Population pharmacokinetic modelling for dose optimization of esomeprazole to treat early-onset preeclampsia

by

MANNA SEMERE GEBREYESUS

SMRMAN003

SUBMITTED TO THE UNIVERSITY OF CAPE TOWN

In partial fulfilment of the requirements for the degree

MPhil (Clinical Pharmacology)

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

1 DECLARATION

I, MANNA SEMERE GEBREYESUS, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. This work has not been reported or published prior to registration for this degree. This thesis has been submitted to the Turnitin module.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

I have used the Harvard referencing style for citations.

Signature: Signed by candidate

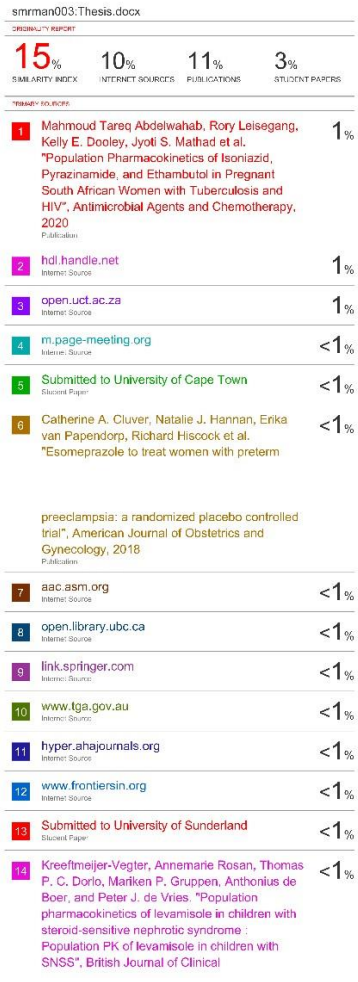
Date: 13/12/2020

2 TURNITIN REPORT

smrman003:Thesis.docx

ORIGINALITY REPORT

15%	10%	11%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS



smrman003:Thesis.docx

ORIGINALITY REPORT

15%	10%	11%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Mahmoud Tareq Abdelwahab, Rory Leisegang, Kelly E. Dooley, Jyoti S. Mathad et al. "Population Pharmacokinetics of Isoniazid, Pyrazinamide, and Ethambutol in Pregnant South African Women with Tuberculosis and HIV", Antimicrobial Agents and Chemotherapy, 2020 Publication	1%
2	hdl.handle.net Internet Source	1%
3	open.uct.ac.za Internet Source	1%
4	m.page-meeting.org Internet Source	<1%
5	Submitted to University of Cape Town Student Paper	<1%
6	Catherine A. Cluver, Natalie J. Hannan, Erika van Papendorp, Richard Hiscock et al. "Esomeprazole to treat women with preterm preeclampsia: a randomized placebo controlled trial", American Journal of Obstetrics and Gynecology, 2018 Publication	<1%
7	aac.asm.org Internet Source	<1%
8	open.library.ubc.ca Internet Source	<1%
9	link.springer.com Internet Source	<1%
10	www.tga.gov.au Internet Source	<1%
11	hyper.ahajournals.org Internet Source	<1%
12	www.frontiersin.org Internet Source	<1%
13	Submitted to University of Sunderland Student Paper	<1%
14	Kreeftmeijer-Vegter, Annemarie Rosan, Thomas P. C. Dorlo, Mariken P. Gruppen, Anthonius de Boer, and Peter J. de Vries. "Population pharmacokinetics of levamisole in children with steroid-sensitive nephrotic syndrome : Population PK of levamisole in children with SNSS", British Journal of Clinical Pharmacology, 2015. Publication	<1%
15	www.clinicaltrials.gov Internet Source	<1%
16	Catherine A. Cluver, Natalie J. Hannan, Erika van Papendorp, Richard Hiscock et al. "Esomeprazole to treat women with preterm preeclampsia: a randomised placebo controlled trial", American Journal of Obstetrics and Gynecology, 2018 Publication	<1%
17	worldwidescience.org Internet Source	<1%
18	"Neonatology", Springer Science and Business Media LLC, 2018 Publication	<1%
19	Gontijo, A. V. L., J. Brillault, N. Gregoire, I. Lamarche, P. Gobin, W. Couet, and S. Marchand. "Biopharmaceutical Characterization of Nebulized Antimicrobial Agents in Rats. 1. Ciprofloxacin, Moxifloxacin and Grepafloxacin", Antimicrobial Agents and Chemotherapy, 2014. Publication	<1%
20	www.tandfonline.com Internet Source	<1%
21	d-nb.info Internet Source	<1%
22	repub.eur.nl Internet Source	<1%
23	Maged M. Costantine, Cande V. Ananth. "The early developments of preeclampsia drugs", Expert Opinion on Investigational Drugs, 2016 Publication	<1%
24	"1st Annual Conference of Midwifery", Walter de Gruyter GmbH, 2020 Publication	<1%
25	"Poster Session Abstracts", Hypertension in Pregnancy, 11/2008 Publication	<1%
26	Submitted to University College London Student Paper	<1%
27	Submitted to Maryville University Student Paper	<1%
28	Zhang, Chao, Paolo Denti, Eric H Decloedt, Yuan Ren, Mats O Karlsson, and Helen McIlleron. "Model-based evaluation of the pharmacokinetic differences between adults and children for lopinavir and ritonavir in combination with rifampicin : Differences between adults and children for lopinavir and ritonavir", British Journal of Clinical Pharmacology, 2013. Publication	<1%
29	Caroline M den Hoed, Ernst J Kuipers. "Esomeprazole for the treatment of peptic ulcer bleeding", Expert Review of Gastroenterology & Hepatology, 2014 Publication	<1%
30	www.dovepress.com Internet Source	<1%
31	mafiadoc.com Internet Source	<1%
32	Elaine Y. L. Blair. "Population pharmacokinetics of raltitrexed in patients with advanced solid tumours", British Journal of Clinical Pharmacology, 4/2004 Publication	<1%
33	Alexander H.C. Chun, Keith Erdman, Yiming Zhang, Ramanuj Achari, John H. Cavanaugh. "Effect on bioavailability of admixing the contents of lansoprazole capsules with selected soft foods", Clinical Therapeutics, 2000 Publication	<1%
34	Johann F. Coetzee. "A Review of Analgesic Compounds Used in Food Animals in the United States", Veterinary Clinics of North America: Food Animal Practice, 2013 Publication	<1%
35	Sven Cnattingius. "Prepregnancy Weight and the Risk of Adverse Pregnancy Outcomes", New England Journal of Medicine, 01/15/1998 Publication	<1%
36	R. Gentry Wilkerson, Adeolu C. Ogunbodede. "Hypertensive Disorders of Pregnancy", Emergency Medicine Clinics of North America, 2019 Publication	<1%
37	Submitted to Manchester Metropolitan University Student Paper	<1%
38	Lien Ngo, Hea-Young Cho, Yong-Bok Lee. "Effects of hydrochlorothiazide and amlodipine on single oral dose pharmacokinetics of valsartan in healthy Korean subjects: Population model-based approach", European Journal of Pharmaceutical Sciences, 2018 Publication	<1%
39	E. Niclas Jonsson, Saila Antila, Lynn McFadyen, Lasse Lehtonen, Mats O. Karlsson. "Population pharmacokinetics of levosimendan in patients with congestive heart failure", British Journal of Clinical Pharmacology, 2003 Publication	<1%
40	Bart van Pinxteren. "Short-term treatment with proton pump inhibitors, H2-receptor antagonists	<1%

and prokinetics for gastro-oesophageal reflux disease-like symptoms and endoscopy negative reflux disease.", Cochrane Database of Systematic Reviews Reviews, 07/19/2006
Publication

41 Gloria Ortiz-Guerrero, Diana Amador-Muñoz, Carlos Alberto Calderón-Ospina, Daniel López-Fuentes, Mauricio Orlando Nava Mesa. "Proton Pump Inhibitors and Dementia: Physiopathological Mechanisms and Clinical Consequences", Neural Plasticity, 2018 <1%
Publication

42 X.-J. Shi. "Association of ABCB1, CYP3A4*18B and CYP3A5*3 genotypes with the pharmacokinetics of tacrolimus in healthy Chinese subjects: a population pharmacokinetic analysis : Association of ABCB1, CYP3A4*18B and CYP3A5*3 genotypes with the PK of tacrolimus", Journal of Clinical Pharmacy and Therapeutics, 10/2011 <1%
Publication

43 Submitted to Kyungpook National University <1%
Student Paper

44 Johnson W. McRorie, Roger D. Gibb, Philip B. Miner. "Evidence-based treatment of frequent heartburn: The benefits and limitations of over-the-counter medications", Journal of the American Association of Nurse Practitioners, 2014 <1%
Publication

45 Submitted to University of Birmingham <1%
Student Paper

46 Submitted to Kwame Nkrumah University of Science and Technology <1%
Student Paper

47 eprints.uniss.it <1%
Internet Source

48 Yoshiaki Shirai, Satoko Ueno, Akira Nakayama, Kiyoko Ikeuchi, Kazuyuki Ubukata, Ryuichi Mihara, Bruce K. Bernard. "Studies of the Toxicological Potential of Capsinoids, XII: Pharmacokinetic Study of Capsinoid-Containing CH-19 Sweet Extract in Rats", International Journal of Toxicology, 2010 <1%
Publication

49 lib.bioinfo.pl <1%
Internet Source

50 www.go-acop.org <1%
Internet Source

51 www.hindawi.com <1%
Internet Source

52 ZHE TANG, Jing Guan, Jingjing Li, Yanxia Yu, Miao Qian, Jing Cao, Weiwei Shuai, Zheng Jiao. "Determination of vancomycin exposure target and individualized dosing recommendations for neonates: model informed precision dosing", Cold Spring Harbor Laboratory, 2020 <1%
Publication

53 med.stanford.edu <1%
Internet Source

54 Natasha de Alwis, Natalie K. Binder, Sally Beard, Tu'uhevaha J. Kaitu'u-Lino, Stephen Tong, Fiona Brownfoot, Natalie J. Hannan. "Novel approaches to combat preeclampsia: from new drugs to innovative delivery", Placenta, 2020 <1%
Publication

55 Boulanger, H.. "Avancees recentes dans la comprehension de la physiopathologie de la preeclampsie et consequences therapeutiques potentielles", Nephrologie & Therapeutique, 200712 <1%
Publication

56 www.page-meeting.org <1%
Internet Source

57 Moherndran Archary, Helen McIlleron, Raziya Bobat, Phillip La Russa, Thobekile Sibaya, Lubbe Wiesner, Stefanie Hennig. "Population Pharmacokinetics of Lopinavir in Severely Malnourished HIV-infected Children and the Effect on Treatment Outcomes", The Pediatric Infectious Disease Journal, 2018 <1%
Publication

58 patents.justia.com <1%
Internet Source

59 Zahra Shahraki, Zohre Sadat Hashemi Bonjar, Forough Forghani, Rezvane Nakhai. "Comparing Neonatal Outcome Following the Use of Ondansetron versus Vitamin B6 in Pregnant Females with Morning Sickness: A Randomized Clinical Trial", Journal of Comprehensive Pediatrics, 2016 <1%
Publication

60 Michael H. Skinner. "Effect of age on the pharmacokinetics of duloxetine in women", British Journal of Clinical Pharmacology, 1/2004 <1%
Publication

61 www.jourlib.org <1%
Internet Source

62 Salahuddin, S.. "Diagnostic utility of soluble fms-like tyrosine kinase 1 and soluble endoglin in hypertensive diseases of pregnancy", American Journal of Obstetrics and Gynecology, 200707 <1%
Publication

63 "Handbook of Nutrition and Pregnancy", Springer Science and Business Media LLC, 2018 <1%
Publication

64 bpspubs.pericles-prod.literatumonline.com <1%
Internet Source

65 Haini Wen, Yixi Liu, Da Xu, Kaijing Zhao, Zheng Jiao. "Population pharmacokinetic modeling of pyrotinib in patients with HER2-positive advanced or metastatic breast cancer", Cold Spring Harbor Laboratory, 2020 <1%
Publication

66 Yewei Chen, Dan Wu, Min Dong, Yiqing Zhu, Jinmiao Lu, Xiaoxia Li, Chao Chen, Zhiping Li. "Population pharmacokinetics of vancomycin and AUC-guided dosing in Chinese neonates and young infants", European Journal of Clinical Pharmacology, 2018 <1%
Publication

67 Bhongsatiern, Jiraganya (JJ), Chris Stockmann, Jessica K. Roberts, Tian Yu, Kent E. Korgenski, Michael G. Spigarelli, Pankaj B. Desai, and Catherine M.T. Sherwin. "Evaluation of vancomycin use in late-onset neonatal sepsis using the AUC24/MIC ≥400 target :", Therapeutic Drug Monitoring, 2015. <1%
Publication

68 ascopubs.org <1%
Internet Source

69 Kirstin Thelen. "Cytochrome P450-mediated metabolism in the human gut wall", Journal of Pharmacy and Pharmacology, 05/2009 <1%
Publication

70 www.endotext.org <1%
Internet Source

71 "Preeclampsia and Angiogenic Imbalance", Annual Review of Medicine, 02/2008 <1%
Publication

72 Hakan Erenel, Nevin Yilmaz, Tayfur Cift, Berk Bulut et al. "Maternal serum autotaxin levels in early- and late-onset preeclampsia", Hypertension in Pregnancy, 2017 <1%
Publication

