcome are aware of this variability.

Infusions of high doses of cytosine arabinoside (30-60 times conventional doses) have recently been used in patients with relapsed and resistant acute leukaemia and may provide a means of overcoming resistance to conventional dose therapy. This therapy is associated with significant toxicity. All studies of high dose cytosine arabinoside have used 3 g/m^2 twice daily over 4-6 days. The dose of cytosine arabinoside has thus been increased dramatically without an evaluation being made of the therapeutic efficacy of intermediate doses. In view of the toxicity associated with high dose cytosine arabinoside therapy,a Phase I and II study of cytosine arabinoside at a dose of $2g/m^2$ twice daily was conducted. Forty patients with acute leukaemia and non hodgkin's lymphoma were studied and it was demonstrated that this may be effective therapy with less central nervous system toxicity than is found at the higher doses.

High dose cytosine arabinoside is generally administered by short infusions given twice daily for several days but the duration of these infusions varies in the reported studies. There are suggestions from the literature that cytidine deaminase might be saturated at high cytosine arabinoside concentrations and that cytosine arabinoside pharmacokinetics at high doses might therefore be non linear and dose dependent. The pharmacokinetics of high dose cytosine arabinoside when administered as a 1 hour infusion or as a 3 hour infusion with or without a loading dose were therefore examined. The result of this study showed that the area under the curve and the initial and terminal half lives of cytosine arabinoside following completion of the infusion were similar during all 3 methods of administration. Furthermore the half lives were also similar to those obtained after completion of a $100mg/m^2$ infusion over 12 hours. There is thus no evidence to suggest dose dependent pharmacokinetics of cytosine arabinoside at these large doses.

The prophylaxis and treatment of central nervous system leukaemia usually requires intrathecal chemotherapy. Patients with neoplastic meningitis may, however, have significant abnormalities of cerebrospinal fluid flow which can prevent even distribution of cytotoxic drugs administered via the lumbar route and may also contribute towards neuro-toxicity. Repeated lumbar punctures are often extremely unpleasant for the patients. It would clearly be to the advantage of the

patients if it were possible to produce prolonged cytotoxic levels of cytosine arabinoside in the cerebrospinal fluid with the same intravenous therapy used to control their systemic disease. Ho et al (1971) and Canellos et al (1979) have demonstrated that cytosine arabinoside crosses the blood brain barrier during continuous intravenous infusions of conventional doses. It seemed likely that the use of high doses of cytosine arabinoside would result in greater cerebrospinal fluid cytosine arabinoside concentrations than conventional dose infusions, and that these might therefore be useful therapeutically in the treatment of central nervous system leukaemia.

The relationship between the cerebrospinal fluid and plasma levels of cytosine arabinoside during high dose infusions was therefore studied in 15 patients with acute myeloblastic leukaemia and in 1 patient with acute undifferentiated leukaemia who had an Ommaya reservoir. These studies demonstrated that significant concentrations of cytosine arabinoside are found in the cerebrospinal fluid during therapy with high dose cytosine arabinoside and that levels are similar during both 1 and 3 hour infusions. The cerebrospinal fluid cytosine arabinoside concentrations at the end of the infusions were higher than the plasma cytosine arabinoside levels obtained during intravenous infusions at conventional doses of 100 to 200 mg/m^2 for 24 hours. Following the completion of the infusions cerebrospinal fluid cytosine arabinoside levels declined much more slowly than those in

plasma . It is likely that in patients treated with high dose cytosine arabinoside at a dose of $3g/m^2$ given every 12 hours cerebrospinal fluid cytosine arabinoside levels greater than 100ng/ml would be maintained almost continuously. This should provide effective therapy for central nervous system leukaemia and overcome any potential maldistribution of cytosine arabinoside in the cerebrospinal fluid in patients with leukaemic meningitis.

The data from the patient with the Ommaya reservoir showed rapid equilibration between plasma and cerebrospinal fluid cytosine arabinoside and suggested that cytosine arabinoside crossed the blood brain barrier as effectively during high dose infusions as at conventional dose infusions. The central nervous system toxicity associated with high dose cytosine arabinoside cannot be explained in terms of the peak levels achieved in the cerebrospinal fluid as these are several hundred times lower than those found following intrathecal cytosine arabinoside administration. It is likely that the central nervous system toxicity is related to the prolonged exposure to relatively high central nervous system concentrations of cytosine arabinoside that occur during this therapy.

