Aluminum acetate solution in the treatment of chronic suppurative otitis media.

Marc A. Thorp

Aluminium acetate solution in the treatment of chronic suppurative otitis media.

Marc A. Thorp

Thesis presented for the Degree of

Doctor of Medicine

in the Department of Otolaryngology

Faculty of Health Sciences

University of Cape Town

October 2005

Supervisors

Professor C. A. J. Prescott*

Professor R. V. Harrison†

*Department of Otolaryngology, University of Cape Town, South Africa.

†Department of Otolaryngology, University of Toronto, Canada.

Declaration

I, Marc Alexander Thorp, claim that this thesis is original work and has not been submitted in any form for a degree at any other university.

Signed: Signed by candidate Date: October 2005

Acknowledgements

Study 1:

Mr. J. Kruger and Dr. S. P. Oliver for their assistance with the bacteriological work.

Dr. S. Isaacs for the statistical analysis.

The Groote Schuur Hospital Pharmacy Department for preparation of the Burow's solution.

Study 2:

Mr. J. Kruger and Dr. S. P. Oliver for their assistance with the bacteriological work.

The Groote Schuur Hospital Pharmacy Department for preparation of the Burow's solution.

Study 3:

Dr. I Gardiner for his assistance in running the clinical study.

Professor C. A. J. Prescott for his advice and assistance.

Dr. S. Isaacs for statistical analysis.

Dr. S. P. Oliver, Mr. J. Kruger and the Red Cross War Memorial Children's Hospital Microbiology Department for the bacteriology.

Mrs. J. Fermor and Ms. C. Hastie for audiometric assessments performed.

The Groote Schuur Hospital Pharmacy Department for preparation of the Burow's solution and the dilutions thereof.

Study 4:

The Medical Neurotology Fellowship of the University Health Network, University of Toronto, Toronto, Ontario, Canada.

The Toronto General Hospital Pharmacy Department for preparation of the Burow's solution.

Professor R. V. Harrison and Mr. R. J. Mount for the use of the facilities at the Auditory Science Laboratory, University of Toronto, Toronto, Canada.

Drs. H. El-Hakim and J. Panesar for their assistance with the laboratory study.

Mr. R. J. Mount for his assistance with the Scanning Electron Microscopy.

Additional Statistical Analysis

Mr. W. Rowe, Department of Mathematics, Grenfell College, Memorial University, Newfoundland, Canada.

Lastly, to Professors C. A. J. Prescott and R. V. Harrison for their supervision, advice, support and encouragement in the preparation of the thesis.

Abstract

Objectives

To determine *in vitro* the efficacy of Burow's solution, in inhibiting growth of common pathogens found in chronic suppurative otitis media, and to determine effective dilutions.

To determine *in vivo* by means of a clinical trial the efficacy of aluminium acetate solution in chronic suppurative otitis media.

To assess by means of an animal model, the safety of these preparations.

Materials and Methods

A range of bacteria commonly encountered in chronic suppurative media were tested against acetic acid and Burow's solution. Thereafter dilutions of Burow's solution were used to determine a minimum inhibitory dilution (MID).

A blinded randomised clinical study was completed to assess clinical efficacy of Burow's solution and dilutions thereof in the treatment of chronic suppurative otitis media. Patients participating were audiologically monitored for signs of ototoxicity.

Finally, a 3.25% aluminium acetate solution and 2% acetic acid were tested against normal saline in adult chinchillas, where distortion product otoacoustic emissions as well as electron microscopy were used to assess ototoxicity.

Results

In a laboratory study, Burows' solution (13% aluminium acetate) was found to be bacteriocidal against all bacteria tested, and whilst 1 – 3% acetic acid showed similar activity it was ineffective against *Staphylococcus aureus*.

Burow's solution (13% aluminium acetate) demonstrated a minimum inhibitory dilution of 1:160 against all bacteria tested except *Staphylococcus aureus* which was inhibited by a 1:80 dilution.

In a clinical study, Burow's solution(13% aluminium acetate) and 3.25% aluminium acetate solution(quarter strength Burow's) were clinically effective whereas 1.3% aluminium acetate solution(1/10 strength Burow's solution) was found not as effective in controlling otorrhoea of chronic suppurative otitis media in 56 ears. Audiologic testing of study patients failed to demonstrate any ototoxicity.

In a laboratory study using a chinchilla animal model, 3.25% aluminium acetate solution and 2% acetic acid were found to be ototoxic using electrophysiologic testing and electron microscopy.

Conclusions

Burow's solution is effective *in vitro* in inhibiting growth of all common pathogens found in chronic suppurative otitis media.

An effective *in vitro* dilution of Burow's solution was determined.

Burow's solution(13% aluminium acetate) was demonstrated at a quarter strength dilution(3.25% aluminium acetate solution), to be ototoxic in an animal model, as was 2% acetic acid.

It remains a highly effective alternative to antibiotic containing ear drop preparations – of which those containing quinolones are currently believed to have the least ototoxic potential. Should quinolone based ear drops be unavailable or bacteriologically ineffective, aluminium acetate solution could be considered for use in chronic suppurative otitis media, provided topical application be discontinued should any alteration in hearing threshold or vestibular function occur.

The first 3 studies were performed in laboratory and clinical settings in Cape Town, South Africa, whilst the fourth was performed in an animal laboratory in Toronto, Canada (these facilities not being available in South Africa).

Contents

1. Introduction

1.1. Thesis and Hypotheses.

1.1.1. Thesis

Aluminium acetate solution ear drops can effectively and safely be used in the treatment of chronic suppurative otitis media.

1.1.2. Hypotheses

1. Topical aluminium acetate ear drop preparations can be demonstrated *in vitro* to have effective inhibiting activity against a range of causative organisms commonly seen in chronic suppurative otitis media.
2. This medication can be shown by means of a randomized clinical trial to be clinically effective in the management of otorrhoea in patients with chronic suppurative otitis media and to be demonstrably free side effects.
3. It can be ascertained *in vivo* in an animal study model that the ear drop preparation is safe for clinical use.

1.2. Chronic Suppurative Otitis Media

Summary

Chronic suppurative otitis media involves mucopurulent discharge from the ear via a tympanic perforation of 3 months duration or longer. The 2 most common causative organisms are Staphylococcus aureus *and* Pseudomonas aeruginosa. *Therapeutic intervention is aimed at resolution of otorrhoea. Treatment includes ear toilette, the application of antiseptic or antibiotic ear drops and systemic antibiotics. Cure is effected by surgical closure of the tympanic defect. This condition carries a definite mortality and a higher but largely unaudited morbidity. Immediate complications relate to anatomical spread of the suppurative inflammation however morbidity includes hearing loss and developmental failure.*

1.2.1. Definition

Chronic suppurative otitis media, also known as chronic otitis media or chronic mucosal otitis media, is most consistently defined as persistent otorrhoea of 3 months duration or longer. By definition a variable sized defect in the pars tensa or pars flaccida is present, with active inflammation of the middle ear or mastoid air cell mucosa and the production of pus or mucopus[1]. For the purpose of this thesis the disease entity will be referred to as chronic suppurative otitis media.

Terminology of chronic suppurative otitis media is not universally agreed upon. The World Health Organisation defines the condition as uni- or bilateral tympanic membrane perforation with otorrhoea present continuously for at least 2 weeks[2]. The most definitive systematic review on chronic suppurative otitis media stretches this definition to 2 – 6 weeks of recurrent or persistent ear discharge[3].

Apart from these chronological definitions, an histological definition involving irreversible tissue disease is outlined by Meyerhoff[4]. The presence of irreversible tissue disease antedates chronological chronic suppurative otitis media. Histological changes range to include increased vascularity of mucosa and submucosa, acute and chronic inflammatory cells, with irreversible changes including granulation tissue formation, bony changes (osteitis, osteoneogenesis, bone destruction), fibrosis of the mucoperiosteum, tympanosclerosis, tympanic perforation, polyp and cholesteatoma formation[5]. Although such a definition has relevance to surgical and post mortem specimens, and provides pathological understanding of the disease process, it does however have limited clinical relevance.

Historically, chronic suppurative otitis media has been divided into *tubotympanic disease* involving perforation of the pars tensa and *atticoantral disease* involving the pars flaccida with retraction pocket formation leading to cholesteatoma formation[6]. Patients with *tubotympanic disease* have been considered as 'safe' in that they are less prone to develop the intracranial complications associated with cholesteatoma[6,7]. Browning has shown in a retrospective study that the risk of intracranial complications is similar in ears with 'unsafe' cholesteatoma, or 'safe' mucosal disease[8]. These definitions have however largely been replaced by chronic otitis media with or without cholesteatoma formation.

1.2.2. Aetiology and Pathogenesis

Poor socioeconomic conditions, including overcrowded housing, poor hygiene and sanitation, and poor nutrition, as well as frequent upper respiratory tract infections are believed to play a role in the development of chronic suppurative otitis media[3,6].

The factors that allow acute infections within the middle ear and mastoid to develop into chronic suppurative otitis media are not clear[7]. The anatomic factors that lead to the development of chronic suppurative otitis media are also not clear, but are believed to be related to the aeration of the middle ear and mastoid cavity via the Eustachian tube[7]. The middle ear is separated from the antrum by the ossicles and by a series of mucosal folds[9]. Oedema and inflammatory granulation tissue associated with chronic infection may block the openings to these mucosal folds leading to further diminution of middle ear and mastoid aeration. Precisely what role adequate aeration of the middle ear plays in prevention of middle ear disease has never adequately been explained. It is known that mucosal clearance mechanisms play a significant role in prevention of respiratory tract infection. It may well be that there is a relationship between middle ear cleft aeration and mucosal clearance from the middle ear cleft via the Eustachian tube.

A few organisms, both *aerobic* and *anaerobic*, have consistently been isolated from the mucopurulent discharge associated with chronic suppurative otitis media. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are most commonly isolated[6,7,10-13], and often prove most difficult to treat, as these organisms have a tendency to develop resistance to systemic antibiotics[14]. *Proteus mirabilis* (non-faecal

type), *Streptococcus pyogenes* and *Escherichia coli* were also commonly isolated[6,7,11,15,16]. *Anaerobic* organisms accounted for 7-50 % of mixed cultures[13,15], and 11 % of pure cultures[15], the commonest organism being *Bacteroides* sp.[6,13,15]. Combination of *aerobic* and *anaerobic* organisms has produced a more marked inflammatory response in experimental animals[6]. The elimination of *anaerobic* organisms from active ears does not necessarily hasten inactivity[17].

1.2.3. Treatment

Modalities of treating chronic suppurative otitis media include ear toilette, the application of antiseptic or antibiotic ear drops, systemic antibiotics and surgery, varying from simple myringoplasty to more extensive tympanomastoidectomy. Although successful surgery remains the only real cure of chronic suppurative otitis media, the discharge and accompanying middle ear inflammation require medical treatment.

As bias remains a common problem facing both the clinician and the researcher, the randomised controlled trial has become the yardstick for measuring the effectiveness of a therapeutic modality[18]. Systematic reviews of clinical therapies increasingly focus on the level of evidence supporting any particular therapy[18]. As information is now easily accessible to physicians and patients alike, the need to remain current becomes increasingly important. Two such evidence-based systematic reviews on the treatment of chronic suppurative otitis media have been published in the last 5 years[2,3]. The database utilized by the Cochrane review[2] dates back to 1996, whereas the Clinical Evidence review[3] database was compiled in 2001 and is therefore more current. Only those studies of sufficient clinical merit (generally randomised controlled trials) are reviewed. The Clinical Evidence review analysed adult and paediatric therapeutic modalities separately, whereas the Cochrane review did not make this distinction. Adult and paediatric literature will be combined in this thesis for greater clarity.

Both systematic reviews emphasized a lack of any real long term follow up in all randomised controlled trials reviewed and that all randomised controlled trials were of brief(7 days to 3 weeks) duration[2,3].

Aural toilet

Aural toilet or ear cleansing varies from microscopically controlled suction debridement to dry mopping with cotton wool swabs. Although no adverse effects were reported, 2 randomised controlled trials[19,20] found no significant difference between aural toilet versus no cleaning. Clinical evidence found no benefit from simple aural toilet alone, but was not strong enough to exclude its benefit[3]. Evidence aside, physical removal of middle ear debris via aural toilet allows greater contact of topically applied ear drops with inflammatory tissue, and hence should, in theory, hasten healing/resolution of otorrhoea.

Topical antibiotics

Antibiotic ear drops have become the most commonly employed preparation in controlling the otorrhoea of chronic suppurative otitis media. Quinolone containing drops are currently popular with no reports of experimental or clinical ototoxicity having been reported to date[3]. However recent data has hinted at ototoxicity of quinolone containing preparations albeit in the artificial setting of outer hair cell culture media[21].

At present 2 small randomised controlled trials show only limited superiority of quinolone based ear drops over placebo[22,23]. Fradis *et al*[22] compared the efficacy of topical ciprofloxacin, topical tobramycin and a 1% aluminium acetate solution which served as a control. As the latter topical antiseptic ear drop was believed probably not to be inert, the control arm of this study was critically negated.

Three randomised controlled trials showed no clear difference of quinolone based ear drops over non-quinolone containing drops[22,24,25].

Two further randomised controlled studies were excluded from the systematic review[3], but are reviewed here, because in the first patients included adults and children[26], and in the second patient age was not included[27]. The first trial showed significantly better clinical cure rates with ciprofloxacin versus gentamicin based ear drops, in the acute stages of chronic suppurative otitis media[26]. This study was particularly unusual as not a single patient was lost to follow up. The second study showed that significantly fewer patients treated with ofloxacin ear drops had active

disease than those treated with neomycin-polymixin B-hydrocortisone ear drops at the end of a 2 week treatment period[27].

Non-quinolone topical antibiotic containing ear drops are generally either aminoglycoside based or contain an assortment of antibiotics and occasionally a steroid as well. Three randomised controlled trials found topical gentamicin to be no better than combination antibiotic ear drops[28-30]. However 2 randomised controlled studies found topical gentamicin plus steroid containing ear drops to be significantly more effective in controlling otorrhoea versus placebo[31,32]. The only paediatric randomised controlled trial found a combined antibiotic ear drop with steroid to be no more effective than dry mopping alone[19]. One randomised controlled study found a significant reduction in otorrhoea in patients treated with gentamicin-hydrocortisone ear drops versus betamethasone containing ear drops alone[33].

Lastly, a randomised non-controlled trial showed that an otic preparation (neomycin-dexamethazone) was significantly more effective when delivered topically as a spray rather than as a customary ear drop[34]. It was reasoned that this was due to improved penetration of the medication through the perforation into the middle ear.

Many variables affect topical therapy, adequacy and frequency of cleaning, method and frequency of ear drop application, size and location of perforation, condition of middle ear mucosa, etc. that are neither addressed nor standardized that conclusions are difficult to reach. However, antibiotic containing preparations are generally more effective in resolving otorrhoea than non-antibiotic containing preparations.

Topical antiseptics

Although more commonly used in the treatment of otitis externa various topical antiseptics have been used in chronic suppurative otitis media. A single randomised controlled trail performed in adults found no significant difference between boric acid and iodine powder, topical antibiotics or systemic antibiotics (prescribed according to individual bacterial sensitivity) in the persistence of activity on otoscopy[17]. A paediatric based randomised controlled study found no significant difference between 2% boric acid in 20% alcohol ear drops versus ear cleansing alone[19]. The same study showed no significant difference between the same antiseptic solution and a topical antibiotic with steroid[19].

An unreviewed non-controlled study showed that irrigation of ears with chronic suppurative otitis media using a 2% acetic acid solution 3 times per week for 3 weeks produced resolution of otorrhoea in 57% of patients[35]. As no audiological investigation was undertaken no comment could be made regarding the otologic safety of acetic acid used in this manner in the middle ear.

Other studies, not randomized or controlled, examining the effectiveness of aluminium acetate solution in the treatment of ear conditions are presented in the section on Burow's solution, under previous research[35-40].

The clinical arm of this thesis[16] was included in the more recent systematic review[3] having fulfilled a set of rigorous exclusion criteria.

Topical steroids

There is no evidence to show that topical steroids are more effective than placebo in the management of chronic suppurative otitis media, in adults or children[3].

Systemic antibiotics

Only limited evidence was found to support systemic antibiotic use in adults and insufficient evidence that systemic antibiotics were useful in the treatment of chronic suppurative otitis media in children[3]. Oral antibiotics were less effective than topical antibiotics, 22 % versus 56% resolution of symptoms at completion of therapy[3,17,41-43]. Additional systemic antibiotics versus placebo, in patients receiving topical antibiotics was found to have no significant difference in resolution of symptoms[41,44].

Surgery

In recent years the need for an evidence-based approach in the practice of otolaryngology has been debated. It has been proposed that otolaryngology and in fact all surgical sub-specialties do not really require an evidence base, because it was felt that there is a fundamental difference between evaluation of medication as opposed to surgical procedures[45]. The counter argument however favours an evidence-based approach in otolaryngology pointing out that to answer questions relating to therapy, randomised controlled trials are required to exclude any particular bias[18]. As a large proportion of chronic suppurative otitis media is treated medically, evidence is required for medical intervention but probably not for surgery. Surgical intervention when successful, can be viewed as an end point in the management of chronic suppurative otitis media.

There are no randomised controlled trials of surgery - tympanoplasty with or without mastoidectomy - versus control of no surgery[3].

Numerous retrospective studies have been performed. One study compared the operated ear versus the unoperated ear in chronic suppurative otitis media[46]. Although hearing deteriorated in both ears, the rate of decline in the operated ear was significantly less. Observational studies have found that surgical success in sealing a perforation varies from 80 – 95%, dependent on patient age, surgeon's technical skill, the state of the middle ear, ossicles and middle ear remnants and the type of surgery performed[1,3].