73 en.wikipedia.org <1%
Internet Source

74 Yali Xiong. "Gadd45a stress signaling regulates sFlt-1 expression in preeclampsia", Journal of Cellular Physiology, 2009 <1%
Publication

75 d-scholarship.pitt.edu <1%
Internet Source

76 pdf.easechem.com <1%
Internet Source

77 scholarworks.gsu.edu <1%
Internet Source

78 Bueters, Tjerk, Christopher Gibson, and Sandra A G Visser. "Optimization of human dose prediction by using quantitative and translational pharmacology in drug discovery", Future Medicinal Chemistry, 2015. <1%
Publication

79 Chao Zhang. "Model-based approach to dose optimization of lopinavir/ritonavir when co-administered with rifampicin : Model of lopinavir/ritonavir with rifampicin", British Journal of Clinical Pharmacology, 05/2012 <1%
Publication

80 D. Hirt, C. Bardin, S. Diagbouga, B. Nacro et al. "Didanosine Population Pharmacokinetics in West African Human Immunodeficiency Virus-Infected Children Administered Once-Daily Tablets in Relation to Efficacy after One Year of Treatment", Antimicrobial Agents and Chemotherapy, 2009 <1%
Publication

81 Cheryl S. W. Li, Kevin Sweeney, Carol Cronenberger. "Population pharmacokinetic modeling of PF-06439535 (a bevacizumab biosimilar) and reference bevacizumab (Avastin®) in patients with advanced non-squamous non-small cell lung cancer", Cancer Chemotherapy and Pharmacology, 2019 <1%
Publication

82 preview-transplantationresearch.biomedcentral.com <1%
Internet Source

83 Eleni Karatza, Sophia L. Markantonis, Andria Savvidou, Anastasia Verentzioti et al. "Pharmacokinetic and pharmacodynamic modeling of levetiracetam: investigation of factors affecting the clinical outcome", Xenobiotica, 2020 <1%
Publication

84 journals.plos.org <1%
Internet Source

85 Hamutal Meiri, George Osol, Irene Cetin, Sveinbjörn Gizurarson, Berthold Huppertz. "Personalized therapy against preeclampsia by replenishing placental protein 13 (PP13) targeted to patients with impaired PP13 molecule or function", Computational and Structural Biotechnology Journal, 2017 <1%
Publication

86 Willem van den Brink, Annette Emerenciana, Francesco Bellanti, Oscar Della Pasqua, Jan Willem van der Laan. "Prediction of thyroid C-cell carcinogenicity after chronic administration of GLP1-R agonists in rodents", Toxicology and Applied Pharmacology, 2017 <1%
Publication

87 uu.diva-portal.org <1%
Internet Source

88 Petra C. van Rijn-Bikker, Oliver Ackaert, Nelleke Snelder, Reinier M. van Hest et al. "Pharmacokinetic–Pharmacodynamic Modeling of the Antihypertensive Effect of Eprosartan in Black and White Hypertensive Patients", Clinical Pharmacokinetics, 2013 <1%
Publication

89 www.umweltbundesamt.at <1%
Internet Source

90 Valérie F. Cosson, Vivian W. Ng, Michaela Lehle, Bert L. Lum. "Population pharmacokinetics and exposure–response analyses of trastuzumab in patients with advanced gastric or gastroesophageal junction cancer", Cancer Chemotherapy and Pharmacology, 2014 <1%
Publication

91 Wiczling, Paweł, Łukasz Kubik, and Roman Kaliszan. "Maximum a posteriori Bayesian estimation of chromatographic parameters by limited number of experiments", Analytical Chemistry <1%
Publication

92 www.essaysauce.com <1%
Internet Source

93 Tse, S. A., R. S. Atayee, J. D. Ma, and B. M. Best. "Factors Affecting Carisoprodol Metabolism in Pain Patients Using Urinary Excretion Data", Journal of Analytical Toxicology, 2014. <1%
Publication

94 Lars Olbe. "A proton-pump inhibitor expedition: the case histories of omeprazole and esomeprazole", Nature Reviews Drug Discovery, 02/2003 <1%
Publication

95 Amel A.F. El-Sayed. "Preeclampsia: A review of the pathogenesis and possible management strategies based on its pathophysiological derangements", Taiwanese Journal of Obstetrics and Gynecology, 2017 <1%
Publication

96 Raju Khatri, Natasha Kulick, Rebecca J.B. Rementer, John K. Fallon et al. "Pregnancy-Related Hormones Increase Nifedipine Metabolism in Human Hepatocytes by Inducing CYP3A4 Expression", Journal of Pharmaceutical Sciences, 2020 <1%
Publication

repositorio.unesp.br

97 <1%
Internet Source

98 Methods in Molecular Biology, 2011. <1%
Publication

99 Sowndramalingam Sankaralingam, Ivan A. Arenas, Manoj M. Lalu, Sandra T. Davidge. "Preeclampsia: current understanding of the molecular basis of vascular dysfunction", Expert Reviews in Molecular Medicine, 2006 <1%
Publication

100 www.nature.com <1%
Internet Source

101 medcraveonline.com <1%
Internet Source

102 "Cardiac Problems in Pregnancy, 4th Edition", Wiley, 2019 <1%
Publication

103 G Nergizoglu. "Circulating endothelial cells in preeclampsia", Journal of Human Hypertension, 04/12/2007 <1%
Publication

104 www.spandidos-publications.com <1%
Internet Source

105 perspectivesinmedicine.cshlp.org <1%
Internet Source

106 Regal, Jean F., Jeffrey S. Gilbert, and Richard M. Burwick. "The complement system and adverse pregnancy outcomes", Molecular Immunology, 2015. <1%
Publication

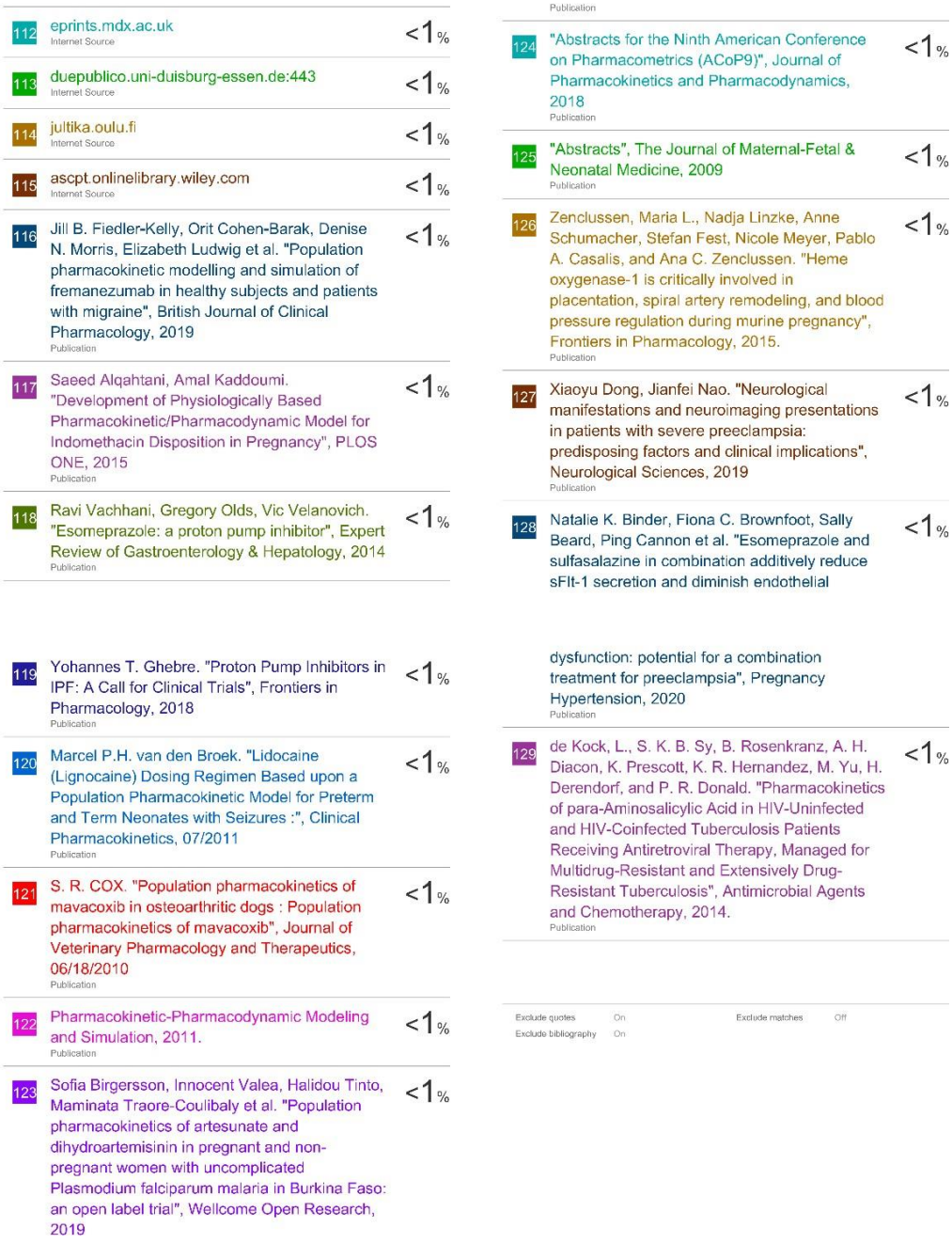
107 www.xfcjwkzazhi.cn <1%
Internet Source

108 www.cambridge.org <1%
Internet Source

109 cnc.cj.uc.pt <1%
Internet Source

110 Rajaa Aouache, Louise Biquard, Daniel Vaiman, Francisco Miralles. "Oxidative Stress in Preeclampsia and Placental Diseases", International Journal of Molecular Sciences, 2018 <1%
Publication

111 Mohamed Abdel-Rehim, Yvonne Askemark, Carina Norsten-Höög, Karl-Johan Pettersson, Magnus Halldin. "Quantification of 4-OH-2,6-Xylidine and its Conjugates in Human Urine Samples Utilising Microextraction in Packed Syringe On-line with Liquid Chromatography and Electrospray Tandem Mass Spectrometry (MEPS-LC-MS/MS)", Journal of Liquid Chromatography & Related Technologies, 2007 <1%
Publication



Supervisor signature

Signature Removed

Student signature

Signature Removed

3 ABSTRACT

Rationale

Esomeprazole is a proton pump inhibitor with preclinical efficacy data showing it lowers concentrations of soluble fms like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng), pathognomonic biomarkers identified in preeclampsia. A randomized controlled trial, Preeclampsia Intervention with Esomeprazole (PIE) trial, was conducted in South African women diagnosed with early-onset preeclampsia to investigate efficacy, but it found no change in clinical outcome or biomarker concentrations. It was hypothesized that the 40 mg daily oral dose was not enough to achieve therapeutic exposure. This study investigated the pharmacokinetics of esomeprazole in patients with early- onset preeclampsia with the aim to optimize the dose for future clinical trials.

Methods

Pharmacokinetic data from ten pregnant patients with early-onset preeclampsia from the PIE trial, median (range) age 30 (21-43) years, weight 98.8 (56-126) kg, and gestational age 29 (26- 31) weeks, were included for model development. In addition, pharmacokinetic data from non- pregnant healthy volunteers consisted of a pooled dataset of 26 male and female subjects, median (range) age of 21 (18-27) years and weight 69 (54-89) kg, who received 40 mg esomeprazole daily. Analysis of the pharmacokinetic data in pregnant patients was performed using nonlinear mixed-effects modelling with allometric scaling on clearance (CL) and volume of distribution (V_d). Metabolite to parent area under the time-concentration curve (AUC_{sulf}/AUC_{eso} and AUC_{hyd}/AUC_{eso}) ratios were compared between pregnant and non-pregnant to assess metabolic changes in pregnancy. Simulations were performed with the model to determine the nonlinear increase in AUC with higher doses and with repeated dosing in the pregnant patients. Simulation results were compared with the preclinical target unbound concentration (0.917 mg/L) and preclinical target unbound AUC_{0-24} (9.29 mg·h/L).

Results

A one compartment pharmacokinetic model with first-order elimination and transit compartment absorption best described the data. Model estimated apparent CL and apparent V_d (95% CI) were 19.2 (14.2-26) L/h and 44.2 (29.9-56.6) L, respectively. Median AUC_{sulf}/AUC_{eso} (IQR) for pregnant patients, 2.00 (1.35-2.61) , was significantly higher than that for non-pregnant subjects on day1, 0.700 (0.636-1.00) , and day5, 1.18 (0.981- 1.58) . Median AUC_{hyd}/AUC_{eso} (IQR) for pregnant

patients, 0.0543 (0.0500-0.0914) , was not significantly different from that of non-pregnant subjects on day5, 0.0777 (0.0569-0.108) but lower than that of non-pregnant subjects on day1, 0.188 (0.156-0.227). Simulation results showed that predicted steady state unbound C_{max} is between 0.0949 and 0.398 mg/L while the predicted unbound AUC_{0-24} in pregnant patients with the highest dose of esomeprazole used clinically, i.e.120 mg BID, is between 0.696 and 2.92 mg·h/L.