The protein binding of cytosine arabinoside has been studied in normal plasma and found to be very low. It is generally considered that the cerebrospinal fluid:plasma and saliva:plasma ratio drug ratios represent a physiological

measure of protein binding. However, the data presented in this thesis showed a cerebrospinal fluid ratio at equilibrium which ranged from 0.12 to 0.25 . Furthermore cytosine arabinoside is a basic drug which might bind significantly to alpha 1 acid glycoprotein. It was thought possible that patients with acute myelogenous leukaemia who are often acutely ill and infected might have raised alpha 1 acid glycoprotein levels at presentation. It was therefore decided to measure saliva:plasma cytosine arabinoside ratios during steady state intravenous infusions of cytosine arabinoside as a further "physiological" measure of cytosine arabinoside protein binding and to complement this with an in vitro assessment of protein binding in serum from patients with acute myelogenous leukaemia at presentation using equilibrium dialysis. In addition the levels of total protein, albumin and alpha 1 acid glycoprotein were measured in these samples.

Cytosine arabinoside protein binding was negligible and was not affected by the dose of drug used. However, the saliva:plasma cytosine arabinoside ratios were not dissimilar to the cerebrospinal fluid:plasma ratios that had previously been determined. There was no correlation between the levels of alpha 1 acid glycoprotein and the extent of cytosine arabinoside protein binding. It is likely that the poor lipid solubility of cytosine arabinoside leads to its relative exclusion from extravascular fluids. These data suggest that the cerebrospinal fluid and saliva:plasma cytosine arabinoside

ratios may not represent a true physiological measure of the unbound fraction of water soluble drugs.

In summary, based on the data presented here, the following conclusions and recommendations can be made:

1) Cytosine arabinoside has a greater anti-leukaemic effect when given by continuous intravenous infusion than when the same dose is given by twice daily intravenous bolus injection. This effect is not masked by the use of combination chemotherapy. The continuous infusion schedule is, however, much more toxic and a reduced dose must be used in order that the patients can tolerate the full course of therapy.

2) High dose cytosine arabinoside has considerable activity in relapsed acute leukaemia and non hodgkin's lymphoma. The use of $2g/m^2$ rather than the conventional $3g/m^2$ may significantly reduce the incidence of central nervous system toxicity, in particular cerebellar toxicity.

3) Subcutaneous bolus cytosine arabinoside is rapidly absorbed and is pharmacokinetically similar to intravenous bolus. Within a few hours the plasma levels are much lower than those found with continuous intravenous infusion.

4) Subcutaneous infusion of cytosine arabinoside is well tolerated and gives equivalent plasma concentrations to continuous intravenous infusion.

5) High dose cytosine arabinoside gives plasma concentrations proportional to the dose administered. There is no evidence of saturation of cytidine deaminase and kinetics are therefore linear and not dose dependent.

6) Cerebrospinal fluid concentrations during high dose cytosine arabinoside therapy are approximately 10% of plasma concentrations, and rise in proportion to the dose of cytosine arabinoside administered. There is rapid equilibration between the plasma and cerebrospinal fluid but the rate of administration of the intravenous infusion does not influence the cerebrospinal fluid concentrations.

Cytosine arabinoside has a much longer half life in the cerebrospinal fluid than in the plasma. This results in cerebrospinal fluid concentrations during high dose intravenous therapy comparable to those obtained in the plasma with conventional dose continuous intravenous infusions.

The use of cytosine arabinoside over the past 15 years, initially as a single agent and more recently in combination with other drugs, has resulted in a dramatic improvement in the prognosis of patients with acute myelogenous leukaemia. Despite extensive experience with the clinical use of cytosine

arabinoside the most effective schedule and route of administration both for systemic and central nervous system leukaemia remain controversial. It is hoped that the clinical and pharmacokinetic data presented in this dissertation will help to answer some of these questions.