In summary, although various ear drop preparations are used to manage the otorrhoea associated with chronic suppurative otitis media, with varying effect, the definitive management remains surgical, patient permitting. Of the ear drop preparations, little difference in effectiveness between antibiotic containing drops and antiseptic ear drops has been proven. Recently the effectiveness and safety profile of quinolone containing ear drops has made these the treatment of choice for the medical management of the otorrhoea of chronic suppurative otitis media.

1.2.4. Complications

In any medical condition, side effects and complications of therapy should ideally be less severe than those of the disease itself. Complications associated with chronic suppurative otitis media can be due to the disease process or the therapy and can be divided into immediate and long term.

Immediate complications are more directly associated with the infective process itself. These may include local complications of facial nerve palsy, labyrinthitis and mastoiditis, or contiguous complications of meningitis, sigmoid sinus thrombosis, extradural empyema or brain abscess. Active chronic suppurative otitis media has been found to be as likely as cholesteatoma to result in intracranial complications[8].

Long term complications relate primarily to hearing and development. Chronic suppurative otitis media is the commonest cause of childhood deafness in the developing world[20]. Hearing impairment in the first 2 years of life impacts on language development[47], which further impacts on education and subsequent development. Hearing loss from chronic suppurative otitis media can either be conductive or sensorineural in nature[6]. Duration and severity of disease has been shown to significantly decrease bone conduction thresholds [48-50] with variable extent[48], whilst others have shown this decrease not to be significant[51]. As sensorineural hearing loss typically is greatest in the basal turn of the cochlea[50], higher frequencies are affected more than low frequency[51]. Cochlear dysfunction in chronic suppurative otitis media is well documented, however concomitant vestibular loss has been poorly documented.

Complications of treatment of chronic suppurative otitis media should be less severe than those of the disease process itself as previously stated. Ototoxicity of systemic antibiotics particularly the aminoglycosides has been well documented in the literature, and is discussed later, as are the effects of topical antibiotics[52-55]. Surgical management of otorrhoea also carries complications, depending on the extent of the surgery. General complications relating to anaesthesia, general medical conditions, wound haemorrhage and wound infection can occur as in all surgical cases. More specific complications relate to the local extent of surgical intervention, depending on whether simple myringoplasty or more aggressive mastoid surgery is required.

1.3. Burow's solution

Summary

The origin of Burow's solution as well as its initial use and details regarding the preparation of 13% aluminium acetate are described. Previous research into the efficacy of this solution in the treatment of otitis externa and chronic suppurative otitis media are discussed.

1.3.1. Historical

Carl August Burow or von Burow, was born on 10 November 1809, in Elbing, Germany, the son of a government secretary. He completed his schooling in Danzig and then moved to the University of Köningsberg, where he was to make most of his contributions to the fields of medicine and surgery. His initial studies were in theology but he later changed to science and medicine, where he studied under the guidance of Ludwig Sachs (1787 - 1848), who had a keen interest in pharmacology. Burow passed his state medical examinations in 1835, and later founded his own clinic specialising in ophthalmology and general surgery. He was made Dozent in 1839 and Extraordinary Professor in 1844 at the University of Köningsberg, a link which he severed in 1859, possibly due to the constraints of his large practice.

Burow is best remembered for his approach to wound healing and the lateral triangle excision he employed to close tissue defects. He published his lateral triangle technique in 1855, showing its usefulness in closing defects of the head and neck after cancer ablations. This technique still carries his name.

Burow's solution as it has come to be known, was initially used in the treatment of open wounds. Burow's treatment of wounds and wound sepsis was contrary to the dogma of the time that viewed the exposure to air as the prime source of putrefaction. He believed in exposure of the wound and liberal irrigation with aluminium acetate. The solution as noted in his original paper was made by adding " eight parts of lead acetate to five parts of alum (cold) and 64 parts of water." The lead component

precipitated leaving aluminium acetate. It is noted that this solution prior to application required filtration[56].

There is no documentation as to whether aluminium acetate solution was used in chronic suppurative otitis media at the time.

1.3.2. Preparation

Burow's solution is a clear, colourless liquid with a faint acetous odour and a sweet taste. It is prepared by adding glacial acetic acid to aluminium subacetate solution and diluting the mixture with water to give a final concentration of approximately 13% aluminium acetate[57].

The British Pharmacopoeia gives a more detailed description of the preparation of Burow's solution[58]. Once 225g aluminium sulphate is dissolved in 600ml purified water, 250ml of acetic acid (33%) and 100g of calcium carbonate is added and mixed with a further 150ml water. This solution is allowed to settle for 24 hours, then filtered, whereafter 45g of tartaric acid is added as a stabilizer.

The chemical equation reads as follows:

$$CaCO_3 + 2H_3C_2O_2 \rightleftharpoons Ca(H_2C_2O_2)_2 + H_2O + CO_2$$

$$3Ca(H_2C_2O_2)_2 + Al_2(SO_4)_3 \rightleftharpoons 3CaSO_4 + 2Al(H_2C_2O_2)_3 + \text{tartaric acid to stabilize}$$

A 3% solution of aluminium acetate has an astringent action[59] whilst a solution of 10% or greater has been shown to have antibacterial and antifungal properties[60].

In the course of this thesis Burow's solution was prepared, in two different centres (Groote Schuur Hospital, Cape Town and Toronto General Hospital), by three different pharmacists and a chemist. On each occasion technical difficulty in preparing the solution was encountered. Each preparation took over 24 hours to complete and required overnight filtration to produce a clear solution.

The issue of the stability of the solution has partly been achieved by the addition of not more than 0.6% of boric acid[57] or tartaric acid[58] and storage below 25°C[58]. On occasion crystallization has occurred in some bottles of Burow's solution, but this disappeared with agitation of the contents. Groote Schuur Hospital Pharmacy arbitrarily assigned a 6 month expiry date on Burow's solution ear drop bottles, despite admission as to not knowing the duration that the solution remained stable.

1.3.3. Applications

Burow's solution and other concentrations have been used primarily in the treatment of various forms of otitis externa.

Politzer makes mention of "*Liquor Burowii*"as early as 1903 for the treatment of dermatitis of the auricle. Boric and acetic acids are described in the treatment of *otitis externa circumscripta* and *diffusa*[61].

Details regarding the constitution of aluminium acetate solution are made as far back as 1959 of the British Pharmaceutical Codex, however indications for use are not disclosed[62].

Jones makes mention of Burow's solution in "External Otitis. Diagnosis and Treatment". He describes how the preparation has been employed by dermatologists in the management of acute allergic dermatitides, and furthermore draws attention to the relative instability of the solution over time[63].

Despite extensive literature searches, including archives from military medicine from both World Wars, no other documentation of the use of aluminium acetate solution was found.

1.3.4. Previous research

Previous research performed on Burow's solution or aluminium acetate solution has been clinical studies, examining effectiveness in the treatment of otitis externa and chronic suppurative otitis media. To the author's knowledge no previous *in vitro* research has been performed on Burow's solution or dilutions thereof.

A poorly randomised but controlled study, showed "aluminium acetate solution BP" (which must be assumed to be 13% aluminium acetate solution) to be as effective as Otosporin® (neomycin-polymixin B-hydrocortisone) ear drops in the treatment of acute otitis externa[38]. No significant difference in the failure rates of either drop preparation was demonstrated although some patients treated with the aluminium acetate solution did complain of discomfort with use.

In a large retrospective study[40] the effects of gauze wicks applied to the external auditory canals of 2008 patients with acute otitis externa were documented[40]. The ear canal was cleaned prior to insertion of a gauze wick soaked in 20% silver nitrate, which was left for 24 hours. This wick was replaced with one saturated with Burow's solution, which was then left *in situ* for a further 2-3 days. Complete cure within 3 days was attained in 1593 (79.3%) patients, whilst a further 398 (19.8%) cases were judged to have had a good response. Only 17 (0.01%) cases did not respond to treatment. The end points of cure, good and poor responses are not clearly defined. As this retrospective study does not appear to include all patients treated and therefore does not appear sequential, it may be fair to assume that selection bias has occurred.

A study examining the use of aluminium acetate solution on chronic suppurative otitis media showed that 8% aluminium acetate solution was as effective as 0.3% gentamicin ear drops in the control of otorrhoea[39]. Criticisms of this randomised controlled study were that the randomization process was not explained and that only 74% of ears completed the trial. The study recommends topical aluminium acetate rather than topical gentamicin "in the initial treatment of otorrhoea on the grounds of cost, avoidance of resistance and toxicity", however fails to monitor any of the included patients for possible toxicity to either ear drop preparation used.

The largest study on Burow's solution in the treatment of chronic suppurative otitis media[36] examined the efficacy of this solution applied to the middle ear by gauze wick in 4281 cases seen in Kinshasa, Zaire. Although Burow's solution is quoted as having a pH of 4.0, the actual strength of solution used in this study is not defined. One therefore has to assume 13% aluminium acetate solution was used. All ears were treated with daily application of cotton wicks soaked with Burow's solution. Treatments were continued for 10 day periods until the ear was dry or until 6 weeks had elapsed. If ears failed to respond after 6 weeks of treatment, they were defined as treatment failures. Therapeutic end points were defined as excellent if the ear was dry in 3 weeks, satisfactory if dry in 6 weeks and improved if the discharge was diminished. A total of 2428 (56.7%) ears achieved excellent results and a further 1146 (26.7%) ears satisfactory results. Treatment failures totaled 285 (6.7%) ears. Adverse reactions in the form of skin excoriation and oedema occurred in 42 (0.1%) patients, who were not included in the results. Monitoring of hearing by audiometry was reportedly performed at onset and completion of therapy, as well as a month after completion of treatment. Although no audiometric data is presented, there were no

reported cases of sensorineural hearing loss occurring during or after the treatment period.

For ease of reference the data from the aforementioned studies as well as some subsequent data is presented in Table 1. Data is displayed from studies employing ear drops as well as ear wicks, in both otitis externa as well as chronic suppurative otitis media. Overall efficacy of therapy, strength of aluminium acetate solution employed and patient population size are the tabulated parameters. The efficacy of therapy in otitis externa (79.3% - 100%) appears to be superior than that achieved in chronic suppurative otitis media (67% - 83.5%).

Table 1: Extrapolated data from clinical trials on the effectiveness of aluminium acetate solution in the treatment of the discharging ear.

	n treated	% solution	% effective
Chronic suppurative otitis media			
Clayton[39] *et al*	42	8	67
Thorp[16] *et al*	26	13	81
	20	3.25	75
	10	1.3	50
Terayama[37] *et al*	6	13	83
Mahoney[36]†	4281	13	83.5
Otitis externa			
Lambert[38] *et al*	62	13*	95
Terayama[37] *et al*	14	13	100
Smathers[40]†	2008	13	79.3

*assumed as 13% is the only concentration described in the British Pharmacopoeia as Aluminium acetate solution BP

†cotton gauze wicks used instead of ear drops

2. Study 1

The antibacterial activity of acetic acid and Burow's solution as topical otologic preparations.

The mode of action of acetic acid and hence Burow's solution is thought to be the alteration of the pH of the external auditory canal and middle ear and hence the bacterial profile and inflammatory response of the mucosal lining[59].

The aim of this study was to determine the antibacterial activity *in vitro* of the three different strengths of acetic acid ear drops and Burow's solution ear drops, available from the hospital pharmacies at Red Cross War Memorial Hospital and Groote Schuur Hospital, against a range of commonly occurring organisms believed to be pathogenic in otitis externa and otitis media.

The South African Ministry of Health[64] guidelines for the treatment of chronic suppurative otitis media have more recently recommended a single ear drop preparation, namely 1% acetic acid for topical therapy.

2.1. Materials and Methods

All otologic pus swab culture results taken from discharging ears at Groote Schuur Hospital from 1992 - 1994 were reviewed and commonly isolated organisms identified[10]. The four most commonly identified organisms, namely: *Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis* and *Streptococcus pyogenes,* were selected for further investigation.

Fresh ear swabs collected from patients with discharging ears seen at the ENT Outpatient Department, Groote Schuur Hospital, were submitted to the laboratory, and plated onto 4 % boiled blood agar and McConkey agar for primary isolation. Colonies of *Pseudomonas aeruginosa, Staphylococcus aureus* and *Proteus mirabilis* were then replated onto Mueller-Hinton agar and *Streptococcus pyogenes* was replated onto Mueller-Hinton + 5% sheep blood agar for sensitivity testing. Inoculation criteria according to NCCLS (National Committee for Clinical Laboratory Standards – U.S.A.) standards were used. Two wells were cut into the agar of each plate and filled with either 1%, 2% or 3% acetic acid or Burow's solution which were prepared by the pharmaceutical services at Groote Schuur Hospital. Plates were then incubated at 35°C for 24 hours and zones of inhibition of organism growth measured and recorded.

A total of 20 strains of each organism were tested against each preparation.

2.2. Results

The results are presented in Table 2 and Figures 1-4.

Growth of all four organisms was inhibited by Burow's solution with average zones of inhibition ranging from 26 – 34mm as shown in Table 2 and the black columns depicting zone of inhibition size in Figures 1-4. Increasing strengths of acetic acid produced increasing zones of inhibition but 1% acetic acid failed to have an effect on the growth of *Staphylococcus aureus* as demonstrated in Figure 4. In general the zones of growth inhibition were smaller with all concentrations of acetic acid than with Burow's solution, particularly for *Staphylococcus aureus*.

Statistical analysis showed that the zones of inhibition from the Burow's solution were significantly greater than those from all strengths of the acetic acid using Analysis of Variance tests($p < 0.001$) for all organisms tested. Further analysis using one way Analysis of Variance (Tukey's pairwise comparison) tests, showed no significant difference in the activity of 1% against 2% acetic acid and 2% against 3% acetic acid, for all organisms tested. Significant difference was shown in the activity of 1% against 3% acetic acid for all organisms tested. The activity of Burow's solution was significantly greater than all strengths of acetic acid for all organisms tested.

Table 2: Mean growth inhibition zone size for each organism tested against each agent.

Organism (20 cultures of each)	1% Acetic acid (SD)	2% Acetic acid (SD)	3% Acetic acid (SD)	Burows soln. (SD)
Pseudomonas aeruginosa	**9.3**	**16.9**	**22.1**	**33.8**
	(7.1)	(7.6)	(6.5)	(5.0)
Proteus mirabilis	**11.2**	**19.0**	**19.9**	**33.5**
	((6.9)	(7.1)	(9.1)	(5.2)
Streptococcus pyogenes	**11.4**	**16.8**	**24.7**	**34.4**
	(3.5)	(3.7)	(2.0)	(3.2)
Staphylococcus aureus	**0.5**	**5.1**	**8.1**	**26.1**
	(3.4)	(5.1)	(8.4)	(4.7)

Range of inhibition zones are shown in Figures 1-4.