Discussion/Conclusion

Model estimated CL/F and V_d/F are higher than values previously reported by other population pharmacokinetic models. AUC_m/AUC_p comparisons showed that esomeprazole metabolism in pregnancy appears to have shifted to the CYP3A4 pathway. This means that the nonlinear AUC increase expected with dose escalation and with repeated dosing are not as significant as in non-pregnant. Simulations indicate that pregnant patients are unlikely to achieve the target concentration and exposure with the highest dose of esomeprazole registered. Further research is necessary to determine the target site of action of esomeprazole in preeclampsia, and the pharmacokinetic metric that correlates with efficacy.

4 ACKNOWLEDGEMENTS

I would like to foremost thank God for enabling me to pursue my master's studies.

I would like to thank A/Prof. Paolo Denti and Dr. Roeland Wasmann for patiently guiding me throughout this entire project. I very much appreciate the countless hours they have spent reviewing my work and teaching me from the beginning until the completion of this project.

I thank Dr. Cathy Cluver and Dr. Eric Decloedt, primary investigators of the team who conducted the Preeclampsia Intervention with Esomeprazole (PIE) clinical trial at the University of Stellenbosch, and Dr. Nicole Hunfeld, primary investigator of the research with the non-pregnant studies from the Netherlands, for giving me the opportunity to work on this project.

I would like to thank Dr. Lubbe Wiesner for his guidance and support during the entire duration of the MPhil program.

I am thankful to everyone at the Pharmacometrics Unit for selflessly helping me through various areas as I worked on this project.

Additionally, I would like to thank my funders UCT and NRF who took off the burden of expenses during this project.

I am also very thankful towards my husband who has been supportive throughout the entire MPhil program.

There are no conflicts of interest to declare.

TABLE OF CONTENTS

5 LIST OF TABLES

6 LIST OF FIGURES

7 ABBREVIATIONS

WHO	World Health Organization
BMI	Body Mass Index
HELLP	Hemolysis, elevated liver enzymes, and low platelet count
DIC	Disseminated intravascular coagulation
µg	Microgram
mL	milliliter
IUGR	Intrauterine growth restriction
GA	Gestational age
PIE	Preeclampsia intervention with esomeprazole
PPIs	Proton pump inhibitors
HIF-1	Hypoxia inducible factor 1
sEng	Soluble endoglin
sFlt-1	Soluble fms like tyrosine kinase 1
TGF-β	Transforming growth factor β
HO-1	Hemoxygenase-1
ET-1	Endothelin-1
eNOS	Endothelial nitric oxide synthase
NO	Nitric oxide
TNF-α	Tumor necrosis factor α
NCA	Noncompartmental analysis
AUC	Area under the curve
GOF	Goodness-of-fit
VPC	Visual Predictive Check
CWRES	Conditional weighted residuals
PRED	Population predictions
IPRED	Individual predictions
CL	Clearance

V_d	Volume of distribution
F	Bioavailability
LLOQ	Lower limit of quantification
BLQ	Below the limit of quantification
CI	Confidence interval

8 BACKGROUND

8.1 Preeclampsia

Preeclampsia is a disorder of pregnancy which is observed after 20 weeks of gestation and resolves within 6 weeks of delivery. The global incidence of preeclampsia is 3% to 8%, and it is estimated to cause 60,000 maternal and 500,000 foetal deaths annually (Marshall et al., 2019). The incidence of preeclampsia is seven times higher in developing countries and preeclampsia-related maternal mortality is also higher, 15% compared to 1.8% or less in developed countries (Ghulmiyyah and Sibai, 2012; Armaly et al., 2018). Maternal risk factors for preeclampsia include: age below 20 years or above 35 years; obesity; first or multiple pregnancies; family history of preeclampsia; preeclampsia in a previous pregnancy; ethnicity (black women are more at risk); history of metabolic, cardiovascular, or autoimmune diseases; assisted pregnancy (e.g. *in vitro* fertilization, artificial insemination), and interval between pregnancies of more than 10 years (English, Kenny and McCarthy, 2015).

8.2 Pathogenesis

In normal pregnancies during placental development (weeks 8 to 20), placental cells called cytotrophoblasts invade the uterine wall and migrate to the spiral arteries, vessels that line that uterus. Cytotrophoblasts differentiate as they migrate to mimic the structure and molecular signaling of vascular endothelial cells which enables them to remodel the endothelial lining and smooth muscle of maternal spiral arteries. As a result, the spiral arteries become low resistance vessels that can handle high volume blood flow to augment blood supply to the placenta and meet the increasing needs of the growing foetus (Figure 1). Proteins released by cytotrophoblasts to facilitate the remodeling process include antiangiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng), and proangiogenic factors such as placental growth factor (PIGF) and vascular endothelial growth factor (VEGF). sFlt-1 concentrations are increased by 20-fold in the maternal circulation in the third trimester of normal pregnancies compared to non-pregnant women (Shibata et al., 2005).

In preeclampsia, migration of cytotrophoblasts into the uterus is shallow and spiral arteries are only partially modified or not modified at all (Figure 1). In some cases, the number of cytotrophoblasts seem to be reduced and in others, cytotrophoblasts do not seem to adequately mimic the properties of endothelial cells. The underlying reason for the altered behavior of placental cells in preeclampsia is unknown, but a combination of genetic (altered differentiation of early trophoblast cells) and immunologic (exaggerated maternal response) factors are implicated (Huppertz, 2008). As a result,

the placenta becomes hypoxic and suffers oxidative stress, releasing inflammatory cytokines and free radicals. Antiangiogenic soluble factors are upregulated in the hypoxic placenta, a process mediated by hypoxia inducible factor 1α (HIF- 1α), and their secretion into the maternal circulation is increased. sFlt-1 and sEng have been reported to be 3-fold higher in the maternal circulation in preeclamptic pregnancies compared to normal pregnancies (Shibata et al., 2005).

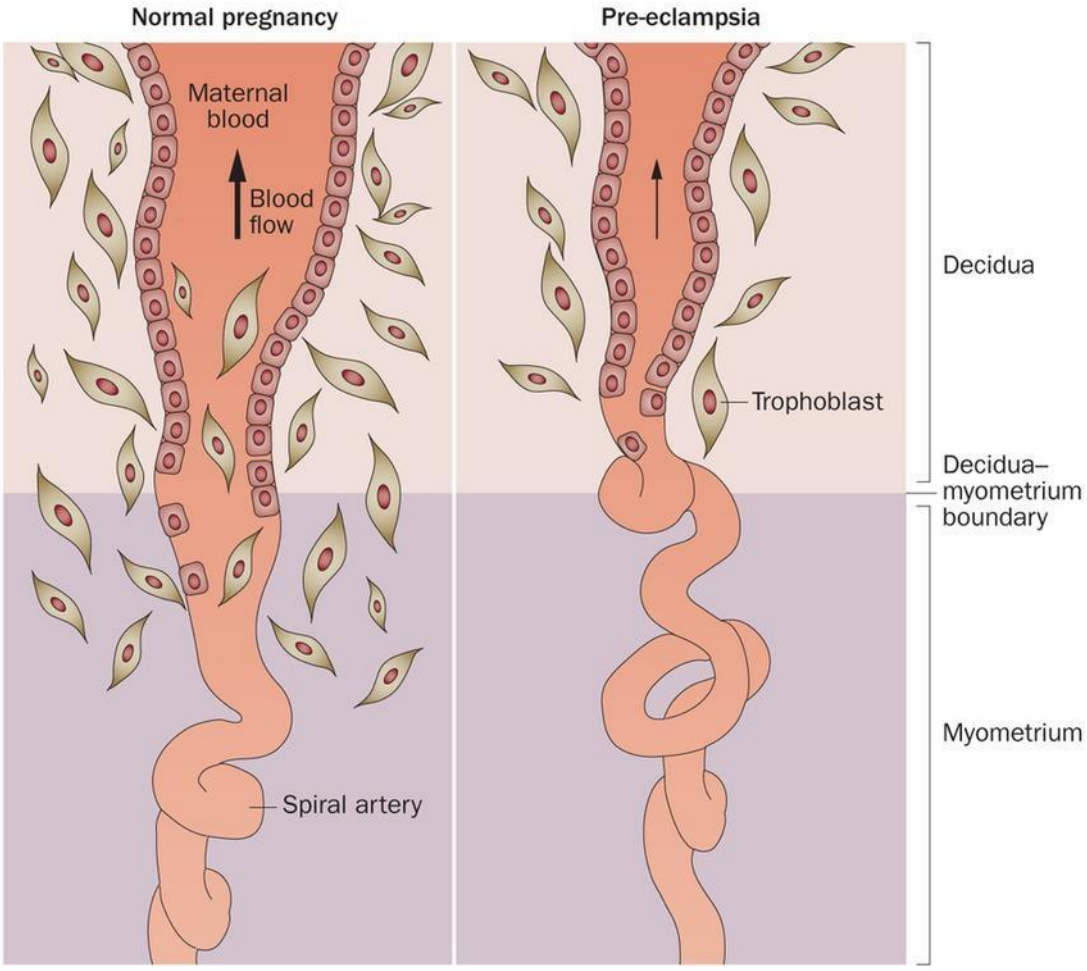


Figure 1. Physiological transformation of maternal spiral arteries in normal pregnancy (left) and preeclamptic pregnancy (right). In preeclamptic pregnancies, trophoblast invasion of the spiral arteries is shallow and does not extend into the myometrium, resulting in lack of adequate transformation (Adapted from (Chaiworapongsa et al., 2014)

sFlt-1 and sEng have been linked to the maternal endothelial dysfunction in preeclampsia in mouse models (Maynard *et al*., 2003; Venkatesha *et al*., 2006). Their concentrations are directly correlated to the severity of preeclampsia and decrease postpartum (Leaños-Miranda *et al*., 2017). sFlt-1 is a circulating splice variant of the vascular endothelial receptor, fms like tyrosine kinase 1 (Flt-1), which binds to VEGF. VEGF and PIGF signalling at the Flt-1 receptor is coupled to endothelial nitric oxide synthase (eNOS) and leads to production of nitric oxide (NO), a vasodilator. sFlt-1 binds to and sequesters VEGF and PIGF, preventing their signalling at their receptors. It also sensitizes vascular endothelial cells to inflammatory cytokines such as tumour necrosis factor-α (TNF- α) and to pro-constrictive factors such as endothelin 1 (ET- 1) leading to vascular inflammation and vasoconstriction. sEng is a circulating splice variant of endoglin, a coreceptor for signalling of transforming growth factor β (TGF- β) and sequesters TGF- β, producing effects similar to that of sFlt-1. PIGF secretion by the placenta is lower in preeclampsia, and its concentration in the maternal circulation is 2.5-fold lower compared to normal pregnancies (Shibata *et al*., 2005). sFlt-1 to PIGF ratio greater than 38 is positively predictive for severity of preeclampsia, in the subsequent 4 weeks since measurement, and reduces the time needed for diagnosis (Zeisler *et al*., 2016; Müller *et al*., 2019).

8.3 Symptoms

The angiogenic imbalance induced by the preeclamptic placenta, i.e. the upregulation of sFlt- 1 and sEng coupled with the downregulation and sequestering of PIGF and VEGF, leads to systemic inflammation, activation of the coagulation system, and endothelial dysfunction in the maternal vasculature. This endothelial dysfunction is marked by a vasoconstrictive state and many organs are affected such as the brain, the kidneys, the lungs, the liver, and the heart. Symptoms indicating onset of preeclampsia include hypertension (≥ 140/90 mmHg) and signs of organ damage, such as proteinuria (≥ 300 mg within 24 h urine) at gestational age (GA) greater or equal to 20 weeks. Other accompanying symptoms such as headaches, visual disturbances, oedema, vomiting, and right upper quadrant abdominal pain could occur. Effects on the foetus include restriction of growth and placental abruption, leading to still birth (Steegers *et al*., 2010). To prevent maternal mortality, preterm delivery might be necessary, and this leads to neonatal complications such as respiratory distress, cerebral palsy, retinopathy associated with prematurity, and even death (Armaly *et al*., 2018).

Preeclampsia is a progressive disorder and symptoms can range from mild to severe. Symptoms of severe preeclampsia include severe hypertension (≥ 160/110 mmHg); severe proteinuria (≥ 5 g within 24 h urine); hemolysis, elevated liver enzymes, and low platelet count (HELLP syndrome); disseminated intravascular coagulation (DIC), and eclampsia (seizures and coma during pregnancy unexplained by other conditions).

Preeclampsia is associated with at least double the risk of long-term maternal complications such as cardiovascular diseases, neurologic diseases, and diabetes mellitus (English, Kenny and McCarthy, 2015).

8.4 Early- and late-onset preeclampsia

In addition to disease severity, morbidity and mortality in preeclampsia are also correlated with GA at which it develops. Preeclampsia is classified as 'early-onset' when it occurs before 34 weeks and 'late-onset' when it occurs after 34 weeks of GA. Early-onset preeclampsia comprises less than 20% of all preeclampsia cases globally. It has higher risk of progressing to severe disease and has poorer foetal outcomes, being more associated with impaired uterine blood flow, foetal growth restriction, and preterm delivery (Huppertz, 2008; Hall, 2016). Late- onset preeclampsia is associated more with maternal morbidity due to metabolic and cardiovascular diseases with less foetal involvement (Huppertz, 2008; Raymond and Peterson, 2011; Armaly *et al*., 2018).