APPENDIX

RAW DATA

RAW DATA

THE PHARMACOKINETICS OF SUBCUTANEOUS CYTOSINE ARABINOSIDE IN PATIENTS WITH ACUTE LEUKAEMIA

CYTOSINE ARABINOSIDE CONCENTRATIONS (ng/ml)

PATIENT	TIME IN HOURS																
	0.05	0.1	0.17	0.25	0.33	0.5	0.75	1	1.5	2.0	2.5	3.0	4.0	6.0	8.0	10.0	12.0
J.A.	4000	1130	748	453	270	176	97	62	40	22	16	17	13	8.5	8.3	8.0	7.5
E.N.	1070	470	242	160	110	77.8	44	28.3	16.5	25.5	10.6	10.5	26	16.6	4.8	3.9	12.4
G.W.	2300	1120	671	377	369	149	95	49	18	26.9	11.2	27	23	23	21	17	21
R.S.	4200	1080	650	445	312	19.6	115	77	33	19	17	15	8.3	5.1	7.5	10.8	9.0
P.W.	5800	2120	755	346	248	121	69.5	42.5	21	14.9	11	10.7	8.5	3.6	7.9	3.7	3.5
Mean	3474	1184	613.2	356	261	144	84.1	51.7	25.7	21.6	13.1	16	15.7	11.3	9.9	8.7	10.6
S.D.	1828	592	212.0	118	96.5	46.5	27.6	18.6	10.2	4.87	3.0	6.7	8.2	8.2	6.3	5.5	6.5

Pharmacokinetics of Cytosine Arabinoside following an Intravenous Bolus (100 mg/m^2)

CYTOSINE ARABINOSIDE CONCENTRATIONS (ng/ml)

PATIENTS	TIME IN HOURS 0.05	0.1	0.17	0.25	0.33	0.5	0.75	1	1.5	2	2.5	3	4	6	8	10	12
J.A.	300	658	988	913	531	294	213	109	54	47.2	41.7	24.2	16.9	8.0	5.1	5.6	7.4
E.M.	596	707	581	575	278	190	165	75	57	40	39	24	15.2	7.8	9.3	11.8	14.9
G.W.	428	1130	1301	1346	898	498	362	229	110	50	40	24	10.1	8.4	24	4.87	13.8
R.S.	360	625	654	743	720	703	558	401	255	145	51.5	59.4	41.7	16.5	16.1	9.9	12.8
P.W.	175	387	513	586	478	263	204	170	49	40	32.5	22	17.5	8.0	6.4	6.7	4.0
Mean	371.8	701.4	807	851	581	389	300	196.8	105	64.4	40.9	30.6	20.2	9.7	12.1	7.7	10.5
S.D.	156	269.3	330	356	237	209	162	128.4	87.4	45.2	6.8	16.0	12.3	3.8	7.8	2.9	4.6

Pharmacokinetics of Cytosine Arabinoside following a Subcutaneous Bolus (100 mg/m^2)

CYTOSINE ARABINOSIDE CONCENTRATIONS (ng/ml)

PATIENT	TIME IN HOURS 0.05	0.1	0.17	0.25	0.33	0.5	0.75	1	1.5	2	4	6	8	10	12
J.A.	2450	790	510	373	225	205	101	80	100	82	71	92	57	76	75
E.M.	645	300	146	115	97	88.5	68	50.5	52	50	73	46	42	40	44
G.W.	-	790	508	285	202	185	133	106	110	126	148	130	115	108	104
R.S.	-	880	229	166	182	143	142	132	93.5	128	117	82	116	80	61
P.W.	-	-	-	-	-	-	-	80	90	105	98.8	87.5	90	92	94
Mean	1547.5	690	348.2	234	176.5	155.3	111	89.7	89.1	98.2	101.5	87.5	84	79.2	75.6
S.D.	(1276)	263	188	116	55.8	51.5	33.6	30.7	22.09	32.7	32.2	29.9	33.5	25.2	24.2

Pharmacokinetics of Cytosine Arabinoside during an Intravenous Infusion (100 mg/m^2) over 12 hours preceded by a Loading Dose