Figure 1: Inhibition zone sizes (mm) for *Pseudomonas aeruginosa.*

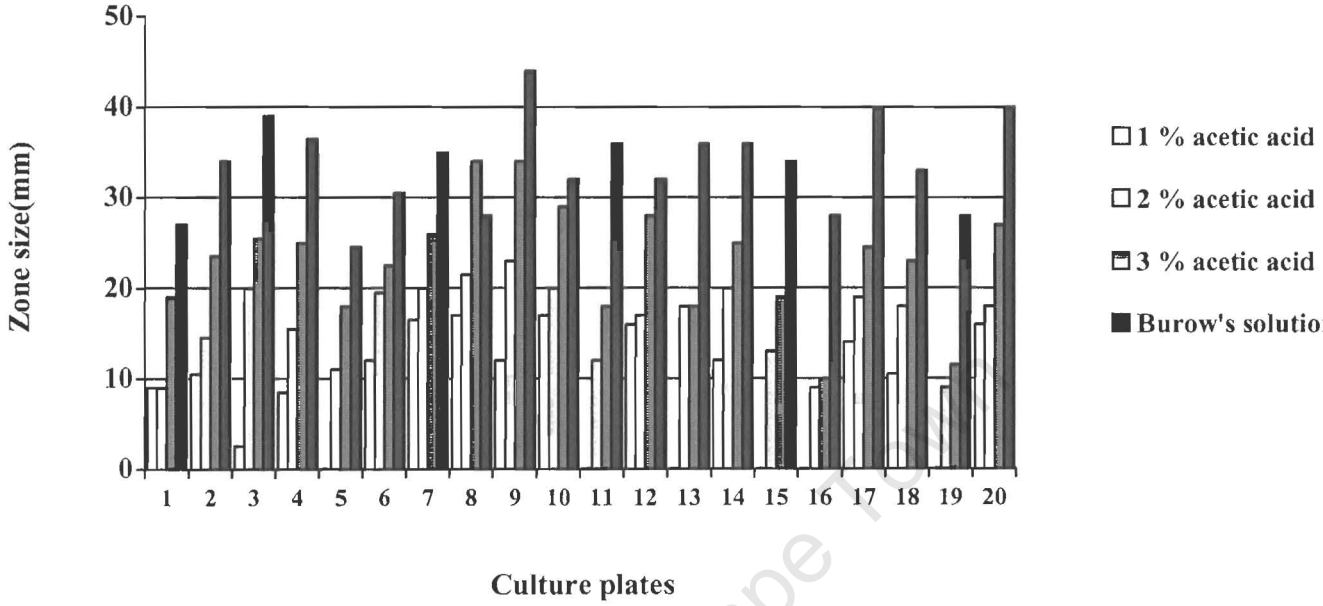


Figure 2: Inhibition zone sizes (mm) for *Proteus mirabilis.*

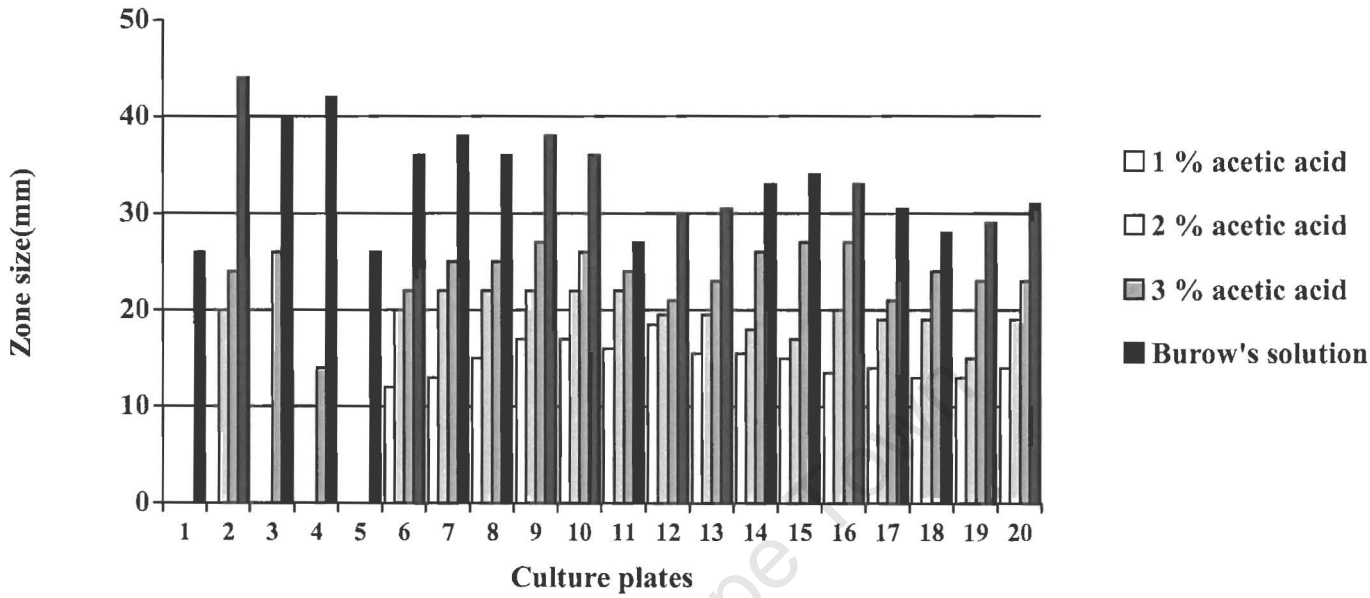


Figure 3: Inhibition zone sizes (mm) for *Streptococcus pyogenes.*

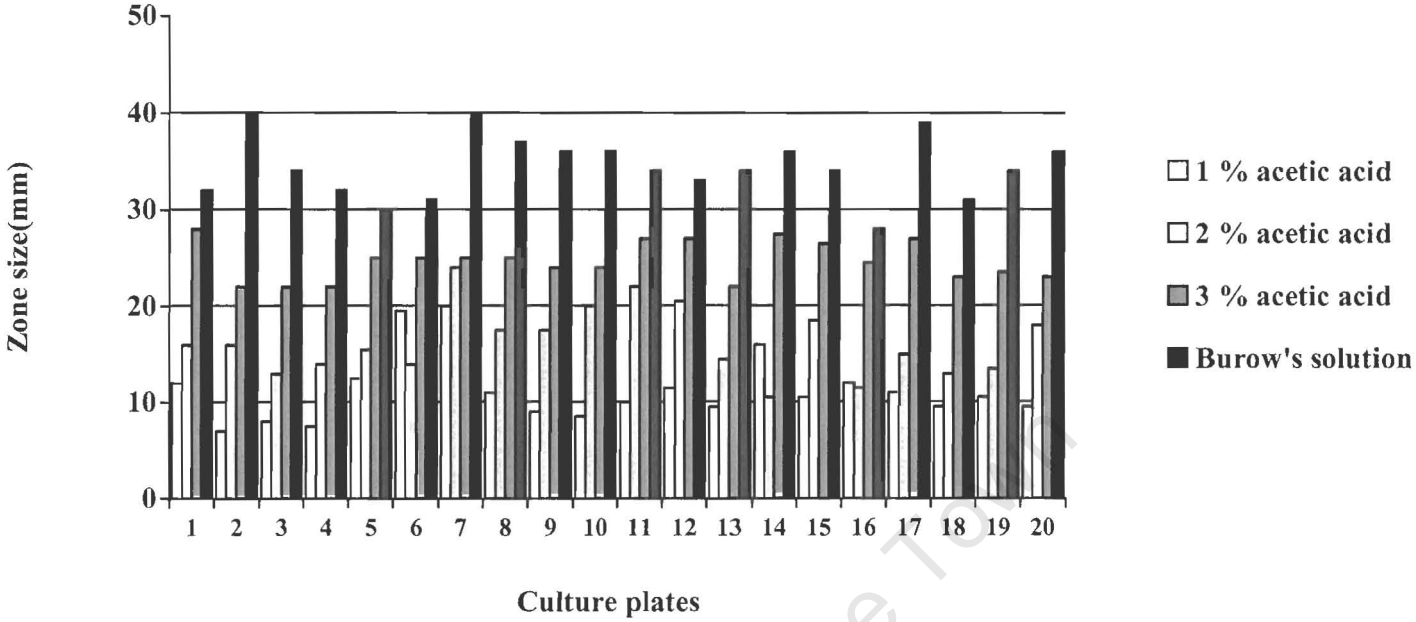
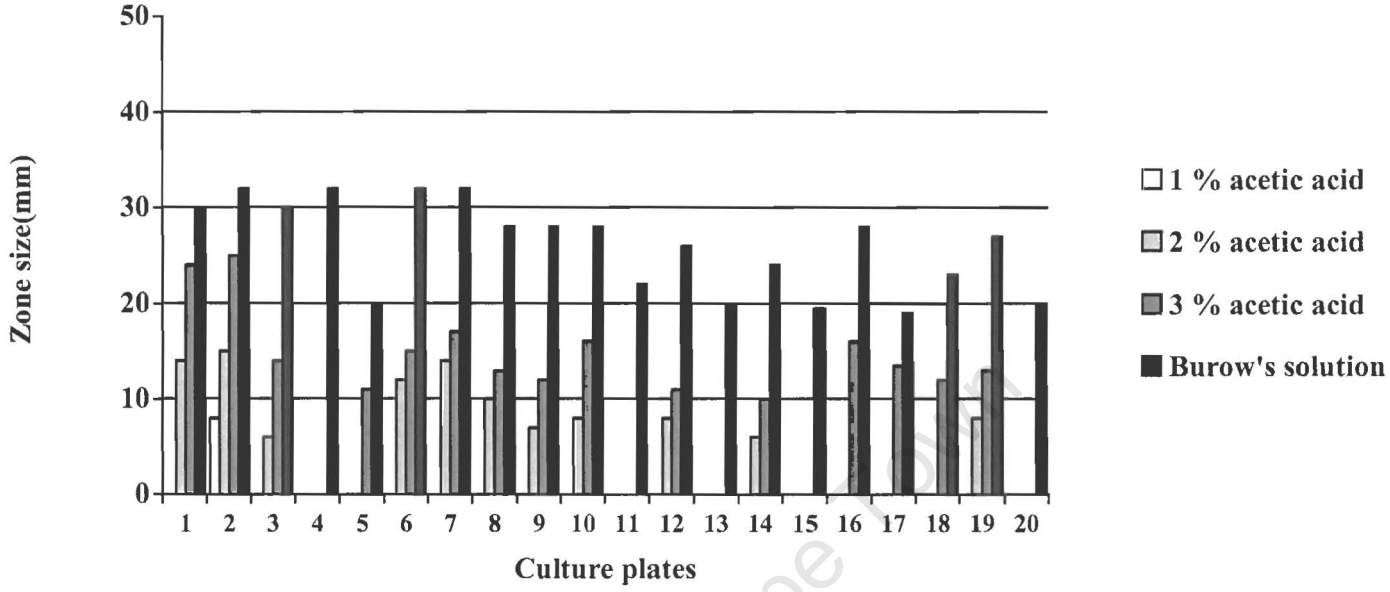


Figure 4: Inhibition zone sizes (mm) for *Staphylococcus aureus.*



3. Study 2

Determination of the lowest dilution of aluminium acetate solution able to inhibit *in vitro* growth of organisms commonly found in chronic suppurative otitis media.

Burow's solution successfully inhibits the *in vitro* growth of *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Proteus mirabilis*[12]. In the subsequent study these organisms together with *Haemophilus influenza* and *Streptococcus pneumonia* were used to establish minimum inhibitory dilutions for organisms commonly occurring in chronic suppurative otitis media, in an attempt to find more dilute preparation which maintains a bacteriocidal capability.

3.1. Materials and Methods

Burow's solution was incorporated into the appropriate agar for each organism tested at doubling dilutions commencing at an initial dilution of 1:40 (0.5ml solution: 19.5ml agar being the initial dilution). 20 fresh isolates of each of the following organisms -- *Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Pseudomonas aeruginosa, Proteus mirabilis* and *Haemophilus influenzae* - were inoculated into BHI (Brain Heart Infusion) broth and incubated for 4 hours, standardised to 0.5 McFarland, inoculated onto respective agar, and then incubated at 35°C for 18 hours. The dilution at which inhibition of organism growth was achieved was then recorded for each organism.

3.2. Results

The average minimum inhibitory dilution for each set of organisms is shown in Table 3.

The minimum inhibitory dilutions for each of the 20 cultures of each particular organism did not vary for that organism. As can be seen from Table 3 the growth *in vitro* of all organisms except *Staphylococcus aureus* was inhibited by a 1:160 dilution of Burow's solution or a 0.08%solution of aluminium acetate. *Staphylococcus aureus* required double the strength of this dilution to inhibit bacterial growth, i.e. a 0.16% solution of aluminium acetate.

Table 3: The minimum inhibitory dilution for organisms tested.

Organism	N	MIC
Staphylococcus aureus	20	1:80
Streptococcus pneumoniae	20	1:160
Streptococcus pyogenes	20	1:160
Pseudomonas aeruginosa	20	1:160
Proteus mirabilis	20	1:160
Haemophilus influenzae	20	1:160

4. Study 3

Burow's solution in the treatment of active mucosal chronic suppurative otitis media: A clinical trial to determine an effective dilution.

Introduction

The previous bacteriological studies on the effectiveness of varying strengths of acetic acid, with Burow's solution added as an interesting aside, suggested that Burow's solution would be a more effective agent for the treatment of chronic suppurative otitis media. They also suggest that diluted Burow's solution would be as effective as full strength Burow's solution. This would add a significant therapeutic advantage as full strength Burow's solution is uncomfortable when applied topically, which could result in poor patient compliance.

Neither an extensive specialist based review of commercially available aluminium acetate based otologic preparations[65], nor a previous study using 8% aluminium acetate solution[39], demonstrated any adverse effects in the treatment of chronic suppurative otitis media.

This study was undertaken to determine whether Burow's solution, or dilutions thereof, is a clinically effective agent in the treatment of chronic suppurative otitis media.

The gold standard of clinical trials and evidence based medicine - the double blind randomized control trial – was not feasible in the setting of this study.

1. The investigator would be the clinician in charge, owing to staffing of the Paediatric ENT outpatient clinic, and therefore would not be fully "blinded".
2. The different strengths of Burow's solution were anticipated (from initial pilot work with full strength and 1:10 dilution Burow's solution) to have obvious differences in patient discomfort.
3. Saline ear drops or aural toilet alone have been shown in the published literature to be ineffective in the resolution of otorrhoea[19,20]. The use of saline ear drops as a "control" could thus not have been justified on clinical grounds.

Therefore the study was designed as a randomised clinical trial, without "placebo" control and not fully blinded, to compare the clinical effectiveness of full strength Burow's solution against dilutions thereof, with periodic review of results with intention to discontinue a particular preparation should it be shown to be clinically ineffective. As Burow's solution applied topically is uncomfortable, determining a dilution that remains clinically effective is of importance to ensure improved patient compliance to therapy. In keeping with the purpose of the earlier studies of *in vitro* determination of effective dilutions of aluminium acetate solution, it was decided to rather test full strength Burow's solution against an effective dilution, the full strength solution acting as a form of control.

4.1 Materials and Methods

All patients with active mucosal chronic suppurative otitis media, presenting to the Otolaryngology outpatient clinic at the Red Cross War Memorial Children's Hospital over a six month period, were assessed for inclusion in the study. Children for inclusion were defined as having active mucosal chronic suppurative otitis media with a defect of the pars tensa, inflamed oedematous middle ear mucosa and a mucopurulent discharge for more than 4 weeks. No effort was made to determine the precipitating factor/s for the current episode of otorrhoea.

Ethical approval was not required for patients being treated in routine clinics with routine medication supplied by the hospital pharmacy, at the time this study was conducted. The only variation introduced was dilution of standard Burow's solution which was not felt to be introducing any potentially harmful effects that would require ethical approval.

Parents/caregivers of the children were informed of the purpose of the study and the methods to be used and then consented to inclusion of their child in the study. The anticipated discomfort of the ear drops was explained, and that they could request withdrawal from the trial at any stage to revert to the other methods of treatment available in the clinic.

Patients were excluded if systemic antibiotics or any topical ear drop preparations had been administered within the preceding 2 weeks, or if there was any evidence of cholesteatoma or aural polyposis.

The ear was thoroughly cleaned with dry mopping prior to a swab of the middle ear being taken for microscopy and culture. The frequency and duration of the discharge was noted. The degree of inflammation was arbitrarily based on the clinician's estimation of erythema and swelling of the middle ear mucosa and the presence of middle ear granulation tissue, and an estimation of the size of the tympanic membrane perforation were recorded.

All patients had pure tone air conduction audiometry performed prior to and following the treatment period. Patients old enough to tolerate headphones had bone conduction thresholds recorded as well.

The ear drops (Solutions A B &C) were prepared in the hospital pharmacy and the pH of each solution was measured following preparation. Each solution was dispensed in an identical ear dropper container, being labeled Solution A, B or C.

Randomisation of patients was effected using a computer generated binary random number sequence (computerized random-number generator[66]), allocating patients to treatment group A or B from a sequential list without randomization restriction[66]. The sequence was set to 100 patients. As the investigating clinician was responsible for the dispensing of the allocated medication, he could not be fully blinded to the allocation sequence, which was recorded on the patient data chart. However the clinical investigator was only one of 3 clinicians involved with patient follow up in the study, the other 2 clinicians being unaware of the ear drop allocation.

Initial and follow up visit assessments were conducted by either the principal investigator or one of 2 other clinicians. Dr I Gardiner (senior registrar) was responsible for the assessment of over 30 of the trial patients, whilst the remainder were assessed by the principal investigator and Professor C. Prescott (consultant and head of unit)[16].

Initial randomisation was between solution A (1.3% aluminium acetate – 1/10 strength Burow's solution) and solution B (Burow's solution – 13% aluminium acetate) with intention to observe clinical response and analyse results should there be any concern regarding either clinical effectiveness or excessive patient discomfort. NOTE: The clinical impression during treatment was that solution A was not proving to be effective in resolving otorrhoea – the clinical indicator of effectiveness of treatment. Therefore after completion of the therapeutic period of the first 30 patients, the results were analysed and the clinical impression was confirmed. It was decided to discontinue treatment with solution A and substitute solution C (3.25% aluminium acetate – ¼ strength Burow's solution) into the initial randomisation sequence for the remainder of the trial.

An arbitrary period of 2 weeks was considered as being sufficient to assess the effectiveness of treatment. Parents were instructed to dry mop the ear prior to insertion of 6 drops of solution three times per day for a 2 week period, or until the discharge had stopped. Delivery of solution to the middle ear was assisted by pulsed tragal pressure following administration of the drops. "Response" would be a dry ear with reduction of mucosal inflammation.

A follow up visit was arranged for 2 weeks after the start of treatment.

If the ear was dry at the follow up visit, the degree of inflammation, audiometric change and any discomfort experienced due to administration of the eardrops was noted. Appropriate follow up arrangements were thereafter made for the child to attend routine ENT clinics.

If at follow up the ear was still discharging a repeat pus swab was taken for culture and the patient placed onto alternative treatment. The degree of inflammation, audiometric change and discomfort of medication were also recorded. Compliance was assessed by the amount of remaining solution combined with the child's response to dry mopping by the clinician. Appropriate follow up arrangements were thereafter made for the child to attend routine ENT clinics.

All data was tabulated and statistical analysis was performed.

4.2. Results

At least half of the patients presenting to the clinic with active mucosal chronic suppurative otitis media had received treatment in the form of topical or systemic antibiotics within the 2 weeks prior to consultation and were not included in the study.

A total of 60 patients and 67 discharging ears were randomised into the trial. As already stated the first 30 patients were randomised to either solution A or solution B, however analysis at this point confirmed the clinical impression of poor effectiveness of solution A. Thereafter the next 30 patients on the randomisation sequence were allocated to receive solution B or solution C to complete the study.

The data of 11 patients was excluded, due to poor compliance in 5 and failure to attend the follow up visit in 6.

Of the 49 patients that fulfilled the trial criteria, 14 were female and 35 were male, with an average age of 5.42 (3.61) years. The data on 56 discharging ears were included for analysis.

Baseline demographic data for each group of patients is shown in Table 4. Neither the ages nor the sex composition of each group varied significantly. Bilateral disease accounted for 14.3% patients completing the clinical study.

The different bacteria isolated and the response of each organism to treatment is shown in Table 5. The two commonest bacteria implicated in chronic suppurative

otitis media - *Staphylococcus aureus* and *Pseudomonas aeruginosa* – are fully responsive to Burow's solution but less responsive to diluted Burow's solution. The number if isolates of the other bacteria are too small for meaningful analysis but appear to have variable response rates.