8.5 Prevention

Preeclampsia cannot be prevented, but severe outcomes can be reduced. Women at risk of preeclampsia are advised to make lifestyle changes before pregnancy, such as dietary changes and smoking cessation to modify metabolic risk factors and adequately manage chronic diseases. Regular prenatal care is needed to detect preeclampsia before it becomes severe. Low dose aspirin, 75 mg per day, 12 to 36 weeks of GA has been recommended for women with one or more risk factors to prevent incidence of severe preeclampsia (Sammour *et al*., 2011). Safety of using aspirin at doses above 100 mg for prevention of severe preeclampsia has not been confirmed (Atallah *et al*., 2017). Calcium supplementation, at doses of 1.5 to 2 g per day from 20 weeks of gestation onwards, has been recommended by the World Health Organization (WHO) to prevent severe preeclampsia, especially in high risk populations (Sammour *et al*., 2011; Omotayo *et al*., 2016) . Both aspirin and calcium reduce vasoconstriction and aspirin stabilizes platelet activity, reducing coagulative complications (U.S. Preventive Services Task Force, 2015).

8.6 Treatment

When mild preeclampsia is diagnosed at less than 32 weeks of GA, hospitalization and expectant management is recommended. This is to maintain balance between managing maternal clinical symptoms and gaining gestation age in order to prevent premature birth. For every day of gestation gained between 23 and 32 weeks, foetal survival is improved by 1% (proteinuria, liver enzymes, platelet count, and symptoms indicating preeclampsia progression, such as severe headaches and visual changes, and foetal assessment for growth and movement are done daily. Patients are treated with antihypertensives if hypertension is severe, with magnesium sulphate to reduce the risk of eclampsia, and with steroids to facilitate foetal lung development. Delivery is indicated at 34 weeks if symptoms are stable, but if there are signs of severity delivery is required even before 34 weeks. For severe preeclampsia diagnosed at 32 to 36 weeks of GA, hospitalization and expectant management is indicated as above, and delivery is at 37 weeks unless foetal/maternal condition is unstable. Although delivery of the placenta is the only curative treatment of preeclampsia, maternal monitoring is continued post- partum since complications might arise (Hall, 2016).

8.7 Proton pump inhibitors for preeclampsia

Proton pump inhibitors (PPIs) are a class of drugs which are used for gastric acid-related disorders. Omeprazole, the first-in-class drug, was first marketed in 1989. All PPIs act by blocking the last step in gastric acid secretion through inhibition of the H+/K+-ATPase enzyme (proton pump) found in the gastric parietal cells (Holt and Howden, 1991; Strand, Kim and Peura, 2017). These drugs have been used in all age groups and safely used by pregnant women for gastrointestinal disorders (Pasternak and Hviid, 2010; Matok *et al.*, 2012). During the search for treatment that could reverse or quench the pathogenesis of preeclampsia, it was identified that PPIs could be potential candidates (Onda *et al.*, 2015, 2017).

In addition to their inhibition of acid secretion, it was observed that PPIs have anti- inflammatory actions (Wandall, 1992; Yoshida *et al*., 2000; Ichikawa *et al*., 2004). It was also shown that one of the PPIs, lansoprazole, upregulated Hemoxygenase-1 (HO-1), an enzyme of the hemoxygenase system, in gastric epithelial cells in rats (Takagi *et al*., 2009). There are reports that the hemoxygenase system could be involved in the pathogenesis of preeclampsia (George and Granger, 2013). HO-1 is induced during oxidative stress and has protective biological functions. In pregnancy, it regulates placental development and reduces production of sFlt-1 and sEng, but its expression might be lower in preeclamptic placental cells (Ahmed *et al*., 2000; Cudmore *et al*., 2007; Ramma and Ahmed, 2014). This led to the hypothesis that HO-1 production inducing drugs could be useful in preeclampsia. However, other reports have found that HO-1 expression is not

lower in preeclampsia, nor does it regulate sFlt-1 and sEng production (BARBER *et al.*, 2001; Tong *et al.*, 2015).

Laboratory studies were conducted to investigate the efficacy of PPIs in preeclampsia (Onda *et al.*, 2015, 2017). Tissues that are known to be sources of antiangiogenic biomarkers namely, isolated cells and explant tissue from the placenta, umbilical vein endothelial cells, and whole blood vessel explants were obtained from women with early-onset preeclampsia at delivery. These tissues were exposed to PPIs at concentrations ranging from 5 to 100 µM. The PPIs investigated were lansoprazole, omeprazole, pantoprazole, rabeprazole, and esomeprazole. Biomarker concentrations were measured in the media after 24 hours (Onda *et al.*, 2017). It was observed that the PPIs tested reduced expression and secretion of these biomarkers concentration-dependently (Figure 2).

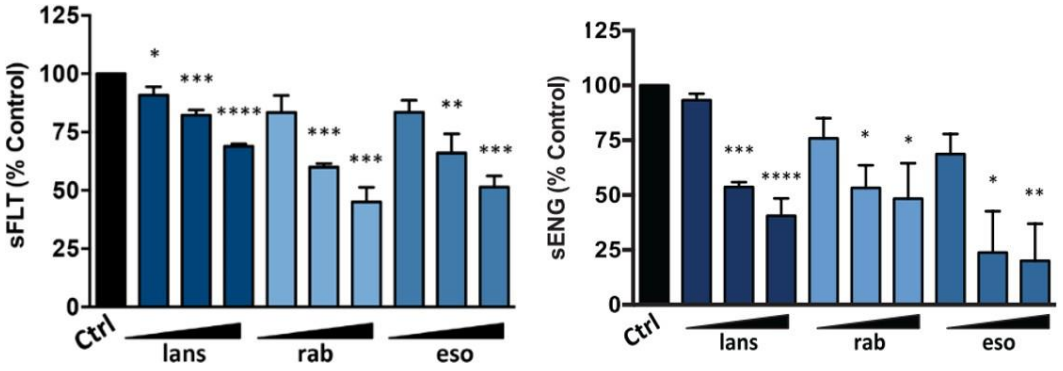


Figure 2. Relative sFlt-1 (left) and sEng (right) concentrations in the media after 24-hour treatment with PPIs administered to primary placental cells. lans= lansoprazole, rab=rabeprazole, eso=esomeprazole. The blue bars indicate 5, 50, and 100 µM concentrations for each drug. Black bar indicates untreated control (Adapted from (Onda et al., 2017))

Esomeprazole was seen to be the most potent of the PPIs tested for reduction of sFlt-1 and sEng. Additional findings of the study (Onda *et al.*, 2017) were that the PPIs tested:

- Upregulated expression of the proangiogenic biomarker vascular VEGF (but did not affect PIGF)
- Vasodilated whole blood vessels from preeclamptic pregnancies in ex vivo experiments
- Decreased blood pressure in mice models of preeclampsia in *in vivo* experiments (through reduction of ET-1 secretion)

- Reduced endothelial dysfunction by reducing secretion of inflammatory factors in *in vitro* experiments.

The underlying molecular mechanisms of these effects, i.e. the targets or receptors at which PPIs act, could not be elucidated in this study. It is the opinion of the authors that these effects are not mediated through an induction of HO-1 although this is what initially led to the investigation into PPIs. These results led to the conclusion that PPIs could indeed be candidates for preeclampsia and that conducting a clinical trial with esomeprazole, the most potent PPI for these effects, would be justified (Tong *et al*., 2015; Onda *et al*., 2017).

A prospective study of a cohort of pregnant women with confirmed or suspected preeclampsia conducted in the Netherlands corroborated the above findings. At the time of blood sampling for biomarker measurement, the women were taking esomeprazole (20 or 40 mg daily dose), omeprazole (10, 20, or 40 mg daily dose), or pantoprazole (20 or 40 mg daily dose). PPI use in this group of patients was associated with lower concentrations of sFlt-1 and sEng as well as lower ET-1 concentrations. There was no effect on PIGF concentrations (Saleh *et al*., 2017).

Another cohort study used data of pregnant women from the Swedish registry to identify if there was association between PPI use and preeclampsia subtypes, i.e. early-onset versus late- onset preeclampsia. The authors reported that there was increased association of PPI use in all trimesters and preeclampsia with term delivery, i.e. delivery after 37 weeks. However, this association was not found for preeclampsia with preterm delivery, i.e. delivery at less than 34 weeks or less than 37 weeks, nor with preeclampsia with small for gestational age (SGA) foetus. PPI use during the third trimester was seen to lower the risk of preeclampsia with preterm delivery, especially delivery before 34 weeks, and with SGA foetus. The authors noted that this could have been because the highest increase in concentrations of antiangiogenic biomarkers (sFlt-1 and sEng) is observed in the third trimester close to disease onset (Hastie *et al*., 2019). These findings pointed out the potential of using PPIs during the third trimester especially for early-onset preeclampsia where preterm delivery is a major concern.

All the above results led to the conclusion that a clinical trial would need to be conducted to confirm the efficacy of PPIs in this group of patients, i.e. whether PPIs could prolong gestation, improve maternal and foetal clinical outcomes, and reduce secretion of antiangiogenic biomarkers.

8.8 Preeclampsia intervention with esomeprazole (PIE) trial

The PIE trial was a phase II double-blind, randomized, placebo-controlled clinical trial conducted in Tygerberg Hospital, South Africa. The aim of this trial was to see if once-daily 40-mg oral esomeprazole would be effective in treating early-onset preeclampsia. 120 pregnant women with GA between 26 and 31 weeks and diagnosed with early-onset preeclampsia received either esomeprazole or placebo (Cluver *et al.*, 2015). Results of the trial showed that there was no significant difference between treatment and placebo arms for prolongation of pregnancy and for maternal/foetal/neonatal outcomes. There was no significant difference in the expression and concentrations of antiangiogenic (sFlt-1 and sEng)/proangiogenic (PGF/VCAM-1)/endothelial dysfunction (ET-1) biomarkers between the two arms (Cluver *et al.*, 2018).

It is unclear why the trial had negative results for all outcomes assessed when previous cohort observational studies in preeclampsia patients had shown that at standard clinical doses PPI use was associated with lower antiangiogenic biomarker concentrations (Saleh *et al.*, 2017). A possible reason for this could be that esomeprazole, at 40 mg dose, is unable to alter the fate of preeclampsia once it has progressed, but there is still a chance that it might do so preventatively, which would need to be further studied (Cluver *et al.*, 2018). The results of the PIE trial are also unexpected, given the preclinical studies by Onda *et al.* (Onda *et al.*, 2017). The authors of the PIE trial believe that by achieving exposure similar to that in the preclinical study by Onda *et al* (Onda *et al.*, 2017) through higher dosing, esomeprazole could show efficacy in this patient group.

Sandrim *et al.* have expressed concerns regarding investigation of PPIs at high doses for preeclampsia, especially for patients who have a cardiovascular risk. Their concern is that PPIs are associated with increased risk of adverse cardiovascular events due to interference with the synthesis and homeostasis of nitric oxide (NO), an endothelium derived factor that relaxes blood vessels (Sandrim, Caldeira-Dias and Montenegro, 2019). However, in the preclinical study investigating efficacy of PPIs for treatment of preeclampsia, Onda *et al* (Onda *et al.*, 2017) had shown that esomeprazole increased activity of the enzyme that synthesizes NO. They have shown that esomeprazole mitigates vascular damage produced by preeclampsia through pathways other than NO, such as by decreasing sFlt-1 and sEng, endothelin-1, and proinflammatory cytokines. They also showed PPIs vasodilated whole blood vessels in ex vivo studies and caused reduction of blood pressure in animal models of preeclampsia (Onda *et al.*, 2017). Additionally, in the PIE trial, there was no evidence that the treatment group had worse blood pressure.

Figure 3 shows summary pharmacokinetic profiles of esomeprazole and its metabolites in 10 patients from the treatment arm of the PIE trial who underwent pharmacokinetic sampling. Esomeprazole geometric mean (95% confidence interval or CI) Area Under the Curve (AUC), 5.88 (2.96-11.68) µmol·h/L, was reported to be similar to that of healthy, non-pregnant subjects, 4.32 (3.04-6.14) µmol·h/L (Hassan-Alin *et al.*, 2000).

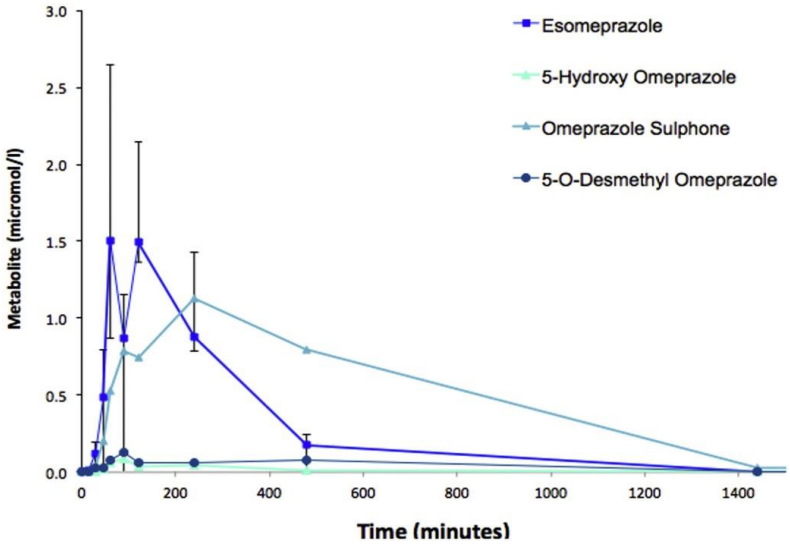


Figure 3. Esomeprazole and metabolite pharmacokinetics (geometric mean with 95% CI error bars) profiles of 10 patients from the PIE trial (Adapted from (Cluver et al., 2018)).