CYTOSINE ARABINOSIDE CONCENTRATIONS (ng/ml)										
PATIENT	TIME IN HOURS 0.05	0.1	0.17	0.25	0.33	0.5	0.75	1	1.5	2
J.A.	39	32	27	27	20	15	13	13	11	9
E.M.	23.5	20	18.3	-	-	13.3	11	10.4	-	-
G.W.	119	129	85	81.3	71	64	43.7	50	32.8	35
R.S.	39.8	-	44	37.8	44.5	27.7	32.2	31.6	28.5	20.5
P.W.	63	50	44	30	25	17.4	13	10.5	9	7.9
Mean	56.8	57.7	43.6	44	40	27.4	22.5	23.1	20.3	18.1
S.D.	37	49	25.6	25.2	23	21.1	14.6	17.4	120	12.6

Pharmacokinetics of Cytosine Arabinoside following completion of an Intravenous Infusion (100 mg/m^2)

CYTOSINE ARABINOSIDE CONCENTRATIONS (ng/ml)

RABBITS	TIME IN HOURS 0	0.08	0.17	0.25	0.33	0.5	0.75	1	1.5	2	2.5	3	4	5	6	8	10	12
A	5	18800	26900	31600	38800	30000	12000	8000	12000	7200	4600	3140	1180	426	226	79	26	20
B	0	14400	30000	26000	43000	44000	30000	-	25000	18500	14000	8900	5500	2900	2100	570	163	83
C	0	14200	22700	26100	31800	22100	19900	17900	15000	8600	5.400	4500	1600	1500	571	279	186	26
Mean	1.7	15800	26533	27900	37866	32033	20633	12950	17333	11433	8000	5513	2760	1608	1542	309	125	43
S.D.	2.8	2600	3663	3204	5658	11090	9022	7000	6806	6159	5211	3010	2382	1240	1337	246	86	34

Subcutaneous Bolus Cytosine Arabinoside (30 mg/kg in Saline) in New Zealand White Rabbits

CYTOSINE ARABINOSIDE CONCENTRATIONS (ng/ml)

RABBIT	TIME IN HOURS 0	0.08	0.17	0.25	0.33	0.5	0.75	1	1.5	2	2.5	3	4	5	6	8	10	12
A	7.5	7200	11550	22250	30500	29500	29000	10800	9130	7550	4300	3100	1290	401	218	77	36	47
B	16.8	22000	30700	33600	35300	36800	40800	29600	18700	11000	8000	6800	3800	2100	1500	400	152	61
C	13	12900	23900	28200	27600	27500	21400	19100	12400	8700	5000	3900	2500	2400	645	170	71	39
Mean	12.4	14033	22050	28016	31133	c31366	30400	19833	13410	9083	5766	4600	2530	1633	787	215	86.3	49
S.D.	4.7	7464	9708	5677	3888	4781	9775	9421	4864	1756	1965	1946	1255	1078	652	166	59.5	11.1

Subcutaneous Bolus Cytosine Arabinoside (30mg/kg in Arachis Oil plus 1 1/2% Aluminium Disterate) in New Zealand White Rabbits

CYTOSINE ARABINOSIDE CONCENTRATIONS (ng/ml)

RABBIT	TIME IN HOURS 0	0.08	0.17	0.25	0.33	0.5	0.75	1	1.5	2	2.5	3	4	5	6	8	10
D	6	27500	35400	43800	56600	43000	45500	36000	23000	17300	11400	9600	4580	1730	1250	540	170
E	4	22900	59300	39000	3830	40000	42000	41500	30000	20500	15700	10600	6800	4100	1900	660	195
F	4.3	30000	33800	44000	50000	45800	39300	27800	21000	15800	9000	5500	3600	1980	475	151	
Mean	4.76	26800	42500	42467	48300	42933	42267	35100	24667	17867	12033	8566.7	4993.3	2603.3	1503.3	558.33	172
S.D.	1.07	3601.4	1370.7	2830.8	9267.7	2900.6	3108.6	6894.2	4725.8	2400.7	3394.6	2702.5	1639.6	1302.2	347.90	93.853	22.068

Subcutaneous Bolus Cytosine Arabinoside (30mg/kg in Saline) in New Zealand White Rabbits

CYTOSINE ARABINOSIDE CONCENTRATIONS (ng/ml)