Of particular note is that the response to 1/10 strength Burow's solution (solution A) – apart from mixed skin flora – appears to be poor but again the number of isolates are too small for meaningful analysis. The poor response of Solution A to *Staphylococcus aureus*, which had only a 33% (2/6) clinical response rate, could be anticipated from the results of the MID study where *Staphylococcus aureus* required double strength 1:80 dilution as MID.

Increasing the dilution strength to quarter strength Burow's solution (Solution C) appears to confer some benefit in response rate but apart from *Pseudomonas aeruginosa(*69.2%) the numbers of isolates are again too small for meaningful analysis.

The pH of Solution A was 3.73, that of Solution B was 3.06 and that of Solution C was 3.50.

The treatment responses to each strength of aluminium acetate dispensed are shown in Table 6. Solution B achieved an 80.7% overall clinical response as evidenced by dry ears at completion of therapy. Solution C achieved a 75% overall clinical response with less continuous discomfort. The overall clinical response for Solution A was 50%. Using a Hypothesis test for Two Proportions the response rate of Solution B

compared to Solution A neared significance(p=0.065), however comparing Solutions B with C or Solutions A with C showed no significant difference. Due to small sample size interpretation of these tests should be viewed with caution.

A shorter average treatment time was noted in patients receiving Solution B compared with patients receiving Solutions C or A. Using a one-way Analysis of Variance of the average treatment time Tukey's pairwise comparison showed a significant difference between the treatment time of Solution B (3.7±1.4 days) and Solution A (6.2±1.3 days). However differences between Solutions B and C and Solutions A and C were not significant.

Not included in this table was the observation that of patients receiving solution B, 3 patients closed their perforations. A further 5 patients developed a crystalline deposit thought to be a precipitant in the external auditory canal although the otorrhoea and inflammation had since resolved. These deposits were easily removed from the external auditory canal.

Analysis of treatment failures is shown in Table 7. For the sake of completeness treatment success numbers are included in the same table in parentheses. Longer duration of otorrhoea, mild as opposed to moderate inflammation and smaller(<25%) perforations as opposed to larger(>50%) perforations seemed more likely to result in treatment failure. As both milder inflammation and smaller perforations predominated it is not therefore surprising that some of these were destined to become treatment failures, however duration of otorrhoea(months as opposed to weeks) remained the one factor likely to result in treatment failure. Patients with prolonged otorrhoea

resulting in treatment failure were likely to have had some degree of mastoid osteitis present, as suggested by Meyerhoff's histological definition of chronic suppurative otitis media[4]. A greater percentage of failures occurred with Solution A(50%) versus Solution B(19.2%) or Solution C(25%). Due to small numbers statistical analysis was not possible.

All 56 ears treated received pre and post study audiological assessment and air conduction pure tone audiological results are shown in Table 8. No deterioration in air conduction thresholds was demonstrated in any patients. In fact, the converse occurred with greatest improvement occurring in patients treated with Solution B, closely followed by Solution C, and less so with patients treated with Solution A. Changes in air conduction thresholds alone cannot indicate any inner ear damage, although this might be suspected should the threshold deteriorate after treatment. This was not observed in any children in this study. Improvements in threshold, when they occurred, probably relate to improvement in the general status of the ear canal, the eardrum and the middle ear and its contents with treatment. Deterioration in bone conduction threshold would be an indication of adverse effects of treatment on the inner ear. It was only possible to undertake bone conduction thresholds on 37 of the 56 ears due to patient compliance with such an investigation, generally in younger patients who could not tolerate headphones. No deterioration to suggest significant hearing loss in any individual patient thresholds could be shown after completion of the treatment period.

Table 4: Baseline demographic data of patients completing therapy.

	Ears	Age	SD	Male	Female	Left	Right	Unilateral	Bilateral
Solution B (Burow's solution)	26	5.00	3.52	16	7	15	11	20	3
Solution C (3.25% aluminium acetate)	20	5.24	3.23	12	5	11	9	14	3
Solution A (1.3 % aluminium acetate)	10	4.60	3.87	7	2	5	5	8	1
Total	56	5.42	3.61	35	14	31	25	42	7

Table 5: Treatment response of organisms cultured from discharging ears at initial visit.

Organism	**n**	**Response to Solution A (%)**	**Response to Solution B (%)**	**Response to Solution C (%)**
Pseudomonas aeruginosa	20	1/2 (50)	5/5 (100)	9/13 (69.2)
Staphylococcus aureus	12	2/6 (33.3)	3/3 (100)	2/3 (66.7)
Proteus mirabilis	6		3/4 (75)	2/2 (100)
Streptococcus pyogenes	2		0/1 (0)	1/1 (100)
Streptococcus pneumoniae	1			1/1 (100)
Haemophilus influenzae	5	0/1 (0)	2/4 (50)	
Escherichia coli	3		3/3 (100)	
Klebsiella oxytoca	2		1/2 (50)	
Providentia	1		1/1 (100)	
Mixed skin flora	4	1/1 (100)	3/3 (100)	

Table 6: Clinical response to each of the treatment solutions.

	n	Dry ears	Response Rate (%)	Mean time taken (days)	Improved inflammn (%)	Initial eardrop discomfort	Continuous discomfort
Solution B (Burow's solution)	26	21	80.7 %	3.8	24 (92.3)	7	19
Solution C (3.25% aluminium acetate)	20	15	75 %	4.9	19 (95)	8	6
Solution A (1.3 % aluminium acetate)	10	5	50 %	6.2	7 (70)	8	1
Total	56	41	74.5 %	4.78	50	23	26

Table 7: Treatment failures in each of the treatment groups. Treatment successes presented in parentheses.

	n	%	Otorrhoea duration		Inflammation severity		Perforation size		
			weeks	months	mild	moderate	0 – 25	25 - 50	>50
Solution B	5	19.2	1	4	4	1	4	1	0
(Burow's solution)	(21)	(80.7)	(7)	(14)	(10)	(11)	(14)	(7)	(0)
Solution C	5	25	1	4	4	1	4	1	0
(3.25% aluminium acetate)	(15)	(75)	(5)	(10)	(6)	(9)	(12)	(1)	(2)
Solution A	5	50	3	2	4	1	4	1	0
(1.3 % aluminium acetate)	(5)	(50)	(3)	(2)	(5)	(0)	(4)	(1)	(0)

Table 8: Mean pure tone air and bone conduction audiological results of treatment groups.

	Initial mean air cond. p.t.a. (SD)	**Final mean air cond. p.t.a.** (SD)	**Mean air cond. p.t.a. improvemnt**	**Initial mean bone cond. p.t.a.** (SD)	**Final mean bone cond. p.t.a.** (SD)	**Overall % audiol improvemnt**
Solution B	**34.4**	**24.7**	**9.67**	**7.3**	**6.5**	**70.8 %**
(Burow's solution)	(8.2)	(5.9)	(6.12)	(2.6)	(2.4)	
Solution C	**36.1**	**28.4**	**8.36**	**8.2**	**8.5**	**63.1 %**
(3.25% aluminium acetate)	(5.7)	(3.6)	(7.38)	(3.1)	(3.4)	
Solution A	**33.1**	**26.8**	**6.33**	**5.0**	**4.5**	**44.4 %**
(1.3 % aluminium acetate)	(9.3)	(6.5)	(4.15)	(0.9)	(3.7)	
Average	**34.0**	**26.3**	**8.49**	**7.0**	**6.5**	**63.5 %**
	(8.8)	(5.6)	(6.17)	(2.6)	(3.2)	

Note: All values in above table in Decibels (dB) except the overall patient audiological improvement which is a percentage score.

p.t.a. – pure tone average.

5. Study 4

The effects of quarter strength Burow's solution (3.25% aliminium acetate solution) and 2% acetic acid on otoacoustic emissions and cochlea hair cells in the chinchilla.

Introduction

Despite no reports of ototoxicity from published studies of the use of aluminium acetate solution in the treatment of suppurative ear disease, and there being no suggestion of ototoxicity in the clinical study conducted, use of topical preparations that can potentially penetrate into the middle ear always raises concern about potential ototoxic effects.

Therefore for completion an animal model study was undertaken to determine whether or not quarter strength Burow's solution was ototoxic potential, since to date no animal studies have been performed to assess the ototoxicity of aluminium acetate solution.

This arm of the study was conducted at the Auditory Science Laboratory, Brain and Behaviour Division, The Hospital for Sick Children, Toronto. Canada.

The chinchilla was selected as the animal model

Clinically measures of cochlear function were obtained using distortion product otoacoustic emission testing during the treatment period.

After completion of the treatment period, scanning electron microscopy was used to assess the general condition of cochlear hair cells.

5.1 Materials and Methods

Six normal hearing adult chinchillas weighing between 580 and 760 g were used. All animal procedures were carried out according to national guidelines issued by the Canadian Council on Animal Care under the supervision of the local (the Hospital for Sick Children) animal care committee.

A solution of 13% aluminium acetate was constituted by the Pharmacy of the University Healthcare Network, Toronto General Hospital. This solution was then diluted with 3 parts water to make a 1:4 dilution or 3.25% aluminium acetate solution (quarter strength Burow's solution). A solution of 2% acetic acid was also prepared. The pH of both solutions and normal saline was recorded prior to application. The pH of the full-strength Burow's solution was 3.42 and that of the quarter-strength solution (3.25% aluminium acetate) was 3.62. The pH of the 2% acetic acid was 2.90 and the normal saline 6.82.

Procedure

All animals were premedicated with atropine sulphate (0.04 mg/kg, i.m.), and then anaesthetised with xylazine (2.5 mg/kg, i.m.) and ketamine hydrochloride (15 mg/kg, i.m.). Distortion product otoacoustic emissions (DPOAE) were recorded twice in each ear. Animals were randomised in a binary fashion to receive the test solution (3.25% aluminium acetate solution or 2% acetic acid) in one ear and control (normal saline solution) in the opposite ear for the duration of the study.

Whilst anaesthetised the mastoid bulla of each animal was punctured with a 22 G hypodermic needle and 0.2ml of control or test solution slowly injected. The animals were allowed to recover and their behaviour observed prior to being returned to holding.

Treatment Schedule

On days 2, 3, 4 and 5, a single transbullar injection of 0.2 ml of 3.25% aluminium acetate solution to the designated ear and injection of normal saline to the opposite side, was performed on animals #1 – #3. Animals #4 - #6 received a single 0.2 ml transbullar dose of 2% acetic acid, with control doses continuing as in animals #1 - #3.

Animal #1 was re-anaesthetised on day 10 and post treatment DPOAEs measured. Animals #2 and #3 were re-anaesthetised on day 18 prior to post treatment DPOAEs being recorded. Animal #4 died on day 1; aetiology unknown. Animals #5 and #6 were re-anaesthetised on day 10 (as in animal #1), prior to post treatment DPOAEs being recorded.

After final DPOAE measurement each animal's cochleas were fixed for microscopic examination.

Otoacoustic Emission Recording

All DPOAEs were measured in the anaesthetised animal in a sound attenuating chamber. For all OAE recordings the seal and position of the ear probe was checked by monitoring the amplitudes of calibration signals within the ear canal. The DPOAEs

were measured twice on each ear (test and control) with the ILO 88/92 device (Otodynamics, London, UK). Parameters for DPOAE recordings were F_1 and F_2 set at 50 dB SPL, with a ratio of 1.2. Amplitudes of the $2F_1 - F_2$ distortion product were recorded at F_1 and F_2 presentations across frequencies (F_2 = 1 – 6 kHz)

Light and Scanning Electron Microscopy

Cochleas were harvested immediately post-mortem. After appropriate dissection gross middle ear cavity morphology was photographically recorded under the light microscope. The round and oval windows were opened and the cochleas perfused with 2.5% glutaraldehyde in 0.1M phosphate buffer. Specimens were post-fixed with 1% OsO_4 and dehydrated in graded ethanols. After critical point drying from CO_2, the specimens were sputter coated with gold and observed by scanning electron microscopy (SEM, Hitachi S-570). Appropriate images of basal, mid-basal and apical turns of the cochlea of the chinchilla were photographically recorded.

5.2. Results

It was observed that within hours of administration of the test solutions all animals displayed vertiginous symptoms of rolling toward the side of injection of the test solution. This only occurred with motion and when suspended by their tails. These symptoms had settled by the third and fourth days of study.

Otoacoustic Emissions

The otoacoustic emissions displayed graphically in Figures 5-7 are the mean value of the two DPOAEs measured in each animal.

DPOAEs shown in Figure 5, are those prior to and following the course of treatment with 3.25% aluminium acetate solution in animals #1 - #3. Obvious differences between the pre and post treatment thresholds of DPOAEs are evident in all 3 animals tested with quarter strength Burow's solution. Little or no response are noted in the post treatment DPOAEs. Animals #2 and #3 were measured at 18 days and animal #1 at 10 days post commencement of therapy. The loss of DPOAE amplitude occurred at all frequencies tested (1 – 6 kHz).

Pre and post-treatment DPOAEs of animals treated with 2% acetic acid are shown in Figure 6. The results of only 2 animals were available post treatment with 2% acetic acid. The loss of DPOAEs in animal #5 at 3kHz was a constant pre therapeutic finding and could not be accounted for. Both animals displayed complete loss of DPOAEs post treatment as observed with the 3.25% aluminium acetate solution. The loss of DPOAE amplitude occurred at all frequencies tested (1 – 6 kHz).

Figure 7 illustrates sample DPOAEs of the control ears in 2 randomly selected animals(animals #1 and #5) prior to and following the course of treatment with normal saline. No change in DPOAEs was seen following the 10 or 18 day courses of treatment with normal saline. Barotrauma induced by the transbullar injection method of application of test fluid to the middle ear of the chinchilla would therefore be unlikely.

Light and Scanning Electron Microscopy

Light microscopy of the middle ear space of the test ear in all animals showed diffuse oedema of the middle ear mucosa, however definition of the round and oval window niches and the anatomical integrity of the ossicular chain was maintained. The authors believe that the degree of oedema and fluid present in the middle ear space could account for the reduction in the DPOAEs of the animals treated with both the 3.25% aluminium acetate solution and the 2% acetic acid, but not the degree of DPOAE loss observed in this study.

Scanning electron microscopy was performed on the cochlea of a single control ear, which, as shown in Photomicrograph 1, demonstrated normal integrity of inner and outer hair cell structure following a 10 day course of transbullar infiltration with normal saline. A single row of inner hair cells can be viewed superiorly and 3 rows of distinctly shaped clusters of outer hair cells inferiorly (Photomicrograph 1).

Scanning electron microscopy performed on the cochleas of ears treated with 3.25% aluminium acetate solution and 2% acetic acid showed similar outer and inner hair cell damage. Photomicrograph 2 shows the damage caused by the 3.25% aluminium

acetate solution was worst in the lower basal turn, with slight improvement in structure through the upper basal turn as seen in Photomicrograph 3, towards the apex of the cochlea shown in Photomicrograph 4. Outer hair cell damage appeared worse than inner hair cell damage. Similar damage was seen throughout the cochleas of animals #5 and #6 treated with 2% acetic acid as demonstrated in Photomicrograph 5.

Figure 5: The DPOAEs measured before (open circle) and on completion of treatment (closed circle) with 3.25% aluminium acetate solution. Data from 3 animals illustrated.

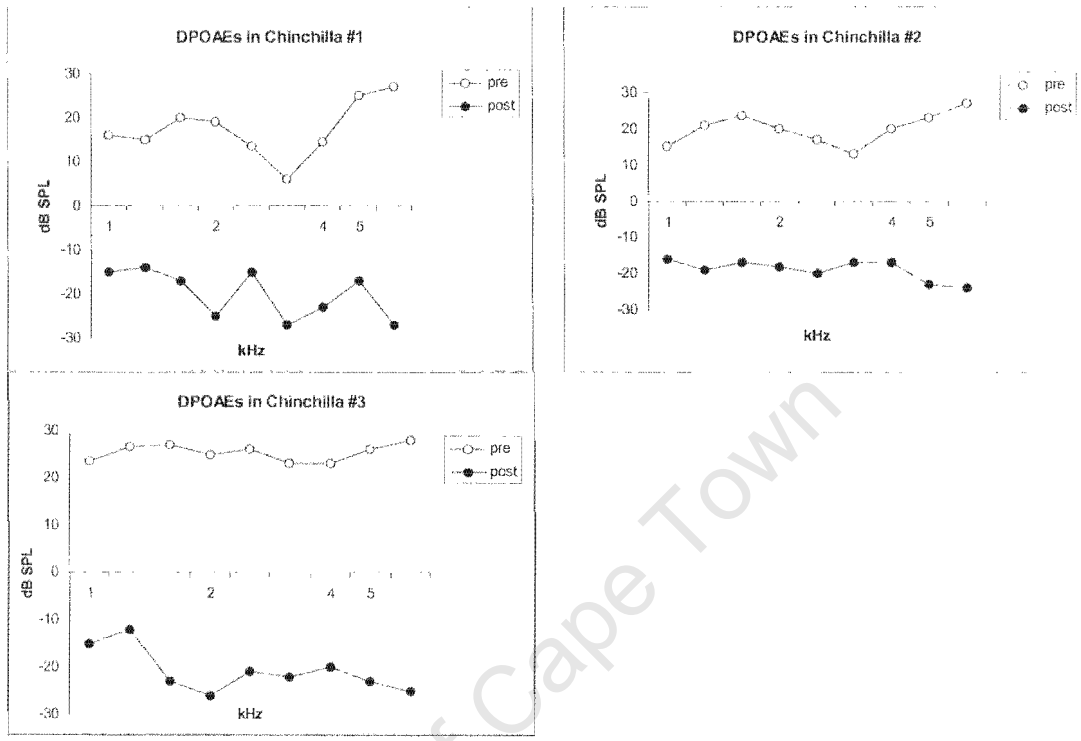


Figure 6: The DPOAEs measured before (open circle) and on completion of treatment (closed circle) with 2% acetic acid. Data from 2 animals illustrated.