This project used pharmacokinetic data from this trial to investigate the population pharmacokinetics of esomeprazole in pregnant patients with early-onset preeclampsia in order to investigate higher doses to be used in future clinical trials.

8.9 Pharmacokinetics of esomeprazole

8.9.1 Introduction

All PPIs have the same mechanism of action for their gastric acid inhibitory effect but differ in their pharmacokinetics and in the extent to which they produce their effect. PPIs are substrates of CYP3A4 and CYP2C19. Differences in pharmacokinetics among the PPIs are due to the extent to which they are metabolized by CYP2C19. For a PPI, interindividual variability in pharmacokinetics is attributed primarily to polymorphism in CYP2C19. Other factors that result in pharmacokinetic variability include meal times in relation to PPI dosing, which affect absorption, and concomitant administration of other drugs, which might also alter absorption or clearance (Andersson, Röhss, et al., 2001; El Rouby, Lima and Johnson, 2018).

8.9.2 Formulation

Esomeprazole is the S-isomer of omeprazole. It is a weak base and is acid labile, hence for oral route it is available in formulations that protect it from the gastric environment. Oral solid dosage forms are available in capsule or multi-unit pellet system (MUPS) tablet formulations. Both formulations contain enteric coated pellets and are expected to disintegrate in the stomach, after which they transport to the small intestine where they dissolve and are absorbed. Esomeprazole is also available for administration by the intravenous (IV) route (Strand, Kim and Peura, 2017; El Rouby, Lima and Johnson, 2018).

8.9.3 Absorption, Distribution, Metabolism, and Excretion

Esomeprazole is a highly albumin-bound drug (97%) with a short half-life of 1 to 1.5 hours. Protein binding is constant at a concentration range of 2 to 20 µM (0.691 to 6.91 mg/L), which is the concentration range it is expected to have in humans. It has fast absorption, achieving a C_{max} of 0.8 to 1.7 mg/L at 1 to 3.5 hours (T_{max}) after a single oral dose of 40 mg without food. On single dosing, its bioavailability is 64% and its V_d is 16 L. 80% of esomeprazole is renally excreted as metabolites, 1% of the parent drug is renally excreted unchanged, and the remaining is excreted by the biliary route (El Rouby, Lima and Johnson, 2018).

Esomeprazole is metabolized by CYP2C19 and by CYP3A4. CYP3A is the predominant CYP subfamily in the small intestine and constitutes ~80% of total intestinal CYP (Dressman and Thelen, 2009). Esomeprazole has higher affinity for CYP2C19 (Michaelis constant or Km = 5µM) than for CYP3A4 (Km = 80 µM) (Andersson, Hassan-Alin, *et al*., 2001). As a result, after single dosing, two-third is metabolized by CYP2C19 to 5-hydroxy and 5-O-desmethyl esomeprazole and one-third by CYP3A4 to esomeprazole sulfone. Esomeprazole sulfone is the most prominent metabolite in plasma and is further metabolized by CYP2C19 to esomeprazole hydroxy sulfone (Figure 4). The metabolites are inactive for gastric acid inhibitory effects, and it is unknown if they have effect in preeclampsia.

CYP2C19 is a polymorphic enzyme. Clearance (CL) of esomeprazole varies by CYP2C19 genotype, with extensive metabolizers having at least 3 times higher CL than poor metabolizers (Dean, 2019). Ethnic differences in frequency of CYP2C19 polymorphisms exist. Asians have been reported to have the highest frequency of poor metabolizer phenotypes, 15 to 25%, compared to Caucasians and Africans, 3 to 5 % (Masimirembwa and Hasler, 1997; Dandara *et al*., 2001).

With repeated dosing, CYP2C19 is inhibited by esomeprazole and by its sulfone metabolite, which results in increased bioavailability and decreased clearance (Andersson, Hassan-Alin, *et al*., 2001; El Rouby, Lima and Johnson, 2018). Esomeprazole concentrations increase, hydroxy and desmethyl metabolite concentrations decrease, and sulphone metabolite concentrations increase (Figure 4).

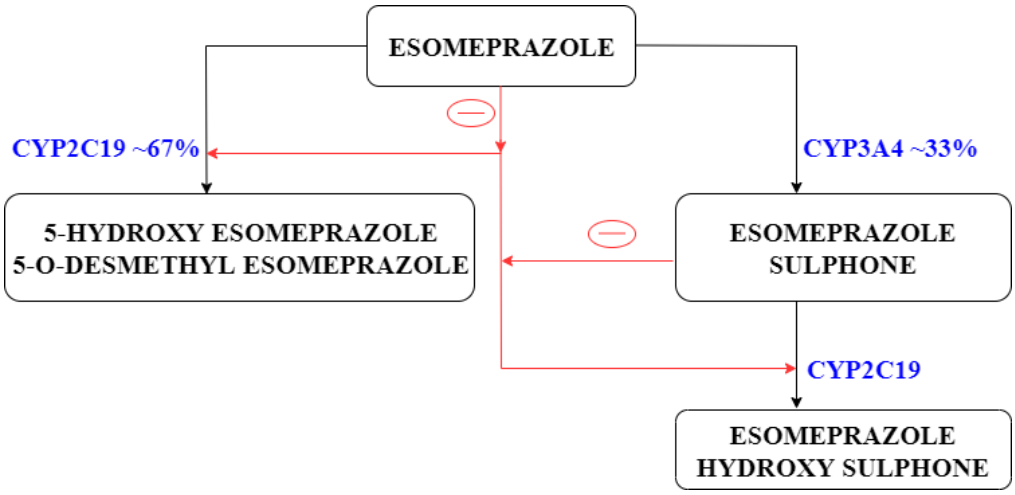


Figure 4. Metabolic pathway of esomeprazole and autoinhibition of CYP2C19 with repeated dosing.

There is little change in AUC with repeated dosing in poor CYP2C19 metabolizers since metabolism is dependent on CYP3A4. On the other hand, AUC increases in extensive metabolizers with repeated dosing (Andersson, Hassan-Alin, *et al*., 2001).

8.9.4 Nonlinear pharmacokinetics and auto-inhibition

The pharmacokinetics of esomeprazole present a nonlinear dose-exposure relationship. As dose increases, AUC increases more than proportionally, which indicates a saturation of clearance and/or first-pass metabolism with increasing doses. Additionally, esomeprazole AUC increases with repeated dosing due to a decrease in clearance and an increase in bioavailability, which is due to autoinhibition of CYP2C19 (Andersson, Hassan-Alin, *et al*., 2001). Most previous studies of esomeprazole pharmacokinetics determined concentrations on day five of repeated dosing, hence day5 will be used in this work to consider the effect of repeated dosing.

In healthy, non-pregnant subjects, with single dosing, AUC increases almost linearly from 5 to 20 mg and nonlinearly from 20 mg onwards which indicates presence of saturation at high doses (Table

1) . With repeated dosing, AUC increases nonlinearly from the lowest dose, i.e. 5 mg (Andersson, Hassan-Alin, *et al*., 2001; Andersson, Röhss, *et al*., 2001).

Table 1. AUC ratios, geometric mean (95% confidence interval), in healthy non-pregnant subjects for day 1 and day 5 of dosing. (Adapted from (Hassan-Alin *et al.*, 2000; Andersson, Röhss, *et al*., 2001)).

	AUC (mg·h/L)		
Dose, oral solution	**Day 1**	**Day 5**	**Day 5/ day 1 ratio**
5 mg	0.100 (0.065 – 0.15)	0.113 (0.0760 – 0.169)	1.14 (0.97 – 1.35)
10 mg	0.225 (0.145 – 0.349)	0.339 (0.228 – 0.504)	1.51 (1.27 – 1.78)
10 mg/5 mg ratio	2.24	2.97	
10 mg	0.225 (0.145 – 0.349)	0.339 (0.0.228– 0.504)	1.51 (1.27 – 1.78)
20 mg	0.508 (0.328 – 0.788)	1.07 (0.722 – 1.59)	2.11 (1.78 – 2.49)
20 mg/10 mg ratio	2.26	3.16	
20 mg	0.51 (0.33 – 0.79)	1.07 (0.72 – 1.59)	2.11 (1.78 – 2.49)
40 mg	1.34 (0.84 – 2.15)	3.22 (1.9 – 5.2)	2.4 (2.38 – 2.42)
40 mg/20 mg ratio	2.64	3.01	

AUC increases nonlinearly for both intravenous and oral formulations with single and repeated dosing as dose increases from 20 to 40 mg, and this increase is higher for oral capsules (Table 2).

Table 2. AUCs, geometric mean (95% confidence interval), in intravenous and oral formulations of esomeprazole. (Adapted from (Andersson, Hassan-Alin, et al., 2001)).

	AUC (mg·h/L)		
Dose, intravenous	**Day 1**	**Day 5**	**Day 5/ day 1ratio**
20 mg	0.922 (0.746–1.13)	1.29 (1.05 – 1.59)	1.39
40 mg	2.35 (1.89 – 2.91)	4.35 (3.63 – 5.22)	1.86
40 mg/ 20 mg ratio	2.55	3.38	
Dose, oral capsule			
20 mg	0.462 (0.352-0.611)	0.881 (0.670 – 1.160)	1.90 (1.72 – 2.09)
40 mg	1.49 (1.05 – 2.12)	3.88 (2.96 – 5.07)	2.59 (2.11 – 3.19)
40 mg/ 20 mg ratio	3.22	4.39	

8.9.5 Effect of food on esomeprazole pharmacokinetics

Food delays and reduces the bioavailability of esomeprazole. It was observed in a study assessing the pharmacokinetics of esomeprazole in fed (dose before 15 min of a meal) versus fasting state (dose before 4 h of a meal) that, under fasting conditions, time to maximum concentration (T_{max}) was faster by half the time compared to T_{max} in fed state while peak concentration (C_{max}) and AUC were higher by ~ 3% and 66%, respectively. This is because esomeprazole is an acid labile drug and a delay in gastric emptying exposes it more to the acidic environment of the stomach, leading to its degradation. The decrease in C_{max} and AUC was greater on single dose than with repeated dosing. This could be due to the increase in gastric pH that occurs with repeated dosing of esomeprazole, which would reduce its degradation, improving its bioavailability (Sostek, Chen and Andersson, 2007). The product label for the capsule formulation of oral esomeprazole recommends that esomeprazole be taken without food while that of the MUPS formulation states that it can be taken with or without food.

8.9.6 Effect of sex on esomeprazole pharmacokinetics

Females have lower body weight than males and are thus expected to have a lower clearance and volume of distribution. In addition to differences in body weight, females have a lower activity of CYP2C19, which is the major metabolizing enzyme for esomeprazole. Consequently, females are expected to have higher AUC and C_{max} for esomeprazole. This has been shown to be the case in a pooled analysis of data from 12 studies. This analysis showed that in females, esomeprazole AUC and C_{max} were 30% higher than males on single dose. With repeated dosing, AUC and C_{max} in females were only 13 to 14% higher and this difference was not statistically significant. The effect of sex on the disposition of esomeprazole has been reported to be clinically insignificant for the gastric acid inhibitory effects (Andersson *et al*., 2001).

8.9.7 Potential effects of pregnancy and preeclampsia on the pharmacokinetics of esomeprazole

Some physiological changes related to pregnancy may affect the pharmacokinetics of esomeprazole. Pregnancy is associated with a 23% increase in total body weight, a 32% increase in total fat mass, and a 41% increase in total body water by the third trimester (Ke, Rostami-Hodjegan, *et al*., 2014). It is expected that, in pregnancy, increase in gastric emptying time by 30% to 50% could cause a lag in absorption, plasma volume expansion by 50% could increase V_d, and decrease in albumin concentration by 20-40% could lead to a larger free fraction and consequently a decrease in total plasma drug concentration of esomeprazole (Costantine, 2014). In preeclampsia, increase in plasma volume in the third trimester is ~13.3% lower than normal pregnancies which could reduce V_d compared to that in normal pregnancies (de Haas et al., 2017) and albumin concentration is further decreased, which could reduce total plasma concentration of esomeprazole even more (Gojnic *et al*., 2004). Additionally, CYP3A4 is upregulated by approximately 2- fold while CYP2C19 activity decreases by 68% due to downregulation during the third trimester which is mediated by pregnancy related hormones including estrogen, progesterone, placental growth hormone, and cortisol (Jeong, 2010; Papageorgiou, Grepper and Unadkat, 2013; Ke, Nallani, *et al*., 2014). It is expected that this might change the metabolic pathway in pregnancy and might affect first-pass and therefore bioavailability. These changes could affect the overall exposure of esomeprazole and influence the outcome of preeclampsia treatment. The pharmacokinetics of esomeprazole in early-onset preeclampsia patients will be characterized in this study by using nonlinear mixed-effects modelling.

8.10 Nonlinear mixed-effects modelling

A model is a mathematical description of the phenomenon under study. In the case of population pharmacokinetics, a model is used to describe how drug concentration varies over time between different individuals in a population. The models used for this are called nonlinear mixed-effect models, as their parameters are determined by fixed effects, whose value is common to the whole population, and random effects, which vary between individuals or occasions. The model is composed of two parts. The first is the structural model, which is a mathematical description of the physiological processes of absorption, distribution, and elimination of a drug in the body. The second part is the variability or stochastic model, which describes the differences in parameters values between individuals and occasions (random effects). Random effects are organised with a hierarchical structure consisting of interindividual variability (IIV), which describes random variability of the pharmacokinetic parameters of an individual relative to the typical population parameters, interoccasion variability (IOV), which describes how these parameters randomly vary between different sampling occasions within an individual, and residual unexplained variability (RUV), which describes any remaining sources of variability between observations and the model predicted values for observations, such as errors in sampling times or assay error. The model estimates the variances of the distributions for the random variables describing IIV and RUV in addition to the typical pharmacokinetic parameters (Mould and Upton, 2013). Typical parameters are estimated in the model taking into account known or identified potential sources of variability, called covariates, such as through allometric scaling (i.e. adding body size descriptors such as weight, fat mass, or fat-free mass) (Anderson and Holford, 2008, 2009).