RABBITS	TIME IN HOURS 0	0.08	0.17	0.25	0.33	0.5	0.75	1	1.5	2	2.5	3	4	5	6	8	10
D	16.5	21300	23000	32800	29900	31000	29000	31500	16100	14000	11500	8830	4750	2800	1560	685	303
E	5.3	5000	13300	23300	14300	46800	25500	10300	16600	12600	8700	7300	4450	2800	2100	943	555
F	17	8200	15300	24300	-	22000	17400	18800	15100	13400	11800	9200	4800	2700	1810	535	189
Mean	12.9	11500	17200	26800	22100	33266.7	23966.7	20200	15933	13333	10667	8443.3	4666.7	2766.7	1823.3	721	349
S.D.	6.6	8636.6	5121.5	5220.2	(11030)	12554	5950.1	10669	763.7	702.3	1709.8	1007.3	189.3	57.7	270.2	206.4	187.3

Subcutaneous Bolus Cytosine Arabinoside (30mg/kg in Arachis Oil plus 4% Aluminium Disterate) in New Zealand White Rabbits

CYTOSINE ARABINOSIDE CONCENTRATIONS (ng/ml)

PATIENT	TIME IN HOURS 0	0.08	0.17	0.25	0.33	0.5	0.75	1	1.5	2	3	4	6	8	10	12
S.J.	18.7	207	-	850	490	337	-	176	103	54	39	20	16	18	8.3	7.5
J.C.	2.5	510	800	750	475	513	272	187	90	64	39	23	10	8	7	5
J.R.	7.9	270	650	650	440	493	297	177	67	46	25	17	11	10	8	6
G.B.	6.3	1100	763	-	530	-	290	220	129	85	36	27	10	8	6.5	4.3
Mean	8.8	521.7	737	750	483	447.6	286.3	196	97.2	62.2	34.7	21.7	11.7	11	7.4	5.7
S.D.	6.9	407	78.1	100	37	96.3	128	20.6	25.8	16.8	6.6	4.3	2.9	4.8	0.8	1.4

Subcutaneous Bolus Cytosine Arabinoside (100 mg/m^2) in Saline

CYTOSINE ARABINOSIDE CONCENTRATIONS (ng/ml)

PATIENT	TIME IN HOURS 0	0.08	0.17	0.25	0.33	0.5	0.75	1	1.5	2	3	4	6	8	10	12
S.J.	12.1	400	430	355	438	375	370	200	158	86	39	26	18	17.5	16	16
J.C.	3.9	815	722	608	633	487	405	372	155	100	41	20	10	3.2	6	4.9
J.R.	7.9	986	950	1150	1000	888	608	403	244	120	57	26	16	16	10	5.5
G.B.	3	591	488	47.5	478	328	143	105	68	26	15	12	9	7	6	5
Mean	6.7	698	647	647	637	519	381.5	270	156	83	38	21	13.2	10.9	9.5	7.8
S.D.	4.1	256	237	350	256	254	190	141	71.8	40	17	6.6	4.4	6.9	4.7	5.4

Subcutaneous Bolus Cytosine Arabinoside (100 mg/m^2) in Arachis Oil Plus 1 1/2% Aluminium Disterate.

CYTOSINE ARABINOSIDE CONCENTRATIONS (ng/ml)

PATIENT	TIME IN HOURS 0	0.08	0.17	0.25	0.33	0.5	0.75	1	1.5	2	3	4	6	8	10	12
S.J.	12	208	273	350	297	332	271	172	81	52	27	20	17	10	10	14
J.C.	1.8	445	508	458	438	418	311	289	123	-	56	37	16.5	8	4.8	3.6
J.R.	10.3	1270	1050	715	473	548	530	513	402	150	60	41	27	20	9	8
G.B.	9.8	-	875	948	875	558	259	148	66	54	23	15	6,8	8.5	9	6
Mean	8.5	641	676.5	617	520	464	342	280.5	168	85.3	41.5	28.2	16.8	11.6	8.2	7.9
S.D.	4.5	557	351	268	248	108	126	16	158	56.0	19.1	12.6	8.2	5.6	2.3	4.4

Subcutaneous Bolus Cytosine Arabinoside (100 mg/m^2) in Arachis Oil Plus 2 1/2% Aluminium Disterate