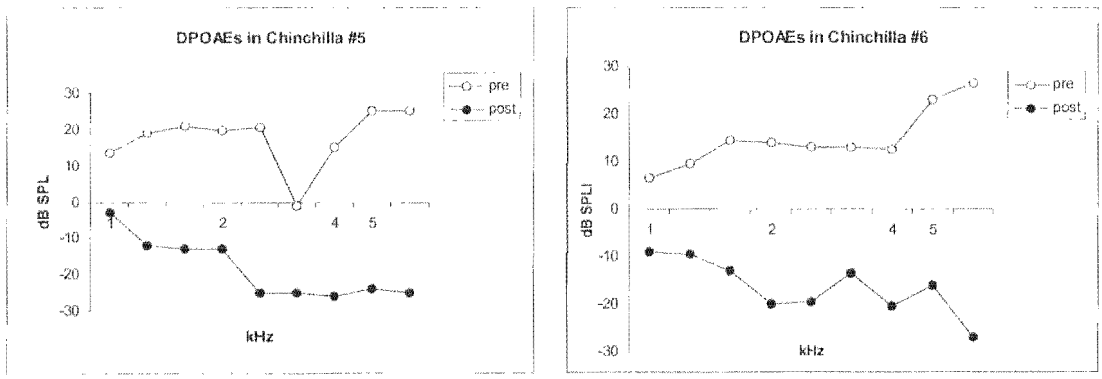
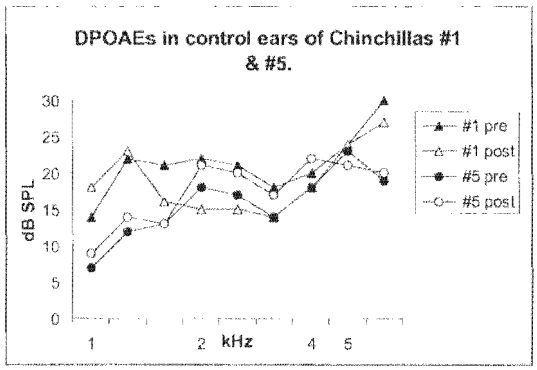
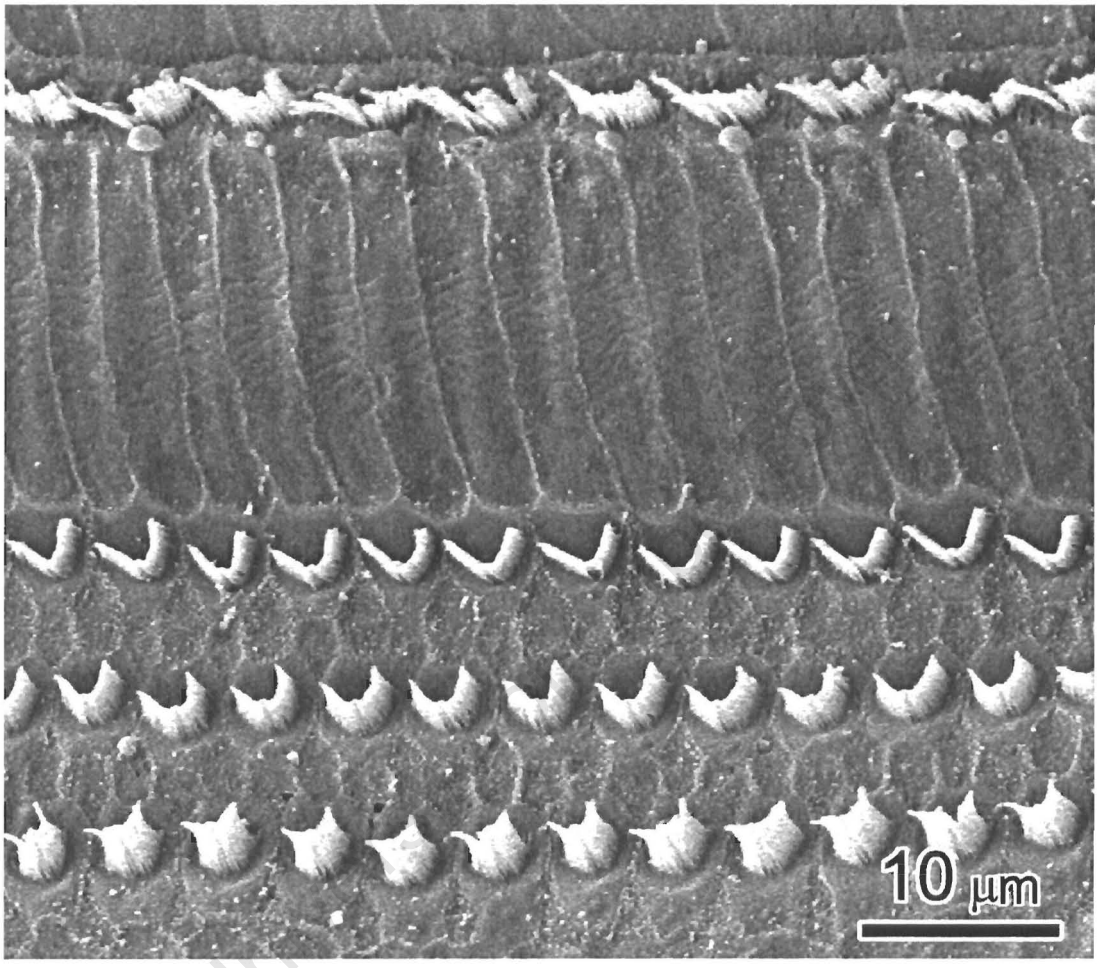


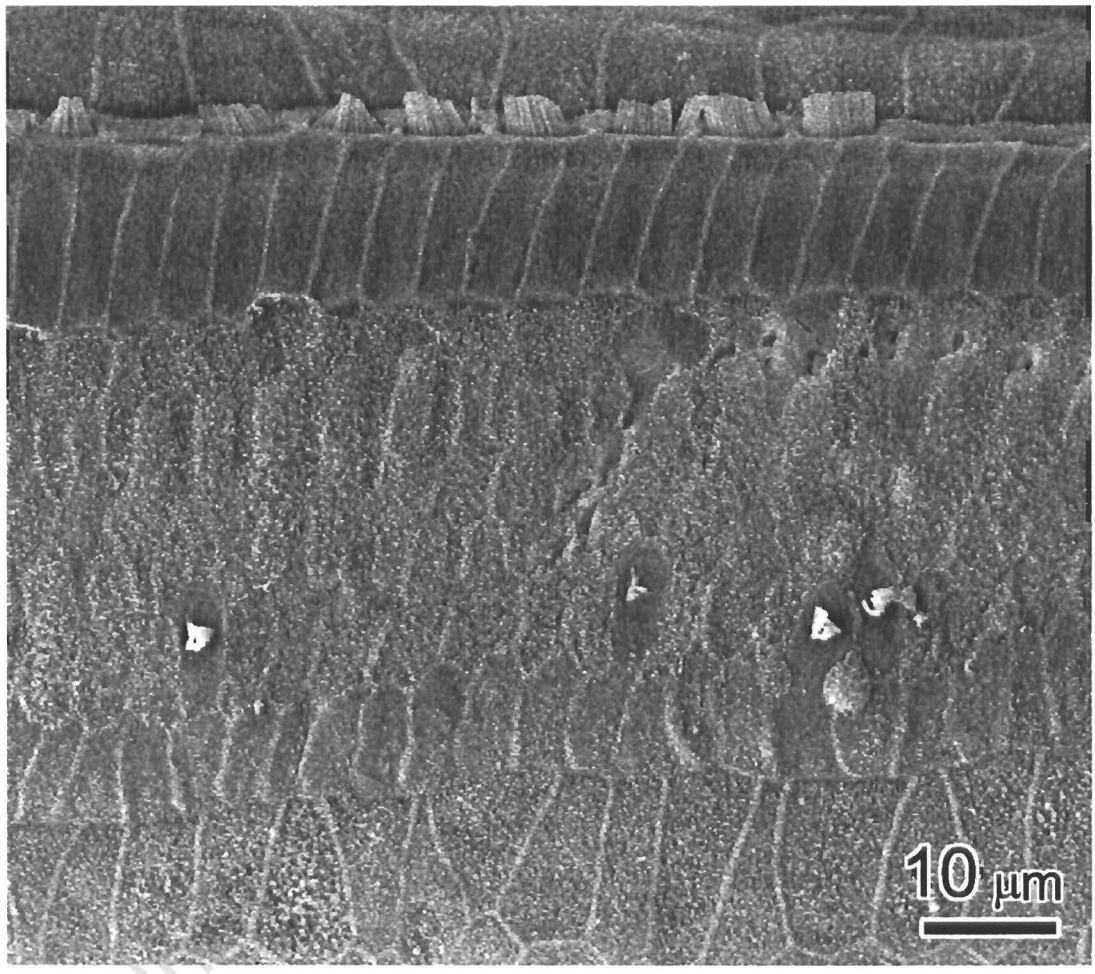
Figure 7: The DPOAEs measured before (open shapes) and on completion of treatment (closed shapes) with normal saline. Data from 2 animals illustrated.



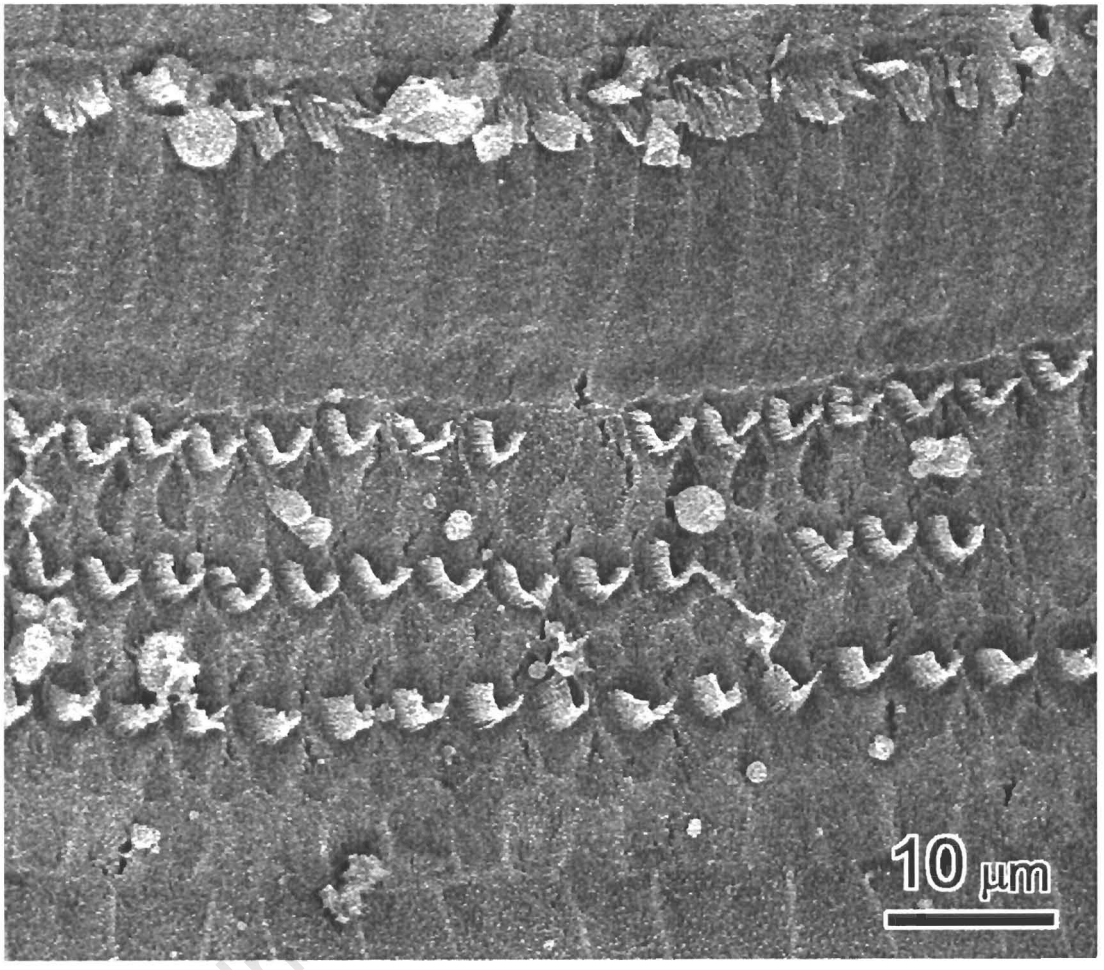
Photomicrograph 1: Scanning electron micrograph demonstrating normal appearing hair cell stereocilia in the control ear of a chinchilla.



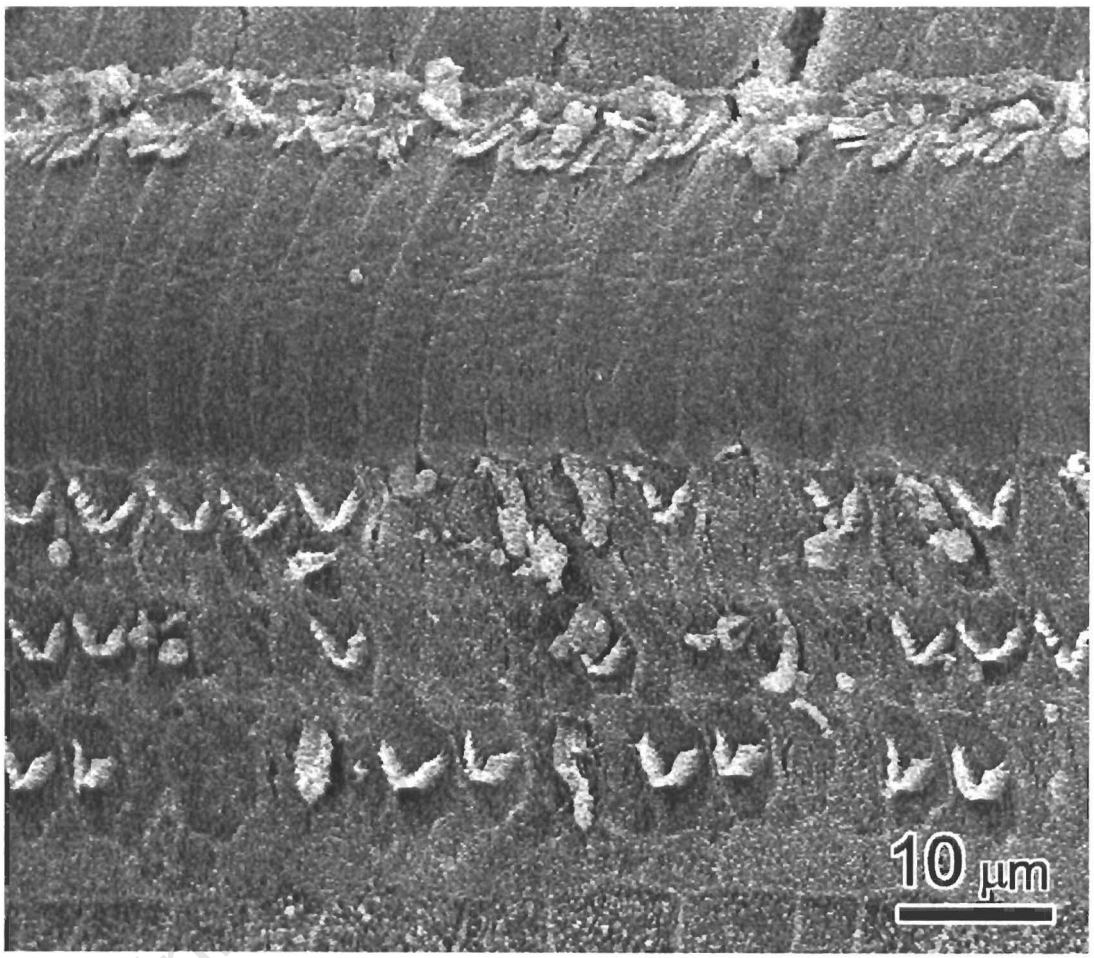
Photomicrograph 2: Scanning electron micrograph of the basal turn of the cochlea of a chinchilla treated with 3.25% aluminium acetate solution demonstrating near total loss of hair cells.