Although the methodology is more complex, population pharmacokinetic modelling offers advantages compared to noncompartmental analysis (NCA). NCA is a model-free pharmacokinetic analysis method in which secondary pharmacokinetic measures (e.g. AUC, C_{max}, T_{max} and half-life) are directly calculated from each subject's profile. Population pharmacokinetic models enable estimation of typical primary parameters for the population of a study as well as the expected and random variability around these parameters while NCA does not consider variability. NCA methods require rich data while model-based methods are more forgiving of sparse data, e.g. often seen in special populations such as children and pregnant women and can be used to describe complex pharmacokinetic processes. The use of a model also allows prediction of new scenarios through simulations making it a useful tool for design of dose regimens (Bulitta and G. Holford, 2014; Barnett *et al.*, 2020). NCA does have some advantages as a tool of pharmacokinetic data analysis. Fewer assumptions are made with NCA, for e.g. there is no assumption made that the underlying pharmacokinetic parameters are log normally distributed. When the pharmacokinetic parameter of

interest for a drug is overall exposure, such as in bioequivalence studies, or metabolite to parent ratios for drugs, NCA can be used adequately since it is a robust method for estimating AUC. Additionally, it can be used to compare the performance of a model through comparisons of the NCA metrics of simulated pharmacokinetic profiles to that of observed profiles (Acharya *et al*., 2016).

9 AIMS AND OBJECTIVES

9.1 Specific Aims

The aim of this study is to characterize the pharmacokinetics of esomeprazole in pregnant patients with early-onset preeclampsia and compare its pharmacokinetics with that of healthy non-pregnant subjects. Additionally, this study aims to predict a dose of esomeprazole to be used for future clinical trials involving early-onset preeclampsia patients.

9.2 Hypothesis

The pharmacokinetics of esomeprazole are expected to be altered in early-onset preeclampsia patients due to pregnancy related physiological and metabolic changes. Changes are expected in bioavailability, volume of distribution, and clearance, and this might necessitate dose adjustments to achieve the same concentration as in non-pregnant.

9.3 Objectives

1. Through population pharmacokinetic modelling, determine the pharmacokinetic parameters of esomeprazole in pregnant preeclamptic patients from the PIE trial and compare how they differ from those of healthy non-pregnant subjects.

2. Through comparisons of the exposures of esomeprazole and metabolites between pregnant and non-pregnant data, investigate how the metabolism of esomeprazole changes between the two groups.

3. Investigate how esomeprazole exposure in pregnant women with preeclampsia compares with drug levels from *in vitro* preclinical studies that showed a potential effect therapeutic effect.

4. Use the developed model to predict exposures at higher doses and to investigate if preclinical effective concentrations or exposures could be achieved.

10 METHODS

10.1 Study population

Pharmacokinetic data for pregnant, early-onset preeclampsia patients were obtained from patients in the treatment arm of the PIE study. Pregnant women who participated in this study were those with single pregnancies diagnosed with early-onset preeclampsia (Cluver *et al*., 2015, 2018). Exclusion criteria included:

- Maternal or fetal compromise that necessitated delivery within 48 hours
- Pregnancies with a suspicion of major fetal anomaly or malformation
- Eclampsia or HELLP syndrome
- Severe hypertension that could not be controlled within 48 hours of admission
- Cerebrovascular event
- Severe renal impairment with creatinine greater than 125 mmol/L

Patients must not have been using PPIs or medications that could interact with PPIs. They must not have had contraindications to the use of PPIs. A comprehensive list of inclusion and exclusion criteria can be found in the PIE study protocol (Cluver *et al*., 2015, 2018).

Pharmacokinetic data for non-pregnant healthy subjects were obtained from two studies conducted by Hunfeld *et al*. which were similar in study design. One of the studies compared the efficacy of esomeprazole versus pantoprazole (eso-panto), while the other study compared the efficacy of esomeprazole versus rabeprazole (eso-rabe) in relation to pharmacokinetics and CYP2C19 polymorphism. These studies were investigator-blind, randomized, two-way cross- over studies. Healthy subjects between ages of 18 and 60 were included. Subjects were excluded if they were pregnant or lactating, if they had used PPIs during the 14 days prior to day one of the study, if they had used antibiotics during the 30 days prior, or if they had any contraindications to the use of PPIs (Hunfeld *et al*., 2010, 2012). Pharmacokinetic data from the two studies of non-pregnant subjects were combined into a pooled dataset for the analysis in this study. Only the data from the esomeprazole arm was used from the control studies, with the aim of investigating a possible change in metabolic pathways during pregnancy. Table 3 summarizes characteristics of the pregnant and non-pregnant studies.

The PIE trial had approval from the South African Medicines Control Council and Health Research Ethics Committee. Patients in the trial gave written informed consent (Cluver *et al*., 2018). Non-pregnant data for this project was obtained from two studies by Dutch researchers (Hunfeld *et al*) and had approval from the institutional review board of the Haga Teaching Hospital, Netherlands. All subjects gave written informed consent (Hunfeld *et al*., 2010, 2012). For this project, there was no need to obtain further ethical approval, since the data received was from clinical trials that had received approval as stated above.

Table 3. Summary of study characteristics of pregnant patients with early-onset preeclampsia (Cluver et al., 2018) and non-pregnant subjects (Hunfeld et al., 2010, 2012).

Study	**Pregnant**	**Non-pregnant**
Formulation	Nexium delayed-release capsules	Nexium MUPS tablets
Dose	Oral 40 mg	Oral 40 mg
Meal	2 hrs post dose	5 mins post dose
Sampling	Day1: pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 24 hrs Day5: pre-dose	Day1 and day5: Pre-dose, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 7, 8 hrs
LLOQ (ESO)	0.001 mg/L	0.026 mg/L
BLQ (ESO)	21	61
LLOQ = lower limit of quantification, BLQ = below lower limit of quantification, ESO = esomeprazole		

10.2 Pharmacokinetic data

Patients in the treatment arm of the PIE trial received a 40 mg daily dose of esomeprazole (delayed release capsules, Nexium®, Astra Zeneca) in fasted state and meals were given two hours after dose. A subgroup of these patients underwent plasma sampling and had samples collected pre-dose and post-dose at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, and 24 hours after the first dose. Pre-dose samples were taken on day5 after repeated daily dosing. Concentrations of esomeprazole and its metabolites (esomeprazole sulfone, 5-hydroxy esomeprazole, and 5-O- desmethyl esomeprazole) were quantified using a validated ultra-performance liquid chromatography-tandem mass spectrometry

method. The lower limit of quantification (LLOQ) was 0.001 mg/L for all analytes (Cluver *et al*., 2015, 2018).

Participants in the non-pregnant studies received a 40 mg daily dose of esomeprazole (MUPS tablets, Nexium®, Astra Zeneca) in fasted state in the morning and meals were given 5 minutes after dose. Plasma samples from both studies were collected pre-dose and post-dose at 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 7, and 8 hours on day1 of dosing. In the eso-panto study, after repeated daily dosing, on day5, samples were collected pre-dose and at 1, 2, 3, 4, 5, 6, 7, and 8 hours post-dose. Concentrations of esomeprazole and its metabolites (esomeprazole sulfone and 5- hydroxy esomeprazole) were quantified by liquid chromatography (HPLC). The LLOQ was 0.026 mg/L for all analytes (Hunfeld *et al*., 2010, 2012).

10.3 Data analysis overview

The initial plan was to model the pregnant data together with the data from healthy non- pregnant subjects in the same model, in order to capture the differences due to pregnancy by performing a test within the model. However, as shown by the profiles in the results section and explained in detail in the discussion section, it was observed during the data exploration that there were unexpected large differences in the absorption of esomeprazole between the two datasets. These seemed unlikely to be caused by pregnancy, but rather due to other differences between the studies. The erratic absorption in the healthy volunteers' dataset is very difficult to describe in a model and it was decided to only model the pregnancy data of esomeprazole and use AUC results (for both esomeprazole and its metabolites) from a non-compartmental analysis for the comparison with the healthy volunteer's data.

10.4 Population pharmacokinetic modelling

A population pharmacokinetic model describing esomeprazole disposition was developed in early-onset preeclamptic pregnant patients. Estimation of parameter values was performed initially with Monolix (version 2019R2, Lixoft®) and the stochastic approximation expectation maximization (SAEM) algorithm. The software NONMEM (version 7.4.2, Icon®) and the algorithm first-order conditional estimation with interaction (FOCE-I) were subsequently used to estimate parameter values, and these were compared with those from Monolix. R (version 4.0.2) was used for data exploration, statistical and graphical analysis, and model diagnostics.

For the population pharmacokinetic model, only pregnant esomeprazole data was used. Pre- dose concentrations, which were used to confirm that the patient had not recently taken esomeprazole and were below the limit of quantification (BLQ), were excluded from the analysis. In Monolix, the M3 method was used whereby the maximum likelihood estimation method is used to fit the model to the observations and for the BLQ values the likelihood that these observations are BLQ is used (Beal, 2001). In NONMEM, the post-dose observations that were BLQ were censored and handled as follows: LLOQ/2 was imputed to the first BLQ value and its additive error was inflated by LLOQ/2 to mitigate the effect of the imputation, then the trailing BLQ values were excluded from the model fit. This is similar to the M6 method. To screen outliers, observations that resulted in unreasonably high residual error in the model were identified by means of goodness-of-fit (GOF) plots and were ignored.

Several structural models were tested to describe the pharmacokinetics of esomeprazole. One- or two-compartment disposition with first-order elimination, and first-order absorption, either with no delay, a lag time, and transit absorption compartment. A log-normal distribution was assumed for the IIVs and the RUV was described using a combined additive and proportional error model. The typical value of relative bioavailability was fixed to 1.

Allometric scaling was applied to apparent clearance (CL/F) and apparent volume of distribution (V_d/F) to account for differences in body size between individuals according to the formula below (Anderson and Holford, 2008, 2009). Pi and Pp are the individual and typical CL/F or V_d/F respectively, while Cov represents the covariates tested, which were weight and fat-free mass, and z is the allometric scaling value which was fixed (0.75 for CL/F and 1 for V_d/F).

$$P_i = P_p \left(\frac{cov}{cov_{standard}}\right)^z \qquad (eq.1)$$

Model selection was based on changes in the objective function value or -2 log likelihood (OFV or -2LL); convergence success of the run; precision of the parameter estimates (relative standard errors, %RSEs); inspection of diagnostic plots such as standard GOF plots, visual predictive checks (VPC); and biological plausibility of the parameter estimates. A VPC plot of the final model was obtained by simulating 500 datasets and plotting the 5th, 50th, and 95th percentiles of the simulated data with the observed data.

10.5 Metabolite to parent AUC ratios (AUC_m/AUC_p)

To investigate possible changes in the metabolic pathways of esomeprazole, the AUCs of esomeprazole, 5-hydroxy esomeprazole, and esomeprazole sulphone were determined using Pkanalix (version 2019R2, Lixoft®) and metabolite to parent AUC ratios were calculated. These values were calculated for the pregnant women day1 data, and non-pregnant adults on day1 and day5 using molar concentrations (micromol·h/L) to correct for differences in molecular weight. AUC ratios of 5-hydroxy esomeprazole to esomeprazole (AUC_{hyd}/AUC_{eso}) comparisons were used to investigate changes in the CYP2C19 pathway while AUC ratios of esomeprazole sulphone to esomeprazole (AUC_{sulf}/AUC_{eso}) for changes in the CYP3A4 pathway. These values were calculated for pregnant women on day1 of treatment and compared with non-pregnant data on day1 to show the effect of pregnancy. Comparisons of pregnant day1 with non-pregnant day5 or non-pregnant day1 with non-pregnant day5 were preformed to show the effect of autoinhibition with repeated dosing. The Mann-Whitney-Wilcoxon test was used to perform these comparisons at a significance level of 0.05. For the overall analysis data, the impact of weight and age on AUC was assessed by linear regression analysis of log transformed AUCs. R was used for the regression analysis, statistical comparisons, and plotting of results.

For the calculation of AUC, data of all analytes, i.e. esomeprazole, 5-hydroxy esomeprazole, and esomeprazole sulphone, from both pregnant and non-pregnant studies were used.

Day1 data (i.e. single dose data of pregnant patients and of non-pregnant subjects) was handled as follows:

- Pre-dose observations were BLQ and were set to 0.
- Post-dose BLQ observations were assumed as 0 if before T_{max} and imputed LLOQ/2 if after T_{max} (Pkanalix-NCA, 2019).
- $AUC_{0\text{-}inf}$ was calculated using log-linear extrapolation to infinity. The extrapolation was performed only if the last concentration (C_{last}) was not BLQ and the area after C_{last} was calculated as $Cl_{ast}/\lambda z$, where λz is the terminal slope of the log concentration versus time profile. Profiles for which the terminal half- life could not be estimated were excluded by the software. If C_{last} was BLQ, no extrapolation was performed and AUC_{last} was used.