CYTOSINE ARABINOSIDE CONCENTRATIONS (ng/ml)

PATIENTS	TIME IN HOURS 0.5	1	1.5	2	3	4	5	6	8	10	12
L.R.	49	64	77	42	66	56	71	87	102	90	64
S.A.	62	84	78	67	59	91	80	102	110	73	105
S.J.	59	73	74	89	101	70	100	88	77	82	85
C.C.	97	102	114	87	92	92	125	102	131	99	108
J.M.	77	126	127	100	142	136	140	129	109	157	137
J.C.	84	84	80	87	93	72	113	98	105	-	145
Mean	71	89	92	79	92	86	105	101	106	100	107
S.D.	17.8	24.7	22.7	20.8	29.4	27.9	26.4	15.2	17.3	33.1	30.5

Intravenous Infusion of Cytosine Arabinoside (100 mg/m^2) over 12 hours

CYTOSINE ARABINOSIDE CONCENTRATIONS (ng/ml)

PATIENTS	TIME IN HOURS 0.5	1	1.5	2	3	4	5	6	8	10	12
L.R.	24	29	50	65	79	80	-	83	71	70	59
S.A.	25	84	82	109	61	104	92	107	147	107	116
S.J.	49	62	65	66	66	53	52	75	75	74	80
C.C.	93	106	116	128	202	86	78	155	76	125	124
J.M.	25	42	110	79	93	111	66	103	50	103	155
J.C.	31	46	66	66	82	84	87	97	86	53	74
Mean	41	62	82	85	97	86	75	103	84	89	101
S.D.	27	28.8	26.4	25.9	52	20.3	16.2	28.1	32.9	27.1	36.3

Subcutaneous Infusion of Cytosine Arabinoside (100 mg/m^2) over 12 hours

RAW DATA

THE PHARMACOKINETICS OF HIGH DOSE CYTOSINE ARABINOSIDE

CYTOSINE ARABINOSIDE CONCENTRATIONS (ng/ml)

PATIENT	TIME IN HOURS 0	0.25	0.5	0.75	1	1.5	2	2.5	3	3.08	3.17	3.25	3.33	3.5	3.75	4	4.5	5	6	8	10	12
R.W.	43	7500	7300	10700	9500	1400	1330	1330	1100	3900	2300	1900	-	1100	737	728	548	257	179	145	53	44
G.M.	38	720	3000	3200	4600	5000	3700	5300	5300	4400	2500	2700	1600	1600	718	527	422	253	192	62	41	23
A.B.	29	3500	4900	-	5800	6200	5500	6000	6100	3100	2300	1400	1300	1800	875	540	263	93	88	43	41	37
G.S.	3.2	2740	6800	-	6050	6560	7000	6500	3150	1370	800	750	400	345	-	185	-	50	34	20	-	-
G.M.	67	7000	5100	-	6800	8200	5300	8400	6700	2400	1800	1200	1000	1000	537	550	361	295	141	121	75	65
H.W.	0	3300	6900	-	11400	10100	13600	13300	9400	-	5400	-	4000	2900	1700	1000	698	455	380	339	300	223
Mean	30	4126	5666	(6950)	7358	6243	6071	6805	5291	3034	2516	1509	1660	1457	913.4	588	458.4	233	169	121	102	78.4
S.D.	25.4	2616	1642	(5303)	2568	2960	4160	3942	2886	1203	1540	745	1380	870	455	268	169.1	146	118	116	111.5	82.2

High Dose Cytosine Arabinoside (2 g/m^2) given as a 3 hour Intravenous Infusion

CYTOSINE ARABINOSIDE CONCENTRATIONS (ng/ml)