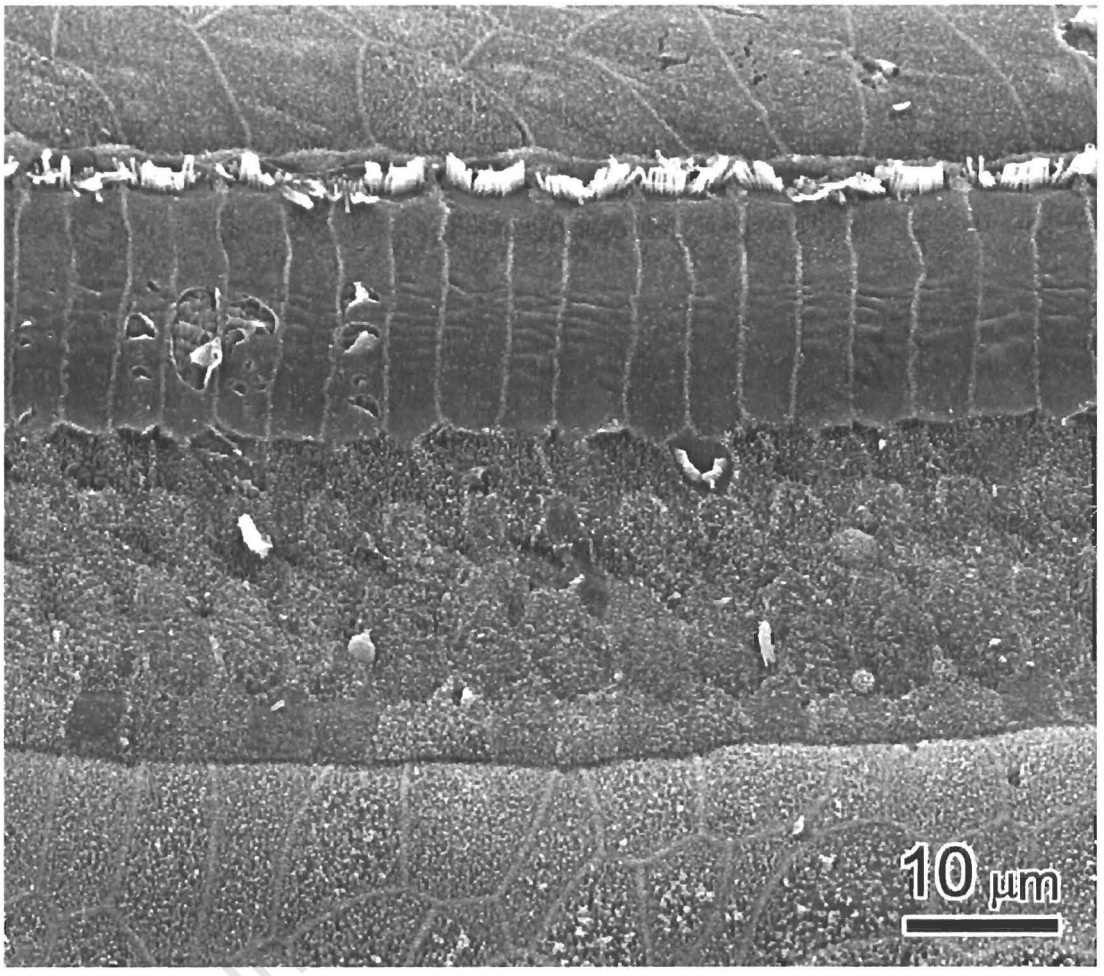
Photomicrograph 3: Scanning electron micrograph of the middle turn of the cochlea of a chinchilla treated with 3.25% aluminium acetate solution demonstrating marked loss of hair cells.



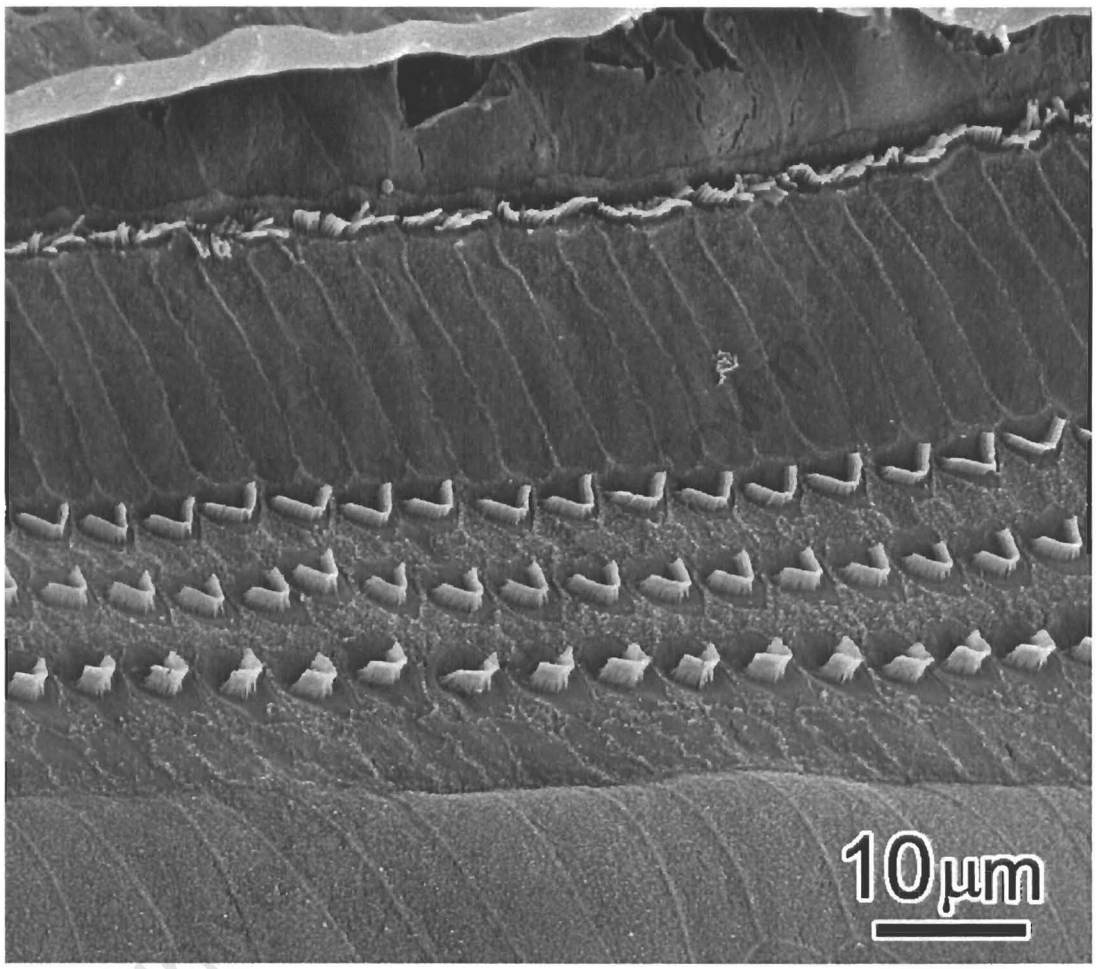
Photomicrograph 4: Scanning electron micrograph of the upper turn of the cochlea of a chinchilla treated with 3.25% aluminium acetate solution demonstrating partial loss of hair cells.



Photomicrograph 5: Scanning electron micrograph of the basal turn of the cochlea of a chinchilla treated with 2% acetic acid demonstrating near total loss of hair cells.



Photomicrograph 6: Scanning electron micrograph of the basal turn of the cochlea of a chinchilla treated with Cipro HC Otic Suspension® (Alcon) demonstrating normal steriocilia of hair cells.



6. Discussion

Summary

The initial 2 hypotheses of this thesis have been successfully proven. The first was to demonstrate the in vitro *effectiveness of aluminium acetate solution against organisms commonly associated with chronic suppurative otitis media.. The second was to demonstrate clinical or* in vivo *effectiveness and safety of the ear drop preparation.*

Aluminium acetate solution ear drops were found in vivo *to cause ototoxic damage to the inner ear of the chinchilla when applied for prolonged periods.*

The non-antibiotic containing ear drops that were available from the pharmacies of Groote Schuur Hospital and the Red Cross War Memorial Hospital – the teaching hospitals of the University of Cape Town – at the time of these studies were:

1. 5% Hydrogen Peroxide ear drops
2. Glycerol and Ichthymmol ear drops
3. Methiolate ear drops
4. 1% acetic acid ear drops
5. 2% acetic acid ear drops
6. 3% acetic acid ear drops
7. Burow's solution ear drops

Ear drops 4 through 7 were selected for study with particular attention given to Burow's solution due to it's *in vitro* effectiveness.

A previous study from the Departments of Otolaryngology and Microbiology, University of Cape Town, investigated the bacterial growth inhibition characteristics of glycerol and ichthammol eardrops[10]. The active ingredient of this preparation, ichthammol, was able to inhibit growth of *Staphylococcus aureus* and *Streptococcus pyogenes*, but was ineffective against *Pseudomonas aeruginosa* and *Proteus mirabilis*.

A previously performed study into the effectiveness of acidic ear drop preparations showed 1-5% acetic acid to be bacteriocidal *in vitro* to a range of organisms commonly encountered in otitis externa, including *Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli, Streptococcus pyogenes and Staphylococcus aureus*[67]. Solutions of acetic acid weaker than 1% were shown not to be bacteriocidal *in vitro*.

This activity was not shown with inorganic acids or the organic acids, lactic and citric acid although the reason for this is not known[67].

6.1. *In vitro* studies

6.1.1. Effectiveness of acetic acid and aluminium acetate solution

Study 1 of this thesis shows that acetic acid inhibits growth of coliform organisms - *Pseudomonas aeruginosa* and *Proteus mirabilis* - as well as gram positive organisms - *Staphylococcus aureus* and *Streptococcus pyogenes* - isolated from discharging ears. As the 2 commonest occurring organisms in chronic suppurative otitis media are *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteriocidal activity against these organisms is important[6,7,10,11,16]. Addition of aluminium to acetic acid to form aluminium acetate appeared to produce an even more effective preparation as demonstrated by the significantly (anova) larger zones of bacterial growth inhibition for all 4 organisms tested.

Although the antibacterial effectiveness of these preparations is thought to be largely due to their acidity, a pH analysis of the solutions used in part 1 of the thesis (1 % acetic acid – pH 2.9; 2 % acetic acid – pH 2.8; 3 % acetic acid – pH 2.7; Burow's solution – pH 3.2) indicated that all strengths of acetic acid tested were more acidic than Burow's solution. The effectiveness of the Burow's solution, though partly due to the acidity of the solution, must lie with the aluminium or with the aluminium acetate. The pH's of full strength Burow's solution and 2% acetic acid used in the animal studies were 3.42 and 2.90 respectively, which were not significantly different from the pH values in the initial work. To the best of the author's knowledge the exact mechanism of action of aluminium acetate solution is unknown, and no published material on this matter exists.

The recent recommendation of the South African Ministry of Health[64] of a single ear drop preparation, namely 1% acetic acid for the treatment of chronic suppurative otitis media appears not to have reviewed this *in vitro* study. The further recommendation of a 1 in 10 dilution of vinegar with boiled water, which would only yield a 0.5% acetic acid solution, would be less effective.

6.1.2. Determining the MID

The methodology used in study 2 followed the guidelines laid down by the NCCLS (National Committee for Clinical Laboratory Standards – U. S. A.) for the determination of minimum inhibitory concentrations (MIC). NCCLS defines MIC as the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in an agar or broth dilution susceptibility test. There are however no established standards for testing antibacterial agents used topically or locally. It was therefore decided to incorporate the Burow's solution, and doubling dilutions thereof, into agar plates until a minimal inhibitory dilution for each organism tested was reached.

Calculation of a MIC from the MID values obtained for Burow's solution is possible provided the active component of the solution is known. However with aluminium acetate solution the bacteriocidal component is unknown, making it more practical to used the MID. Thus due to the nature of Burow's solution, the determination and expression of a MIC was problematic, hence the results were expressed as minimum inhibitory dilutions.

Testing in serum or broth was not possible due to repeated occurrence of a precipitate seen in preliminary testing, presumed to be denatured protein in the broth caused by the activity of the acid.

Although this study suggests that a 1:80 to 1:160 dilution of Burow's solution would be effective in inhibiting growth of common causative bacteria seen in chronic

suppurative otitis media, it was not possible to test this in the laboratory in a mucopurulent environment such as would be found in the clinical situation. The precipitates that occurred with attempted MIC testing in broth, thought to be due to protein denaturation in lowered pHs, suggested that less dilute preparations of aluminium acetate solution would be required in the oedematous and purulent environment of the chronically suppurating middle ear cleft. Consequently the strength of aluminium acetate solution would likely need to be increased in the clinical management of chronic suppurative otitis media.

Another factor to be considered in the clinical setting is the more dilute the solution applied to the middle ear cleft, the less the acidity and hence local discomfort to the ear, which would improve patient tolerance and hence compliance. The fact that most organisms were inhibited by a dilution of 1:160 suggests that a more dilute solution than 13 % aluminium acetate may be clinically effective. As in the preceding laboratory study where 1% acetic acid was disproportionately less effective against *Staphylococcus aureus* than more concentrated solutions, *Staphylococcus aureus* required a slightly stronger dilution of aluminium acetate to inhibit growth(inhibited to a dilution of 1:80), which correlates with the smaller inhibition zone sizes seen in the initial *in vitro* study.

Moroxella catarrhalis, reported to be one of the three commonest organisms found in acute otitis media[68], recurrent otitis media and hence, by implication, chronic suppurative otitis media, is not commonly found in our population[68]. A few samples of *Moroxella catarrhalis* were however tested. All had an MID of 1:160 of Burow's solution.

6.2. Clinical Trial

Although this clinical study was completed a few years prior to randomised control trial criteria becoming available, attempts have been made to report as recommended by the Consolidated Standards of Reporting Trials (CONSORT) statement[66]. Three main issues require explanation.

1. Randomisation.

   The randomization sequence generation was fully explained as was allocation concealment in the trial methodology. As ear drops were in identical containers, clinicians involved in the study were blinded, with the exception of the clinical investigator were unaware of the ear drop allocation.

2. Intention to treat.

   Due to the small numbers involved, data of those patients completing the study was analysed as the response to medication of defaulter and non compliant patients could only be surmised. However if defaulters and non compliant patients were included on an intention to treat basis, the results were markedly different due largely to small sample size.

	Response	Included	Response rate	Defaulters	Non compliant	ITT response
Solution B	21	26	80.7%	2	4	63.6%
Solution C	15	20	75%	1	0	71.4%
Solution A	5	10	50%	3	1	35.7%

3. Power.

   As an acceptable difference in response rates to varying strengths of aluminium acetate solution is unknown, the ability to calculate the sample size necessary to

achieve acceptable power in this study is not possible. However if a statistically significant(statistical power of 0.95 - or beta error level) difference in response rate of 5% between Solution B and C were to be shown, with an alpha error level of 0.05, a further 1500 patients would need to be randomised to achieve this. If intention to treat data was used, 1050 further patients would be required to prove that Solution C was significantly more effective than Solution B.

A evidence based systematic review emphasised that the results of the relative success of each preparation strength of aluminium acetate in the clinical trial of this thesis could have occurred by chance, as the numbers in each group were small[3].

Full strength Burow's solution proved most effective in resolving otorrhoea but due to its greater acidity was the most uncomfortable of the concentrations of aluminium acetate to use (Table 4). As compliance to therapy is important, the quarter strength Burow's solution, though slightly less effective, proved to be more acceptable to the paediatric population used in this study. The discomfort experienced with Solutions A and C disappeared shortly after application and with resolution of the otorrhoea, whereas the duration and intensity of discomfort of full strength Burow's solution was considerable.

The success rates for clearing otorrhoea of 75 - 80% in this clinical study with both full and quarter strength Burow's solution compared well with the reported success rates of aminoglycoside[20,69] and quinolone[41,70] containing preparations. These ranged from 39%[17] – 87%[28,30] for the gentamicin containing ear drop preparations to 47%[24] - 95%[22] for the quinolone containing ototopicals.

Comprehensive review of the literature reveals that the effectiveness of aluminium acetate solution as an ear drop in the treatment of chronic suppurative otitis media has only been studied once before[39]. This double-blind, randomised prospective clinical trial warrants assessment. A total of 139 ears were entered into the study of which 102 (74%) completed therapy. As the percentage completing the study does not surpass 80% this valuable study does not feature in evidence-based clinical reviews[2,3]. Patients were randomised, method not revealed, to receive either topical antibiotic ear drops, 0.3% gentamicin sulphate or 8% aluminium acetate solution for 21 days, with assessments at day 9 and day 21. The condition of the ear was evaluated on a scoring system at commencement and each assessment thereafter. Discharging ears included otitis externa, which made up 64% of ears, and chronic suppurative otitis media from mastoid cavities as well as central tympanic perforations. Although the overall success of the 8% aluminium acetate solution was similar to that of the 0.3% gentamicin ear drops, namely 67% and 68% respectively, only 4 ears with chronic suppurative otitis media received the aluminium acetate solution. Of these 4 ears only 2 (50%) showed improvement. The second arm of this study investigated the bacterial resistance to the test agents *in vitro*. No resistance to aluminium acetate solution was encountered, however 12 of 60 ears treated with 0.3% gentamicin ear drops showed resistant organisms at the onset of the study. Only 2 of these 12 failed to improve on therapy, with a single case of resistance to gentamicin emerging in the trial period. The authors conclude that a topical antiseptic rather than a topical antibiotic preparation be used in the initial management of otorrhoea on the grounds of cost, avoidance of resistance and toxicity. The final part of this statement cannot be validated as this trial failed to monitor audiologic or vestibular parameters. Furthermore the trial failed to include follow up data, this being a common criticism

and failing with all studies on the medical management of chronic suppurative otitis media[2,3,31].

Browning[31] however makes the point that these results are not a true reflection of the effectiveness of the medication, as relapse rates at 4 – 6 weeks post therapy show approximately 43% of inactive ears becoming active again. Critically the clinical study of this thesis fails in that there was no interval follow up of patients to ascertain long term effectiveness of medication.

Assessment of the treatment failures showed that the only qualitative feature that had any bearing on response was the duration of the otorrhoea. Duration of discharge of 3 months or more proved most difficult to resolve. The degree of inflammation and perforation size had no effect on the outcome. Statistical analysis of treatment failures was not possible due to the small size of the groups.

Audiological assessment failed to demonstrate any evidence of ototoxicity at the completion of the treatment period in this study. However audiological function was not measured at any period beyond completion of the trial. The large Zairian retrospective study using cotton wicks soaked in Burow's solution[36] failed to demonstrate a single case of ototoxic sensorineural hearing loss. Patients were exposed to the aluminium acetate continuously via the wick for up to 6 weeks duration without any noted ill effects.

Vestibular testing at the completion of therapy, although not performed, would have been helpful to ascertain if any vestibular compromise occurred. No reports of vestibular compromise in trial participants were noted.