Day5 data (i.e. steady state data of non-pregnant subjects) were handled as follows:

- There were no BLQ observations.
- $AUC_{0\text{-}24}$ were calculated using the linear up log down method. Since the non-pregnant sampling schedule was up to 8 hours, a 24-hour sample was included in the day5 dataset of the non-pregnant subjects and assumed to have same concentrations as the pre-dose samples.

10.6 Translation from *in vitro* to *in vivo* target

To determine a target concentration to achieve in preeclampsia patients, some considerations had to be made to translate the preclinical concentrations to concentrations in patients. The *in vitro* target esomeprazole concentration of 50 µM (17.3 mg/L) was chosen based on the preclinical study by Onda *et al*. This was the lowest concentration amongst those tested at which a significant difference from control was observed for the biomarker lowering effect of esomeprazole in the *in vitro* studies (middle bar for esomeprazole in Figure 2).

Only unbound drug is expected to interact with the target and be pharmacologically active, so the expected protein binding difference between in the *in vitro* preclinical experiments and the clinical levels in pregnant women has to be considered.

Esomeprazole is 97% protein bound to human serum albumin (HSA) (Andersson, Röhss, *et al*., 2001; Rabbani and Ahn, 2019). The fraction unbound (f_u) in the *in vitro* culture, in which fetal calf serum was assumed to be the only source of bovine serum albumin (BSA), needed to be calculated. Equation 2 was used to calculate f_u (Schalkwijk, Greupink and Burger, 2017) where K_d is the binding constant (in µM) and Bmax is the maximum binding capacity (in µM).

$$f_u = \frac{K_d}{Bmax + K_d} \qquad (eq.2)$$

A concentration of 16 g/L was used for the protein content of the culture based on information from the Sigma Aldrich certificate of analysis for fetal calf serum used in the preclinical study. Esomeprazole binds one-to-one with albumin, thus Bmax for esomeprazole in the culture was taken as 16 g/L. The Sigma Aldrich information also provided the molecular weight of BSA as 66430.3 g/mol. The K_d value for BSA to esomeprazole could not be obtained from literature, but a value could be found for K_d for BSA to omeprazole at temperature of 35°C: 13.5 µM (Deepa, Kabir and Amran, 2016). Since esomeprazole has been reported to have a K_d value for HSA similar to omeprazole (Pawar *et al*., 2017), it was assumed that the use of omeprazole K_d for BSA would suffice for this rough estimation.

10.7 Unbound *in vitro* esomeprazole AUC over 24 hours incubation

Esomeprazole is not stable in plasma. Its degradation half-life during the *in vitro* incubation was assumed to be 8 hours based on stability information on esomeprazole product label (Nexium 40 mg label) at the same temperature and pH as in the *in vitro* culture.

To calculate exposure of unbound esomeprazole over the 24 hours of *in vitro* incubation ($fAUC_{0-24}$) and adjusting for the degradation, the following steps were taken:

The degradation rate constant, k, was derived from the degradation half-life using equation 3 ($t_{1/2}$ = 8 hours).

$$K = \ln(2) / t_{\frac{1}{2}} \qquad (eq. 3)$$

The unbound starting concentration, C_0, was calculated using equation 4.

$$C_0 = target\ concentration \times f_u \qquad (eq. 4)$$

Equation 5 was used to calculate the concentration after 24 hours, C_{24}.

$$C_t = C_0 \cdot e^{-k \cdot t} \ with\ (t = 24\ h) \qquad (eq. 5)$$

AUC of esomeprazole in the incubation was calculated using equation 6. This formula is valid for exponential degradation/decay.

$$fAUC_{0-24} = (C_0 - C_{24})/K \qquad (eq. 6)$$

These calculations provided an equivalent AUC level to target in patients. The preclinical target to be compared with exposure in pregnant patients is going to be either the instantaneous concentration as C_{max} or the 24-hour exposure as AUC, i.e. the calculated $fAUC_{0-24}$.

10.8 Unbound *in vitro* esomeprazole C_{max}

The lowest esomeprazole concentration which was found to have effect in the *in vitro* incubation, i.e. 50 µM or 17.3 mg/L, was taken for comparison with C_{max} of the pregnant patients. This was adjusted for protein binding by multiplying with the fraction unbound (f_u) calculated from equation 2.

10.9 Unbound pregnant esomeprazole AUC

The preclinical unbound AUC during the time of incubation, i.e. $fAUC_{0-24}$, was compared to the median $fAUC_{0-24}$ for a single dose in the pregnant patients, or the simulated AUC_{0-24} at steady-state. To calculate the unbound AUC of the pregnant data from the PIE study ($fAUC_{0-24}$), the median AUC_{0-24} was multiplied by the estimated fu in pregnancy. In non-pregnant healthy subjects, albumin binding of esomeprazole is 97%, however Plasma albumin concentrations are lowered by ~31% in the third trimester (Ke, Rostami-Hodjegan, *et al*., 2014) and are expected to be in the range of 25 to 35 g/L. In preeclampsia, concentrations below 30 g/L have been observed (Gojnic M, Petkovic S, Papic M, 2004). Among reports for plasma albumin concentration during the third trimester in preeclampsia are (mean ± standard deviation) 25.5 ± 2.8 g/L (Benoit and Rey, 2011) and (mean ± SEM) 26.79 ± 4.68 (Dai *et al*., 2017).

Assuming average plasma albumin concentration, of 25 g/L in pregnant early-onset preeclampsia patients, f_u was estimated using equation 2. Esomeprazole binds one-to-one with albumin, thus Bmax was taken as 25 g/L. A molecular weight of HSA of 66000 g/mol and K_d value for HSA to esomeprazole of 20 µM were used (Pawar *et al*., 2017).

10.10 Simulations

Simulations were performed using the model developed for pregnant patients with early-onset preeclampsia to predict exposures for higher doses of esomeprazole in these group of patients with single and repeated dosing. Three scenarios were simulated to investigate exposures for the highest dose of esomeprazole which has been reported to be used clinically, i.e. 120 mg BID (McKeage *et al*., 2008). Two main assumptions needed to be made for these simulations:

(i) the dose exposure increase for higher doses of esomeprazole, and (ii) the increase in exposure from single dose to steady state.

The scenarios simulated were as follows:

- Scenario one assumed (i) linear (or dose proportional) increase in AUC with dose escalation and (ii) no change in AUC with repeated dosing.
- Scenario two assumed (i) maximum increase in AUC with dose escalation and (ii) maximum increase in AUC with repeated dosing.
- Scenario three was a compromise between the first two scenarios and possibly a best guess estimation of exposures for pregnant patients.

11 RESULTS

11.1 Pharmacokinetic data

From the treatment arm of the clinical trial involving pregnant patients with early-onset preeclampsia, i.e. the PIE trial, 10 patients who underwent pharmacokinetic sampling were included in this analysis. From the healthy volunteer eso-panto and eso-rabe studies by Hunfeld *et al*., a pooled dataset contributed 26 subjects. The non-pregnant group included participants of both sexes, 65% of whom were female. The median weight of pregnant patients was 98.8 kg and that of non-pregnant subjects was 69 kg. Summary characteristics of subjects from both studies are shown in Table 4.

The pregnant data consisted of 96 observations of esomeprazole. All pre-dose observations and ~13% of post-dose observations were BLQ. During modelling, 10% of post-dose observations were identified as outliers by means of GOF plots, resulting in unreasonably high residual error in the model, and were subsequently ignored.

The non-pregnant data consisted of 266 day1 observations for the 26 subjects. For 19 of these subjects, day5 samples were also available, consisting of 135 observations. All pre-dose observations and ~13% of post-dose observations were below the limit of quantification.

Table 4. Summary of subject characteristics of pregnant patients (Cluver et al., 2018) and non- pregnant healthy subjects (Hunfeld et al., 2010, 2012).

Parameter	**Pregnant (Cluver *et al.*) (n = 10)**	**Non-pregnant (Hunfeld *et al.*) (n = 26)**
Age, years, median (range)	30 (21-43)	21 (18-27)
Weight, kg, median (range)	98.8 (56-126)	69 (54-89)
BMI, kg/m^2, median (range)	37.6 (21.6-47.9)	21.4 (18.3-27.5)
Gestational age, weeks, median (range)	29 (26-31)	-
No. of males (%) No. of females (%)	0 (0) 10 (100)	9 (35) 17 (65)
Genotype (no.) Poor metabolizer HetEM HomEM	N/A	 1 11 14
Race (no.) Caucasian Black	 3 7	 24 2
HetEM = heterozygous Extensive Metabolizer HomEM = Homozygous Extensive Metabolizer		

11.2 Exploratory data analysis

Day1 pharmacokinetic profiles of esomeprazole and its metabolites, esomeprazole sulfone and 5-hydroxy esomeprazole, from the pregnant group are presented in Figure 5. In the pregnant patients, absorption is fast with peak concentrations around 2 hours. The profiles are regular, although some double peaks during absorption can be seen for patients 4 and 5. Unexpected high concentrations of esomeprazole and its metabolites could be observed at the last sampling time point for patient 8 and only for the hydroxy metabolite for patient 6. Concentrations for esomeprazole are mostly larger than that of its metabolites, except for patient 6 and patient 10, in whom esomeprazole sulfone peak concentrations appear to be higher than that of the parent drug.

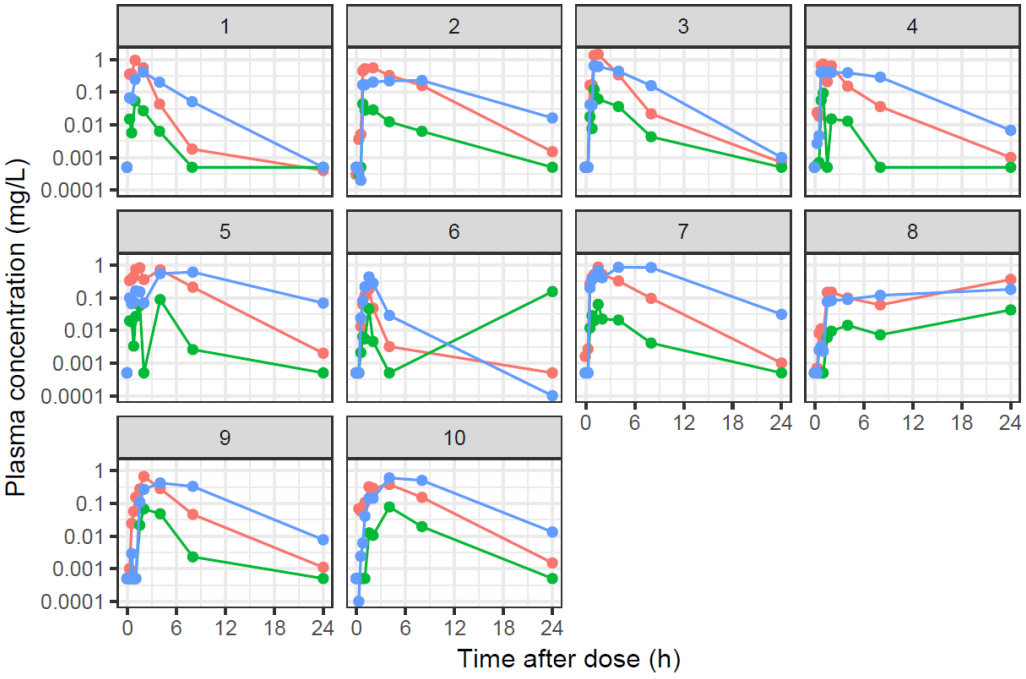


Figure 5. Concentration-time day1 profiles of pregnant patients with early-onset preeclampsia. Red, green, and blue profiles are of esomeprazole, and its hydroxy and sulfone metabolites, respectively.

Day1 pharmacokinetic profiles of esomeprazole and its metabolites, esomeprazole sulfone and 5-hydroxy esomeprazole, from the non-pregnant group are presented in Figure 6. Absorption is much slower in these profiles, with some subjects seemingly having no absorption for up to 3 to 4 hours and some subjects showing pronounced double peaks.

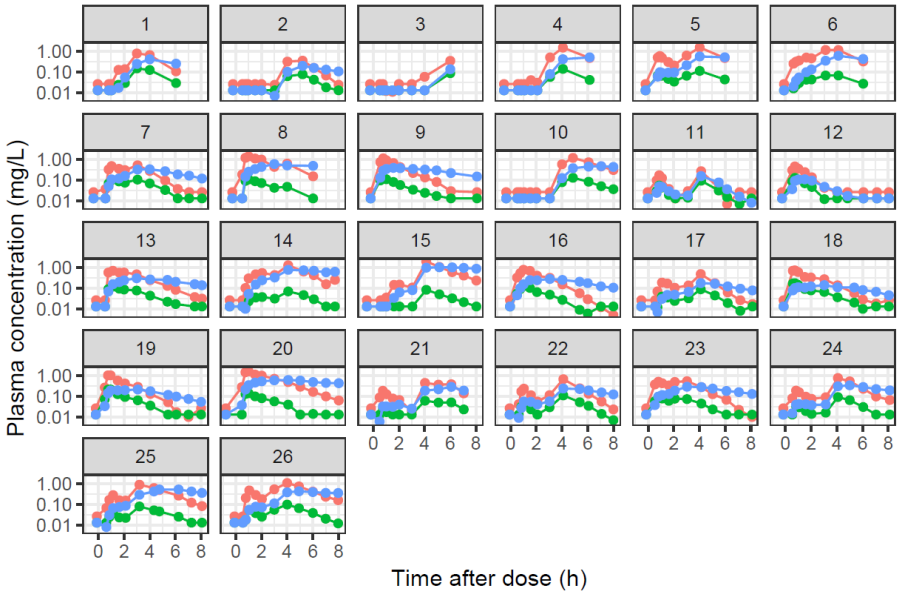


Figure 6. Concentration-time day1 profiles of healthy, non-pregnant subjects. Red, green, and blue profiles are of esomeprazole, and its hydroxy and sulfone metabolites respectively. Observations that are below the limit of quantification are plotted as half the lower limit of quantification.