PATIENT	TIME IN HOURS 0	0.25	0.5	0.75	1	1.08	1.17	1.25	1.33	1.5	1.75	2	2.5	3	4	5	6	8	10	12
R.W.	31	25300	26600	23000	11100	6300	4000	2700	2700	1700	1300	679	336	255	139	133	109	36	28	50
G.M.	1.6	18700	19300	23600	27200	11900	10000	7200	5300	4500	2300	1500	633	360	200	140	82	75	25	18
A.B.	0	12400	15700	18900	20100	12800	10500	7400	6000	4300	2800	1700	765	277	130	90	64	63	28	19
G.S.	17.8	22900	27600	27300	21500	5000	2880	1680	820	420	275	235	115	100	55	-	3.9	22	26	-
G.M.	84	28300	25300	27300	26100	10600	5700	3300	3400	2800	1400	1500	393	456	235	-	138	81	-	-
H.W.	-	18100	29700	36000	36300	17800	17100	-	10300	8900	6200	3400	1600	603	677	449	476	381	350	-
Mean	26.88	20950	24033	26016	23716	10733	8363	4456	4753	3770	2379	1502	640	341.8	239	203	151.3	109	91.4	29
S.D.	34.36	5710	5382	5806	8406	4650	5287	2660	3290	2952	2065	1088	522	174	223	165	162	134	144	18

High Dose Cytosine Arabinoside (2 g/m^2) given as a 1 hour Intravenous Infusion

TIME IN HOURS	PATIENTS R.W.	G.Mo.	A.B.	G.S.	G.M.	H.W.	Mean	S.D.
0	33	40	49	0	1.8	0	20.6	22.5
0.08	19700	21000	28500	44000	8300	22700	24033	11797
0.17	19700	13400	22900	21400	10000	13100	16750	5258
0.25	12100	12300	9500	15000	5500	9900	10716	3228
0.33	9300	11500	9400	10100	6100	6600	8833	2083
0.5	10300	7900	8800	6800	5900	6400	7683	1658
0.75	6700	5100	6400	6000	3900	3000	5183	1474
1	5900	4900	5300	7500	5800	3800	5533	1227
1.5	6900	5400	5300	7100	2100	3700	5083	1914
2	5500	5100	5200	7700	5800	4600	5650	1082
2.5	6800	6100	5200	7100	3400	5500	5683	1334
3	6300	7000	5800	7300	4400	6200	6166	1025
3.08	2500	3900	5200	1410	1400	-	2882	1652
3.17	2000	3200	4400	951	950	2500	2333	1340
3.25	1600	1600	2300	630	790	-	1384	680
3.33	1300	2000	1700	497	833	1500	1305	557
3.5	890	1300	1200	375	514	895	862	365
3.75	610	758	675	325	370	563	550	170
4	419	537	495	305	295	457	418	99.5
4.5	207	354	328	-	208	-	274	77.8
5	-	222	205	129	200	-	189	41
6	138	249	106	56	137	187	145.5	66.5
8	78	54	69	34	105	120	76	31.8
10	66	57	47	29	76	132	67	35.3
12	70	24	37	-	57	135	64.6	43.1

CYTOSINE ARABINOSIDE CONCENTRATIONS (ng/ml)

High Dose Cytosine Arabinoside (2 g/m^2) given as a 3 hour Intravenous Infusion Preceded by a Loading Dose

Cytosine Arabinoside Concentrations (ng/ml)

PATIENT	TIME IN HOURS 0.08	0.17	0.25	0.33	0.5	0.75	1	1.5	2	2.5	3	3.08	3.17	3.25	3.33	3.5	3.75	4	4.5	5	5.5	6
G.W.	16100	6900	5100	5400	4280	3380	3100	3370	3480	-	3280	-	1830	1240	898	729	283	235	112	51	43	76
M.C.	18350	10570	7100	6220	5950	5650	5200	5180	4910	-	4350	1650	1040	778	630	467	242	171	78	57	44	56
G.O.	-	13000	-	7200	2310	2060	2000	1650	1600	2140	-	-	1610	626	403	251	151	143	118	82	21	70
M.M.	19200	9400	6100	4900	2900	3200	2500	4000	2900	3200	2600	1500	944	671	370	306	143	125	78	69	60	92
R.L.	5630	4400	3900	2800	3100	2800	2500	2150	2370	2510	2430	1570	993	851	603	384	215	148	69	51	45	35
Mean	14820	8854	5550	5104	3708	3418	3160	3270	3052	2616	2960	1573	1283	835	581	427	207	164	91	62	43	66
S.D.	6265	3320	1370	1569	1443	1347	1282	1470	1248	538	883	75	407	248	212	187	60	43	22	13	14	21