Specialist otolaryngologists' prescribing habits in the use of ototopical preparations have been assessed in both the United Kingdom[71] and in the United States of America[65]. Lancaster *et al*[71] found that 93% of otolaryngologists responding to a questionnaire (reply rate of 308/525), used aminoglycoside containing ear drops in the management of chronic suppurative otitis media. Only 48% believed these ear drops to be ototoxic in the presence of a tympanic membrane perforation. A similar but far larger study in the United States[65](reply rate of 2235/7463) used ototopicals in the presence of a draining perforation (84.1%), drainage through a ventilation tube (93.7%) or in an open draining mastoid cavity(92.8%). Eighty percent agreed with the statement "The risk of ototoxicity of otitis media is as great, or greater than the risk for ototoxicity of an ototopical preparation". Only 3% had ever directly witnessed irreversible inner ear damage that was unquestionably related to the use of an ear drop preparation. Of the preparations identified as possibly ototoxic 31.6% believed Cortisporin suspension® was responsible, 23.7% believed that gentamicin was responsible and 10.5% identified other agents. Interestingly the only preparations felt never to have been responsible for inner ear damage included Domeboro® which is an aluminium acetate based antiseptic and VoSol® which has 2% acetic acid as its active agent. Although this data by no means justifies the use of acetic acid or aluminium acetate solution in chronic suppurative otitis media, it does represent the largest collection of peer based information on the ototoxicity of ear drop preparations available in the current literature.

Since the publication of the clinical study at Red Cross War Memorial Hospital (study 3) utilizing aluminium acetate solution[16], further work employing Burow's solution has been published. A recent Japanese-based study[37] examined the effects of full strength Burow's solution on the purulent drainage of chronic otitis externa and chronic suppurative otitis media including chronically discharging mastoid cavities. A total of 25 ears were included. All ears with chronically draining mastoid cavities were improved (72% cured), whilst all ears with chronic otitis externa, chronic fungal otitis externa and chronic granular myringitis were cured. Of the 6 ears with chronic suppurative otitis media 4 were cured of otorrhoea (cure defined as a dry middle ear, without redness, not requiring further treatment), whilst one showed improvement and one showed no change. No signs or symptoms of ototoxicity were encountered. Hearing levels were monitored by means of pre- and post treatment audiograms.

6.3. Safety versus Ototoxicity

To date most work has investigated the ototoxicity of systemically administered drugs than topically applied preparations[54,72]. Published literature on the toxicity of topical otologic preparations consists of *in vitro* and *in vivo* studies. To date most work unfortunately has involved *in vivo* animal studies. It is unknown how applicable these animal model studies are to the treatment of human patients.

The 3.25% aluminium acetate and 2% acetic acid solutions were both found to be topically ototoxic in the study performed on the chinchilla. The degree of hair cell loss seen on electron microscopy was extensive, but not as extensive as that seen with aminoglycoside antibiotics on the same animal model[73]. The fact that no cochlear hair cell destruction was seen in the control ears, and that these ears tested normally with distortion product otoacoustic emissions, would appear to indicate that this was a true ototoxic effect of the agents and not an experimental method artefact.

With regard to extrapolation of this finding to the human ear, the drainage of the middle ear of the chinchilla is thought to be less effective than in humans, adding to the period of exposure to the round window membrane. The test solution is likely to have been in continuous contact with the round window membrane for the full 10 to 18 day period. Whether or not the results of this animal study can be extrapolated to the human ear is uncertain. Further testing in larger primates would closer resemble the response of a human inner ear to aluminium acetate solution.

6.3.1 Substances tested

Numerous substances, some commonly found in ear drop preparations, and others not, have been used in various animal test models. Some have been found to be ototoxic and others not.

1. Sterile water[74].

Direct perilymphatic perfusion of chinchilla cochleas with sterile water caused total ablation of inner and outer hair cells ahead of other cells due to a presumed osmotic effect.

2. Propylene glycol[75,76].

Both studies performed on guinea pigs showed hearing loss following installation of propylene glycol in to the middle ear. Vernon *et al*[75] felt damage to be conductive in nature, whereas Morizono *et al*[76] concluded the hearing loss to be sensorineural in nature and that the absence of morphological hair cell damage did not indicate normal function.

3. Ethanol [77].

Irreversible decline in endocochlear potentials occurred in guinea pigs exposed to 70% ethanol, whilst those exposed to 35% ethanol demonstrated reversible decline.

4. Povidone-iodine preparations[78].

Povidone-iodine scrub applied to the round window in the chinchilla, was more ototoxic than povidone-iodine solution. A 1:4 dilution of 1% povidone-iodine solution showed substantial ototoxic effect whereas a 1:10 dilution did not.

5. Acetic acid[79].

Application of VoSol(2% acetic acid + 3% propylene glycol) and 2% acetic acid to the round window membrane produced significant decreases in endocochlear potential and endolymphatic pH in the chinchilla. Unfortunately this study did not include microscopic visualization of the organ of Corti to assess the extent of damage. A 2 % acetic acid solution was found to be 4 times as ototoxic as Cortisporin®[14]. Interestingly, physiologic saline at pH 2.7 produced no loss of sensory hair cells in guinea pigs[80].

6. Corticosteroid solution[81].

Triamcinolone diacetate was found not to significantly alter the compound action potential in chinchilla auditory nerves when tested against a control of Ringer's solution.

7. Bacterial toxins[82].

Penetrability of the round window membrane by *Escherichia coli* endotoxin and *Staphylococcus aureus* exotoxin was greater than normal but less than that seen in otitis media.

8. Antimycotics[83].

Clotrimazole, miconazole and tolnaftate appeared to be safe in a guinea pig model. Nystatin did not yield conclusive results whilst gentian violet had potential for severe damage.

9. Antibiotics.

A number of different antibiotics have been shown to be ototoxic, whilst others have been shown to be safe, in animal models. Early microscopic work without electrophysiologic monitoring performed on guinea pigs showed hair cell loss with aminoglycosides, tetracyclines and chloramphenicol[80,84]. Penicillin and carbenicillin were shown to have no deleterious effect[80], however topical ticarcillin ear drops with or without clavulanic acid (Timentin® - SmithKline Beecham – ticarcillin+clavulanic acid) produced showed extensive loss of hair cells on electron microscopy[85]. Ceftazidime (Tazicef® - SmithKline & French) however showed minor reversible ototoxic damage in outer hair cells in the basal turn of the cochlea in chinchillas[86]. Cortisporin Otic Suspension®, (polymixin B, neomycin, hydrocortisone and propylene glycol) is one of the most commonly used ototopicals by specialists in North America[65], has been shown in numerous animal models to be ototoxic[14,55,87-89]. It was shown to be significantly more ototoxic in the chinchilla than the baboon, polymixin B being considerably more toxic than neomycin[89]. Cortisporin® has been included in a series of studies assessing cytotoxicity to isolated outer hair cells[21,90].

More recent studies have focused on the quinolones, finding both ofloxacin and ciprofloxacin to be safe in animal models ranging from rodents to primates[70,91,92].

Table 9: Quantitative ototoxicity as demonstrated by hair cell loss in the guinea pig[80].

Compound	**Degree of hair cell loss**
0.9% Sodium chloride	0
Amphotericin B	0
Bacitracin	0
Benzylkonium chloride	+++
Carbenicillin	0
Chloramphenicol	+
Chlortetracycline HCl	++
Chlorhexidine acetate	+++
Colimycin	+++
Distilled water	0
Gentamicin sulphate	+++
Gramicidin	+
Griseofulvin	++
Iodochlorohydroxyquinolone	++
Nystatin	0
Oxytetracycline HCl	++
Propylene glycol	++
Tetracycline HCl	++

Hair cell loss:
+ minor
++ moderate
+++ major

6.3.2 Animal models

Various animal models have been used in ototoxicity studies. Rodents, including rats, guinea pigs and chinchillas, prove to be popular as they are cheap and easy to house, whereas primates (monkeys and baboons), although anatomically more similar to humans have higher maintenance costs and are hence less often employed. Differences between animal models and the diseased human middle ear space can be divided into anatomic and physiologic[53].

Anatomic

1. The position of the round window niche and the oval window in the chinchilla and guinea pig is very prominent in the middle ear space. Any test substance placed in the middle ear will therefore have prolonged contact with the round window membrane. By contrast the human round window membrane is deeply recessed and protected from fluids in the middle ear[14,55,93].
2. The thickness of the human round window membrane is 6 – 10 times that of its rodent counterpart. The round window membrane in the chinchilla being approximately 16µm thick versus a normal human round window membrane being 60 - 65µm thick[94]. The round window membrane in mammals are similar, although the cellular structure in the human is more dense[53,95]. It is composed of 3 layers, (1) an outer cellular layer with an underlying basement membrane, (2) a middle connective tissue layer containing fibroblasts, collagen, elastin, vessels and nerves, and (3) an inner mesothelial layer[94-96]. The round window membrane in the baboon lies between that of a human and rodents (approximately 30µm), and

studies performed have shown considerably diminished experimental ototoxicity in this animal model.

3. Most human ears have a mucosal membrane similar to the round window membrane in structure[94] that spans the bony margins of the round window niche. This pseudomembrane is not encountered in the middle ear of any other test animal[94] including primates. Therefore in most humans ototopical medication would never come into contact with the round window membrane[53].

Physiologic

1. In the setting of chronic suppurative otitis media, mucosal oedema with or without mucopurulent exudate would provide a significant barrier between ototopical medication in the middle ear and the round window membrane[97,98]. Membrane thickness is greatest in patients with chronic suppurative otitis media, with maximal involvement of the epithelial layer of the round window membrane and the subepithelial space[95]. Reduced permeability of the round window membrane is thought to be due to residual effusion overlying the membrane, membrane thickening and the presence of mucosal membranes and granulation tissue within the round window niche[96]. The round window membrane has been shown however to be permeable to antibiotics, local anaesthetics, toxins, tracers and albumin[96].
2. Reduced permeability of the round window membrane may be due to increased thickness[95] and a deposition of collagen in the middle ear space as a response to chronic suppurative otitis media[87].
3. An hypothesis has been proposed that because the round window membrane carries an anionic charge, the presence of negatively charged substances from

neutrophil lysosomes could neutralise the membrane charge and decrease the permeability to heavily cationically charged substances such as neomycin and gentamicin[14,98].

The temporal bone is the single largest skull bone in the chinchilla due to the size of the mastoid bulla, which despite its size is paper thin facilitating easy sharp perforation[99]. The large mastoid bulla is reliably palpable below the postauricular skin[99]to such a degree that repeat puncture invariably traverses the original bony defect. Only one other published study employed the same method of delivery of test substance to the middle ear, namely needle perforation of the mastoid bulla of the chinchilla[88]. The degree of cochlear hair cell destruction with aluminium acetate solution in this study was similar to that of the Cortisporin Otic Suspension[88]. In a baboon study Cortisporin Otic Suspension showed considerably less destruction, hair cell loss being confined to the basal turn only.

A study employing the same animal model and technique, performed in the same animal laboratory as the animal study in this thesis[100], showed no change in otoacoustic emissions or evidence of hair cell damage in the cochlea when Cipro HC Otic Suspension® (Alcon) was compared with saline in control ears. Of particular interest is that Cipro HC has a pH of 4.6 and has an acid base of acetic acid and sodium acetate. The pH of 3.25% aluminium acetate however varied from 3.5 – 3.62, making it approximately 10 times stronger in acidity than Cipro HC. The pH alone may have accounted for the cochlear damage sustained in this sensitive animal setting.

6.3.3 Qualitative versus Quantitative testing

Most animal studies are qualitative in nature, however a few studies have attempted to quantify the ototoxic risk[21,80,84]. Stupp *et al*[84] observed in guinea pigs that the ototoxicity of the aminoglycosides was directly proportional to the drug concentration in the endo- and perilymph of the inner ear. Polymixins, chloramphenicol and the tetracyclines only exhibited toxic effects when administered to the middle ear and not when administered systemically[84]. Parker and James[80] using a guinea pig model were able to quantify hair cell loss for a range of antibiotics, antifungals and antiseptics. The hair cell loss was assessed after 10 days of test compound administration by light microscopy and divided into minor, moderate and major hair cell loss. No electrophysiologic testing was completed. Results are shown in Table 9(p90).

A more recent study assessed the relative ototoxicity of common otic preparations by a method of direct exposure of cochlear outer hair cells harvested from chinchillas[21]. Isolated outer hair cells were then exposed to dilutions of test substance in isoosmolar conditions and recorded by inverted microscopy. Parameters measured were average time to cell death and average percentage change in cell length. Both parameters confirmed ototoxicity in increasing magnitude with Tobradex® (0.3% tobramycin + 0.1% dexamethasone), Cipro HC® (0.2% ciprofloxacin + 1% hydrocortisone), Gentacidin® (0.3% gentamicin), Ciloxin® (0.2%ciprofloxacin) and Floxin® (0.2%ofloxacin) being least toxic, and Cortisporin® (0.35% neomycin + polymyxin B + 1% hydrocortisone), VoSol® (2%acetic acid + 3% propylene glycol) and Acetasol HC (2% acetic acid + 1% hydrocortisone) being significantly more toxic[21]. All substances tested caused destruction of cells in such an isolated environment. Any

protection afforded to the cochlea was removed in this study, and hence isolated cytotoxicity was observed. It would appear that the effects of acid media are significantly greater on isolated outer hair cells than in anatomically intact animal model testing. It would be worth recalling that exposure of hair cells to sterile water resulted in immediate cell death[74].

The ototoxic effect of aluminium acetate solution in a primate model, would be the next logical step in assessment of the ototoxic potential of aluminium acetate solution.

Vestibolutoxicty of ototopical preparations have only recently been studied in animal models comparing auditory and vestibular evoked potentials using a fat sand rat model[101,102]. Using normal saline as a control, 0.3% gentamicin and 0.5% chlorhexidine, abolished both vestibular and auditory evoked potentials in all animals, whereas 70% ethyl alcohol had effects in only some animals and 10% povidone-iodine did not appear to affect vestibular evoked potentials[102].

There is mounting evidence that both vestibulotoxicity and cochleotoxicity can occur in patients treated with antibiotic containing and especially aminoglycoside based topical otic preparations, especially when used for prolonged periods[52,103-106]. However the opinion is that despite widespread use of ototopical preparations, ototoxic sensorineural hearing loss is rarely induced[107].

The vestibulotoxic effects of intratympanic gentamicin are well documented for the treatment of the incapacitating vertigo associated with Menière's disease[52]. The doses however tend to be somewhat higher for chemical ablation (24mg/ml) versus that

found in ototopical preparations[52]. Despite this ototopical drops have been used for chemical ablations in Menière's disease[108].

To date no reports of ototoxicity from topical antiseptics have appeared in the published literature.

7. Conclusions

A number of conclusions can be drawn from these studies.

1. Acetic acid and Burow's solution are effective in inhibiting (bacteriocidal) *in vitro* growth of the bacterial pathogens commonly associated with chronic suppurative otitis media.
2. Dilutions of aluminium acetate solution as low as 1.3% remain effective *in vitro* against bacterial pathogens commonly associated with chronic suppurative otitis media.
3. Other clinical studies using antibiotic containing ear drops have shown response rates similar to this study using either full strength (13% aluminium acetate solution) or quarter strength (3.25%aluminium acetate solution) Burow's solution. This would appear to indicate that such preparations could be a useful alternative. Ototoxicity is always of concern when using topical preparations to treat chronic suppurative otitis media, but of the clinical trial participants there were no reports or audiological evidence of ototoxicity.
4. Quarter strength Burow's solution (3.25% aluminium acetate solution) has been shown to be ototoxic to the chinchilla. With regard to extrapolation of this finding to the human ear, the drainage of the middle ear of the chinchilla is thought to be less effective than in humans, adding to the period of exposure to the round window membrane. The test solution is likely to have been in continuous contact with the round window membrane for the full 10 to 18 day period. Whether or not this means that the results of this animal study can be extrapolated to the human ear is uncertain. Further testing in larger primates would closer resemble the response of a human inner ear to aluminium acetate solution.

Until this is done use of this preparation in chronic suppurative otitis media cannot be recommended unless the same precautions are applied as with use of commercially available antibiotic containing preparations, especially those containing gentamicin.

If so used quarter strength Burow's solution (3.25% aluminium acetate solution) would appear to be a cheap and effective alternative to antibiotic containing ear drops in the management of chronic suppurative otitis media. Of these antibiotic containing ear drops, those containing quinolones are currently believed to have the least ototoxic potential, which in many countries remain prohibitively expensive.

8. References

1. Browning GG. Medical management of chronic mucosal otitis media. *Clinical Otolaryngology* 1984;9:141-44.
2. Acuin J, Smith A, Mackenzie I. Interventions in chronic suppurative otitis media. In: *The Cochrane Library*. Oxford: Update Software; 2001.
3. Acuin J. Chronic suppurative otitis media. In: Clinical Evidence. London: BMJ Publishing Group; 2002. p. 458-65.
4. Meyerhoff WL. Pathology of chronic suppurative otitis media. *Annals of Otology, Rhinology and Laryngology* 1988;97 Suppl131:20-24.
5. Schucknecht HF. Pathology of the Ear. Cambridge, Mass.: Harvard University Press; 1974.
6. Mills RP. Management of chronic suppurative oitis media. In: Scott-Brown's Otolaryngology. Booth JB, editor 6 th ed. Oxford: Butterworth-Heinemann; 1997. p. 3/10/1-11.
7. Chole RA, Choo MJ. Chronic Otitis Media without Cholesteatoma. In: Otolaryngology - Head and Neck Surgery. Cummings CW, Frederickson JM, Harker LA, editors 3 rd ed. Philadelphia: Mosby-Year Book; 1997. p. 3034-46.
8. Browning GG. The unsafeness of safe ears. *Journal of Laryngology and Otology* 1984;98:23-26.
9. Proctor B. The development of the middle ear spacesand their surgical significance. *Journal of Laryngology and Otology* 1964;78:631-2.

10. Nilssen ELK, Wormald PJ, Oliver S. Glycerol and ichthammol:medicinal solution or mythical potion? *Journal of Laryngology and Otology* 1996;110:319-21.

11. Sweeney G, Picozzi GI, Browning GG. A quantitative study of aerobic and anaerobic bacteriain chronic suppurative otitis media. *Journal of Infection* 1982;5:47-55.

12. Thorp MA, Kruger J, Oliver S, *et al*. The antibacterial activity of acetic acid and Burow's solution as topical otological preparations. *Journal of Laryngology and Otology* 1998;112:925-28.

13. Yuen AP, Chau PY, Wei WI. Bacteriology of chronic suppurative otitis media: ofloxacin susceptibility. *Journal of Otolaryngology* 1995;24:206-08.

14. Morizono T. Toxicity of ototopical drugs: animal modeling. *Annals of Otology, Rhinology and Laryngology* 1990;Supplement 148:42-5.

15. Erkan M, Aslan T, Swuk E, *et al*. Bacteriology of chronic suppurative otitis media. *Annals of Otology, Rhinology and Laryngology* 1994;103:771-74.

16. Thorp MA, Gardiner IB, Prescott CAJ. Burow's solution in the treatment of active mucosal chronic suppurative otitis media: determining an effective dilution. *Journal of Laryngology and Otology* 2000;114:432-36.

17. Browning GG, Picozzi GL, Calder IT, *et al*. Controlled trial of medical treatment of active chronic otitis media. *British Medical Journal* 1983;287:1024.

18. Browning GG. Is there an evidence base for the practice of ENT surgery? *Clinical Otolaryngology* 1998;23:1-2.

19. Eason R, Harding E, Nicholson R, *et al.* Chronic suppurative otitis media in the Solomon Islands: a prospective, microbiological, audiometric and therapeutic survey. *New Zealand Medical Journal* 1986;99:812-15.

20. Smith AW, Hatcher J, MacKensie IJ, *et al.* Randomised controlled trial of treatment of chronic suppurative otitis media in Kenyan schoolchildren. *Lancet* 1996;348:1128-33.

21. Jinn TH, Kim PD, Russell PT, *et al.* Determination of Ototoxicity of Common Otic Drops Using Isolated Cochlear Hair Cells. *Laryngoscope* 2001;111:2105-08.

22. Fradis M, Brodsky A, Ben-David J, *et al.* Chronic otitis media treated topically with ciprofloxacin or tobramycin. *Archives of Otolaryngology - Head and Neck Surgery* 1997;123:1057-60.

23. Kasemsuwan L, Clongsuesuek P. A double-blind, randomised prospective trial of topical ciprofloxacin versus normal saline solution in the treatment of otorrhoea. *Clinical Otolaryngology* 1997;22:44-46.

24. Llorente J, Sabater F, Maristany M, *et al.* Multicenter comparative study of the effectiveness and toleranceof topical ciprofloxacine (0.3%) versus topical gentamicine (0.3%) in the treatment of chronic suppurative otitis media without cholesteatoma. *An Otorrinolaringol Ibero Am* 1995;5.

25. Miro N, Perello E, Casamitjana F, *et al.* Controlled Multicenter study on chronic suppurative otitis media treated with topical applications of ciprofloxacin 0.2% solution in single dose containers or combination of polymyxin B, neomycin, and hydrocortisone suspension. *Otolaryngology Head and Neck Surgery* 2000;23:617-23.

26. Tutkun A, Ozagar A, Koc A, *et al*. Treatment of chronic ear disease - Topical ciprofloxacin versus topical gentamicin. *Archives of Otolaryngology - Head and Neck Surgery* 1995;121:1414-16.

27. Tong MC, Woo JK, van Hasselt CA. A double-blind comparative study of ofloxacin ear drops versus neomycin-polymixin B-hydrocortisone otic drops in the medical management of chronic suppurative media. *Journal of Laryngology and Otology* 1996;110:309-14.

28. Gyde MC. When the weeping stopped: an otologist views otorrhea and gentamicin. *Archives of Otolaryngology* 1976;102:542-6.

29. Gyde MC. A double-blind comparative study of trimethoprim-polymixin B versus trimethoprim-sulfacetamide-polymixin B otic solutions in the treatment of otorrhoea. *Journal of Laryngology and Otology* 1981;95:251-59.

30. Gyde MC, Norris D, Kavalec EC. The weeping ear: clinical re-evaluation of treatment. *Journal of International Medical Research* 1982;10:333-34.

31. Browning GG, Gatehouse S, Calder IT. Medical management of active chronic otitis media: A controlled study. *Journal of Laryngology and Otology* 1988;102:491-95.

32. Picozzi G, Browning GG, Calder I. Controlled trial of gentamicin and hydrocortisone ear drops in the treatment of active otitis media. *Clinical Otolaryngology* 1983;8:367-68.

33. Crowther J, Simpson D. Medical treatment of chronis otitis media: steroid or antibiotic with steroid eardrops. *Clinical Otolaryngology* 1991;16:142-44.

34. Connolly AP, Picozzi GL, Browning GG. Randomized trial of neomycin/dexamethasone spray vs drop preparation for the treatment of active chronic mucosal otitis media. *Clinical Otolaryngology* 1997;22:529-31.

35. Aminifahrsidmehr N. The management of chronic suppurative otitis media with acid media solution. *American Journal of Otology* 1996;17:24-25.

36. Mahoney JL. Mass Management of Otitis Media in Zaire. *Laryngoscope* 1980;90:1200-08.

37. Terayama Y, Takizawa M, Gotouda H, *et al.* Effects of Burow's Solution as an Ear Drop on Intractable Chronic Suppurative Diseases of the External Ear Canal and Middle Ear. *J Otolaryngol Jap* 2003;106:28-33.

38. Lambert I. A comparison of the treatment of otitis externa with 'Otosporin' and aluminium acetate. *Journal of the Royal College of General Practitioners* 1981;31:291-94.

39. Clayton M, Osborne J, Rutherford D, *et al.* A double-blind, randomized, prospective trial of a topical antiseptic versus a topical antibiotic in the treatment of otorrhoea. *Clinical Otolaryngology* 1990;15:7-10.

40. Smathers CR. Chemical treatment of External Otitis. *South Med J* 1977;70:543-45.

41. Esposito S, D'Errico G, Montanaro C. Topical and oral treatment of chronic otitis media with ciprofloxacin. *Archives of Otolaryngology - Head and Neck Surgery* 1990;116:557-59.

42. Esposito S, Noviello S, D'Errico G, *et al.* Topical ciprofloxacin versus intramuscular gentamicin for chronic otitis media. *Archives of Otolaryngology - Head and Neck Surgery* 1992;118:842-44.

43. Yuen P, Lau S, Chau P, *et al.* Ofloxacin eardrop treatment ifor active chronic suppurative otitis media: prospective randomised study. *American Journal of Otology* 1994;15:670-73.

44. Picozzi G, Browning GG, Calder I. Controlled trial of gentamicin and hydrocortisone ear drops with or without systemic metronidazole in the treatment of active otitis media. *Clinical Otolaryngology* 1984;9:305.

45. Maran AG, Molony NC, Armstrong MW, *et al.* Is there an evidence base for the practice of ENT surgery? *Clinical Otolaryngology* 1997;22:152-57.

46. Coletti V, Fiorino FG, Indelicato T. Surgery vs natural course of chronic otitis media. Long term hearing evaluation. *Acta Otolaryngologica* 1991;111:762-68.

47. Lewis N. Otitis media and linguistic incompetence. *Achives of Otolaryngology* 1976;102:387-90.

48. Dumich PS, Harner SG. Cochlear function in chroinc otitis media. *Laryngoscope* 1983;93:583-86.

49. English GM. Chronic otitis media as a cause of sensorineural hearing loss. *Archives of Otolaryngology* 1973;98:18-22.

50. Paparella MM, Morizono T, Le CT, *et al.* Sensorineural hearing loss in otitis media. *Annals of Otology, Rhinology and Laryngology* 1984;93:623-29.

51. MacAndie CM, O'Reilly BF. Sensorineural hearing loss in chronic otitis media. *Clinical Otolaryngology* 1999;24:220-22.

52. Marais J, Rutka JA. Ototoxicity and topical eardrops. *Clinical Otolaryngology* 1998;23:360-7.

53. Roland PS. Clinical ototoxicity of topical antibiotic drops. *Otolaryngology Head and Neck Surgery* 1994;110:598-602.

54. Scott PM, Griffiths MV. A clinical review of ototoxicity. *Clinical Otolaryngology* 1994;19:3-8.

55. Wright CG, Meyerhoff WL. Ototopical agents: efficacy or toxicity in humans. *Annals of Otology, Rhinology and Laryngology* 1988;Supplement 131:30-32.

56. Goldwyn RM. Carl August Burow. *Plastc and Reconstructive Surgery* 1984;73:687-90.

57. Dollery S. Therapeutic Drugs. London: Churchill Livingstone; 1991. p. A62-A63.

58. British Pharmacopoeia. London: HMSO; 1993. p. 765.

59. Martindale W. The Extra Pharmacopoeia. London: Pharmaceutical Press; 1990. p. 777.

60. Leyden JJ, Kligman AM. Aluminium chloride in the treatment of symptomatic athlete's foot. *Archives of Dermatology* 1975;111:1004-10.

61. Politzer. A textbook of the Diseases of the Ear for students and practitioners. Philadelphia: Lea Brothers & Co.; 1903. p. 844.

62. British Pharmaceutical Codex. London: The Pharmaceutical Press; 1959. p. 1091.

63. Jones EH. External Otitis. Diagnosis and Treatment. Springfield, Illinois: Charles C Thomas; 1965. p. 27-37, 160.

64. Health SADo. Guideline for the prevention of hearing impairment due to otitis media at clinic level. In.

65. Lundy LB, Graham MD. Ototoxicity and ototopical medications: a survey of otolaryngologists. *American Journal Otology* 1993;14:141-6.

66. Moher D, Schultz KF, Altman DG. Revised recommendations for improving the quality of reports of parallel group randomized trials 2001. In; 2001.

67. Jones EH, McLain PG. Does acid pH inhibit bacterial growth in the external ear canal. *Laryngoscope* 1961;71:928-36.

68. Stenfors LE, Raisanen S. Quantitative analysis of the bacterial findings in otitis media. *Journal of Laryngology and Otology* 1990;104:749-57.

69. Kenna MA, Bluestone CD, Reilly JS, *et al.* Medical management of chronic suppurative otitis media without cholesteatoma in children. *Laryngoscope* 1986;96:146-51.

70. Dohar JE, Alper CN, Rose EA, *et al.* Treatment of chronic suppurative otitis media with topical ciprofloxacin. *Annals of Otology, Rhinology and Laryngology* 1998;107.

71. Lancaster JL, Makura ZG, Porter G, *et al.* Topical aminoglycosides in the management of active mucosal chronic suppurative otitis media. *Journal of Laryngology and Otology* 1999;113:10-12.

72. Wright A, Forge A, Kotecha B. Ototoxicity. In: Scott Brownes Textbook of Otolaryngology. p. 20/1-20/36.

73. Chen JM, Kagiki A, Hirakawa H, *et al.* Middle ear instillation of gentamicin and streptomicin in chinchillas: morphologic appraisal of selective ototoxicity. *Journal of Otolaryngology* 1999;28:121-28.

74. Harrison RV, Mount RJ, Hirakawa H. Total ablation of cochlear haircells by perilymphatic perfusion with water. *Hearing Research* 1997;110:229-33.

75. Vernon J, Brummett R, Walsh T. The ototoxic potential of propylene glycol in guinea pigs. *Archives of Otolaryngology* 1978;104:726-29.

76. Morizono T, Paparella MM, Juhn SK. Ototoxicity of propylene glycol in experimental animals. *American Journal of Otolaryngology* 1980;1:393-99.

77. Morizono T, Sikora MA. Ototoxicity of ethanol in the tympanic cleft in animals. *Acta Otolaryngologica* 1981;92:33-40.

78. Morizono T, Sikora MA. The ototoxicity of topically applied povidone-iodine preparations. *Archives of Otolaryngology* 1982;108:210-3.

79. Ikeda K, Morizono T. The preparation of acetic acid for use in otic drops and its effect on endocochlear potential and pH in inner ear fluid. *American Journal of Otolaryngology* 1989;10:382-5.

80. Parker FL, James GW. The effect of various topical antibiotic and antibacterial agents on the middle and inner ear of the guinea-pig. *Journal of Pharmacy and Pharmacology* 1978;30:236-9.

81. Ikeda K, Morizono T. Effect of ototopic application of a corticosteroid preparation on cochlear function. *American Journal of Otolaryngology* 1991;12:150-3.

82. Ikeda K, Morizono T. Changes of the permeability of round window membrane in otitis media. *Archives of Otolaryngology - Head and Neck Surgery* 1988;114:895-97.

83. Tom LW. Ototoxicity of common topical antimycotic preparations. *Laryngoscope* 2000;110:509-16.

84. Stupp H, Kupper K, Lagler F, *et al.* Inner ear concentrations and ototoxicity of different antibiotics in local and systemic application. *Audiology* 1973;12:350-63.

85. Jakob T, Wright CG, Robinson K, *et al.* Ototoxicity of topical ticarcillin and clavulanic acid in the chinchilla. *Archives of Otolaryngology - Head and Neck Surgery* 1995;121:39-43.

86. Brown OE, Wright CG, Edwards LB, *et al.* The ototoxicity of ceftazidime in the chinchilla middle ear. *Archives of Otolaryngology - Head and Neck Surgery* 1989;115:940-2.

87. Ikeda K, Morizono T. Round window membrane permeability during experimental purulent otitis media: altered Cortisporin ototoxicity. *Annals of Otology, Rhinology and Laryngology* 1990;99:46-48.

88. Wright CG, Meyerhoff WL. Ototoxicity of otic drops applied to the middle ear in the chinchilla. *American Journal of Otolaryngology* 1984;5:166-76.

89. Wright CG, Halama AR, Meyerhoff WL. Ototoxicity of an ototopical preparation in a primate. *American Journal of Otology* 1987;8:56-60.

90. Russell PT, Church CA, Jinn TH, *et al.* Effects of common otic preparations on the morphology of isolated cochlear outer hair cells. *Acta Otolaryngologica* 2001;121:135-39.

91. Barlow DW, Duckert LG, Kreig CS, *et al.* Ototoxicity of topical otomicrobial agents. *Acta Otolaryngologica* 1995;115:231-5.

92. Brownlee RE, Hulka GF, Prazma J, *et al.* Ciprofloxacin. Use as a topical otic preparation. *Archives of Otolaryngology - Head and Neck Surgery* 1992;118:392-6.

93. Meyerhoff WL, Morizono T, Wright CG, *et al.* Tympanostomy tubes and Otic drops. *Laryngoscope* 1983;93:1022-27.

94. Schachern PA, Paparella MM, Duval AJ, *et al.* The human round window membrane. *Archives of Otolaryngology* 1984;110:15-21.

95. Sahni RS, Paparella MM, Schachern PA, *et al.* Thickness of the human round window membrane in different forms of otitis media. *Archives of Otolaryngology - Head and Neck Surgery* 1987;113:630-34.

96. Schachern PA, Paparella MM, Goycoolea MV, *et al.* The permeability of the round window membrane during otitis media. *Archives of Otolaryngology - Head and Neck Surgery* 1987;113:625-29.

97. Goycoolea MV, Paparella MM, Goldberg B, *et al.* Permeability of the round window membrane in otitis media. *Archives of Otolaryngology* 1980;106:430-33.

98. Goycoolea MV, Paparella MM, Juhn SK, *et al.* Oval and round window changes in otitis media. Potential pathways between middle and inner ear. *Laryngoscope* 1980;90:1387-91.

99. Browning GG, Granich MS. Surgical anatomy of the temporal bone in the chinchilla. *Annals of Otology* 1978;87:875-82.

100. Kaplan DM, James AL, Thorp MA, *et al.* Ototoxicity of Cipro HC Otic Suspension in the chinchilla. *in press*.

101. Sichel JY, Eliashar R, Plotnik M, *et al.* Assessment of vestibular ototoxicity of ear drops by recording of vestibular evoked potentials to acceleration impulses. *American Journal of Otology* 2000;21:192-5.

102. Perez R, Freeman S, Sohmer H, *et al.* Vestibular and Cochlear Ototoxicity of Topical Antiseptics Assessed by Evoked Potentials. *Laryngoscope* 2000;110:1522-27.

103. Bath AP, Walsh RM, Bance ML, *et al.* Ototoxicity of topical gentamicin preparations. *Laryngoscope* 1999;109:1088-93.

104. Hui Y, Park A, Crysdale WS, *et al.* Ototoxicity from ototopical aminoglycosides [see comments]. *Journal of Otolaryngology* 1997;26:53-6.

105. Longridge NS. Topical gentamicin vestibular toxicity. *Journal of Otolaryngology* 1994;23:444-46.

106. Tommerkup B, Moller K. A case of profound hearing impairment following the primary use of framycetin eardrops. *Journal of Laryngology and Otology* 1984;98:1135-37.

107. Linder TE, Zwicky S, Brandle P. Ototoxicity of ear drops: a clinical perspective. *American Journal of Otology* 1995;16:653-7.

108. Kaplan DM, Hehar SS, Bance ML, *et al*. Intentional ablation of vestibular function using commercially available topical gentamicin-beatmethasone eardropsin patients with Meniere's disease: further evidence of topical eardrop ototoxicity. *Laryngoscope* 2002;112:689-95.