Plasma Pharmacokinetics of High Dose Cytosine Arabinoside (1 g/m^2) given as a 3 hour Infusion Preceded by a Loading Dose

Cytosine Arabinoside Concentrations

PATIENT	TIME IN HOURS 0	0.25	0.5	0.75	1	1.08	1.17	1.25	1.33	1.5	1.75	2	2.5	3	3.5	4
M.P.	1	5600	11900	12400	10300	-	4700	3950	2900	1370	950	497	195	151	90	64
R.W.	27	10700	12100	12300	8400	4400	2400	2030	1500	1400	618	518	177	124	118	114
A.B.	1.1	7500	10600	20100	8900	4600	3300	3300	1500	1350	633	610	224	184	140	115
J.P.	0	5900	11800	-	12200	5500	4400	2200	1500	1200	813	293	203	147	90	106
P.H.	3.9	6700	7700	7500	8100	4100	3600	2300	2200	1200	708	675	185	84	62	57
Mean	6.6	7280	10820	13075	9580	4650	3680	2756	1920	1304	744	518	196	138	100	91
S.D.	11.4	2049	1840	5211	1690	602	914	831	626	96	138	145	18	37	29	28

Plasma Pharmacokinetics of High Dose Cytosine Arabinoside (lg/m^2) given as a 1 hour Intravenous Infusion

CYTOSINE ARABINOSIDE CONCENTRATIONS (ng/ml)

Patient	Time in Hours .25	.5	.75	1	1.5	2	2.5	3	3.08	3.17	3.25	3.33	3.5	3.75	4	5	6	8	10	12
J.Sp Dose 1	3630	5200	5600	5500	5300	4970	4300	6106	3000	1350	1260	760	626	324	260	---	80	44	21	16
5	5400	7600	6100	5600	4900	6100	5800	6200	1950	1380	1350	752	765	292	157	120	64	120	50	39
11	3530	4100	3800	3700	3500	3600	5400	6000	1050	710	592	420	434	298	150	90	58	37	27	31
J.S. Dose 1	4400	5300	7700	7400	8100	10500	8800	10100	3500	3000	2300	1600	1300	700	463	177	91	48	34	24
5	6400	7500	7000	6500	7900	9100	10900	8900	4100	2400	1400	1200	870	---	401	185	63	38	29	17
11	595	7100	7900	7200	9800	9200	11400	6900	3300	2200	1970	1270	1200	745	535	158	81	73	--	--

Plasma Pharmacokinetics of High Dose Cytosine Arabinoside ($2g/m^2$) Given as a 3 Hour Infusion during Dose 1, 5 AND 11.

CYTOSINE ARABINOSIDE CONCENTRATION (ng/ml)

PATIENT	TIME IN HOURS																				
	.08	.17	.25	.33	.5	.75	1	1.5	2	2.5	3	3.08	3.17	3.25	3.33	3.5	3.75	4	4.5	5	5.5	6
S.J.	24900	23400	13500	11900	10200	10800	1300	7100	6000	8200	7200	1800	1810	1160	1060	989	495	333	255	145	119	101
J.C.	16000	-	11800	10200	6700	6800	6600	6500	6900	5400	6300	3400	2600	1700	1700	777	665	462	200	-	138	93
R.B.	36500	32100	20200	20800	25200	25000	14700	15700	15000	16800	16400	4400	3500	2970	2720	1430	918	710	541	314	255	265
J.S.	42300	27700	13200	14800	10100	10200	10300	9400	9400	10700	8900	4800	3200	1600	2050	1400	875	619	609	382	-	277
R.W.	50600	19200	18900	13600	9800	9000	8100	8400	8900	9300	9200	5500	3300	-	-	1200	1600	1400	355	272	231	222
Mean	34060	25600	15520	14260	12400	12360	9400	9420	9240	10080	9600	3980	2882	1857	1882	1159	910	704	392	278	186	191
S.D.	13761	5551	3762	4048	7301	7230	3273	3687	3510	4230	3986	1435	687	778	693	277	421	414	178	99	67	89

Plasma Pharmacokinetics Of High Dose Cytosine Arabinoside ($3g/m^2$) Given As a 3 Hour Infusion Preceded By a Loading Dose

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