On day5, as shown in Figure 7, double peaks are not seen for profiles from the same subjects shown in Figure 6.

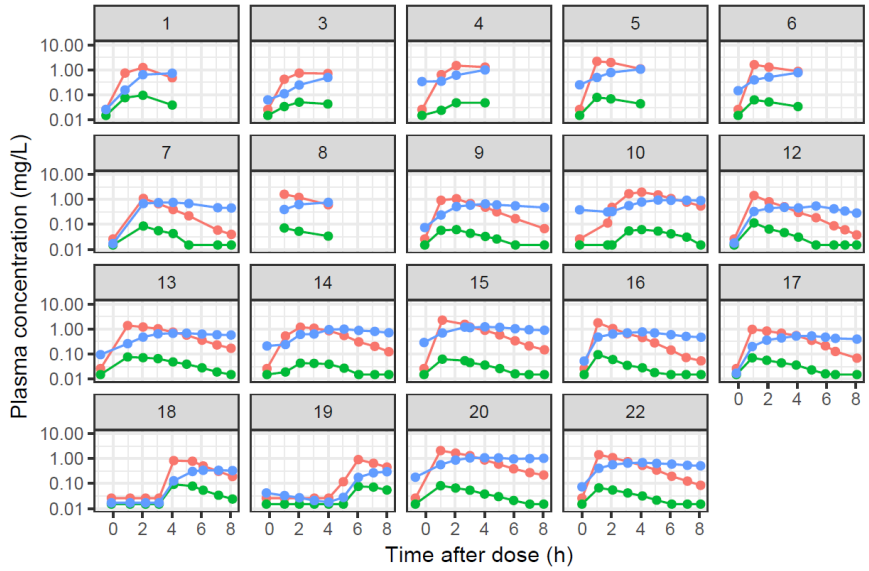


Figure 7. Concentration-time day5 profiles of healthy, non-pregnant subjects. Red, green, and blue profiles are of esomeprazole, and its hydroxy and sulfone metabolites respectively. Observations that are below the limit of quantification are plotted as half the lower limit of quantification.

11.3 Model description

Day1 esomeprazole data of the pregnant patients was best described by a one compartment disposition model with first-order elimination and transit absorption compartments. A schematic of this model is presented in Figure 8.

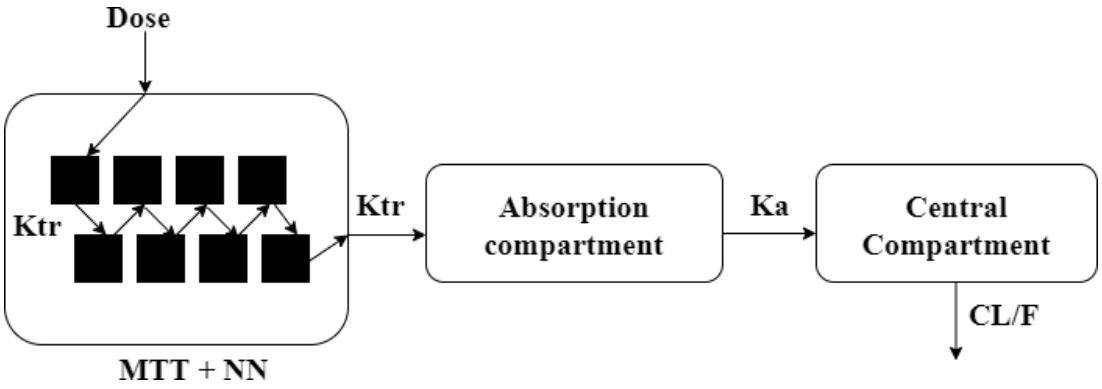


Figure 8. Schematic of esomeprazole model in pregnant patients with early-onset preeclampsia. MTT= mean transit time, NN = number of transit compartments, Ktr = transfer rate between transit compartments, Ka = absorption rate constant, CL/F = apparent clearance.

Testing the inclusion of a second compartment did not improve the model. There was an improvement of the OFV with transit absorption compartment model (a decrease in OFV of 7.5 points compared to lag absorption (P-value < 0.05)). The number of transit compartments (NN) was estimated to a large number and was fixed to 50 (without resulting in significant worsening of fit) to make the parameter estimates more stable (Savic *et al*., 2007). Adding allometric scaling as weight on apparent clearance and volume of distribution improved the OFV by 10.5 points compared to no scaling. Using fat-free mass as an alternative descriptor of body size did not improve the fit. The results of parameter estimates from NONMEM were compatible with those from Monolix. The final model parameter estimates in the model are presented in Table 5.

Table 5. Parameter estimates of esomeprazole model for pregnant patients with early-onset preeclampsia.

Description	**Unit**	**Typical value (95% CI)[a]**	**RSE%**
Absorption rate constant	h^{-1}	1.54 (0.486-10.4)	54
Clearance[b]	L/h	19.2 (14.2-26.0)	15
Volume of distribution[b]	L	44.2 (29.9-56.6)	13
Bioavailability	-	1 FIXED	-
Number of transit compartments	-	50 FIXED	-
Absorption mean transit time	h	0.503 (0.378-0.668)	13
Inter individual variability			
KA (%CV)[c]	%	269.2 (87.6-2389.5)	29
CL (%CV)[c]	%	41.2 (12.4-58.4)	32
MTT (%CV)[c]	%	37.3 (13.4-56.9)	30.1
Residual error			
Proportional error	%	34.2 (24.8-41.1)	14
Additive error	mg/L	0.015 (0.00158-0.0337)	39

[a]95% confidence intervals (CI) were obtained by bootstrap (n=1000)

[b]Allometric scaling was used for clearance and volume of distribution (by weight); the typical values reported are for the median weight of 98.8 kg as reported in Table 4.

[c]IIVs were assumed to be log-normally distributed and are reported as approximate %CVs.

11.4 Model Evaluation

GOF plots for the final model are shown in Figure 9. There is no apparent pattern for the conditional weighted residual versus time and conditional weighted residual versus population predictions and points are scattered around the line of unity for the observed versus population predicted values as well as observed versus individual predicted values. These plots do not highlight any misspecification in the model. A VPC for the final model is shown in Figure 10, showing that the 5th, 50th, and 95th percentiles of the data agree with the 95% confidence interval of the simulations for each percentile.

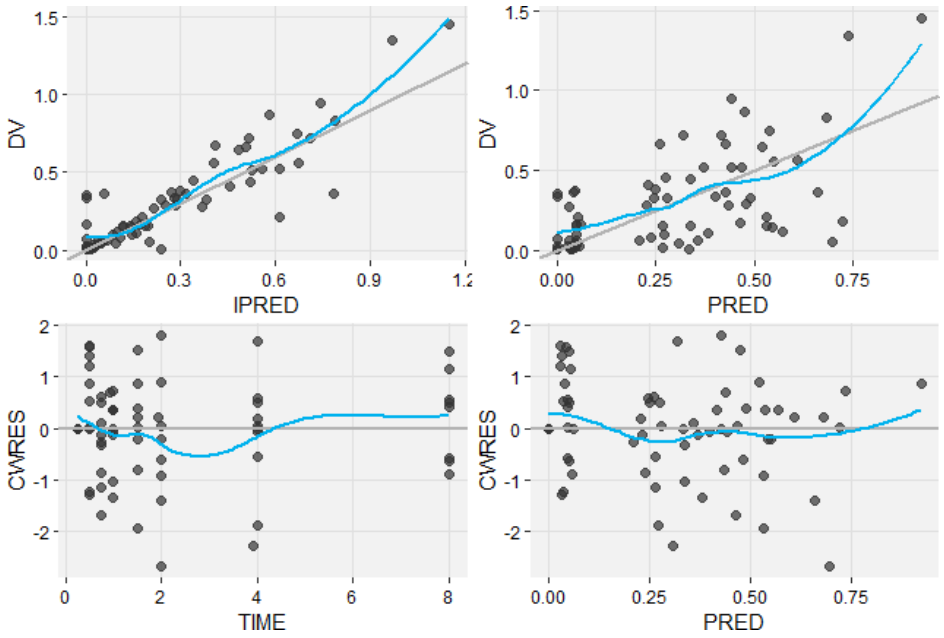


Figure 9. Goodness-of-fit plots for final model. DV = observations, IPRED = individual predictions, PRED = population predictions, CWRES = conditional weighted residuals. Grey line is line of identity and blue line is a smooth line to indicate general trend.

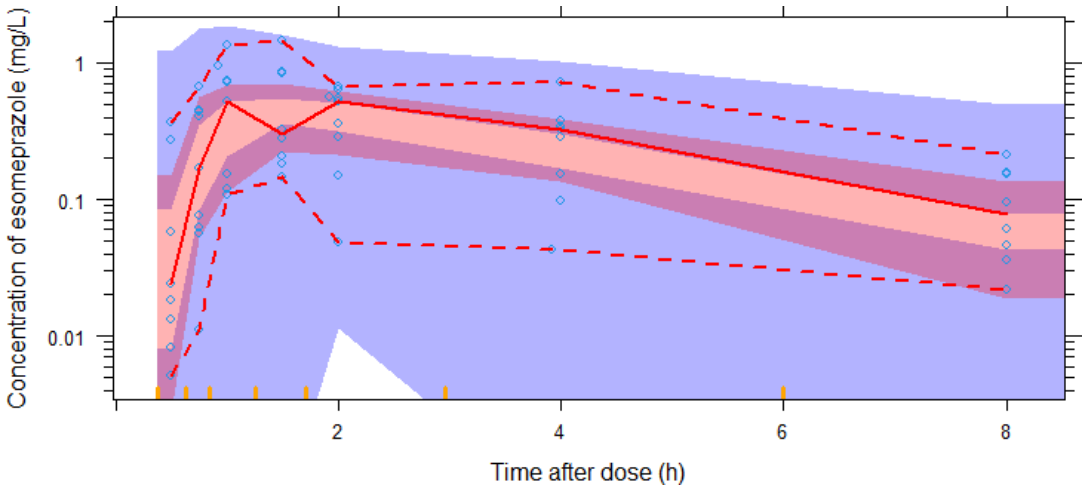


Figure 10. Visual predictive check for final model. Blue dots are observations, red lines are 5th, 50th, and 95th percentiles for the observations, and shaded areas are respective simulated 95% confidence intervals for each percentile. Yellow ticks at the bottom indicate bins.

11.5 Exposure comparisons between pregnant and non-pregnant

A comparison of the AUCs between pregnant and non-pregnant was performed to get some insight into how the metabolic pathways may change due to pregnancy (day1 pregnant and non-pregnant comparisons) and autoinhibition (day1 pregnant and day 5 non-pregnant comparisons). Table 6 shows AUC values for esomeprazole and AUC_m/AUC_p values for pregnant and non-pregnant. Profiles for which the terminal half-life could not be calculated were excluded by the software and thus some comparisons had fewer profiles. Due to these exclusions, the sub-optimal sampling schedule and other differences between the studies, we did not aim for this comparison of AUC values to be a fully quantitative analysis, but rather as an opportunity to get a glance at the trends in how exposure differs and identify possible metabolic pathway changes between pregnant and non-pregnant.

Table 6. Exposure and metabolite to parent exposure ratio comparisons between pregnant patients (Cluver et al) and non-pregnant subjects (Hunfeld et al). Number of profiles included in each study are shown below each value.

	Median (interquartile range)		
Study	**AUC_{eso} mg·h/L**	**AUC_{hyd}/AUC_{eso}**	**AUC_{sulf}/AUC_{eso}**
Pregnant day1	2.52 (1.85-3.05)	0.0543 (0.0500-0.0914)	2.00 (1.35-2.61)
No of subjects	7	7	7
Non-pregnant day1	1.94 (1.50-3.02)	0.188 (0.156-0.227)	0.700 (0.636-1.00)
No of subjects	20	14	14
Non-pregnant day5	6.25 (4.38-8.69)	0.0777 (0.0569-0.108)	1.18 (0.981-1.58)
No of subjects	17	17	17
AUC_{eso} = esomeprazole exposure, AUC_{hyd}/AUC_{eso} = 5-hydroxy esomeprazole to esomeprazole ratio, AUC_{sulf}/AUC_{eso} = esomeprazole sulphone to esomeprazole ratio			

Day5 esomeprazole AUC of non-pregnant subjects was significantly larger (p-value < 0.05) than the esomeprazole AUC of both day1 pregnant patients and day1 non-pregnant subjects. Day1 esomeprazole AUC of pregnant patients was not significantly different from that of day1 non-pregnant subjects (p-value > 0.05) (Figure 11). The AUC comparisons were adjusted for weight (r = -0.197, p-value > 0.05) and age (r = -0.138, p-value > 0.05) through linear regression analysis and were significant for day5.

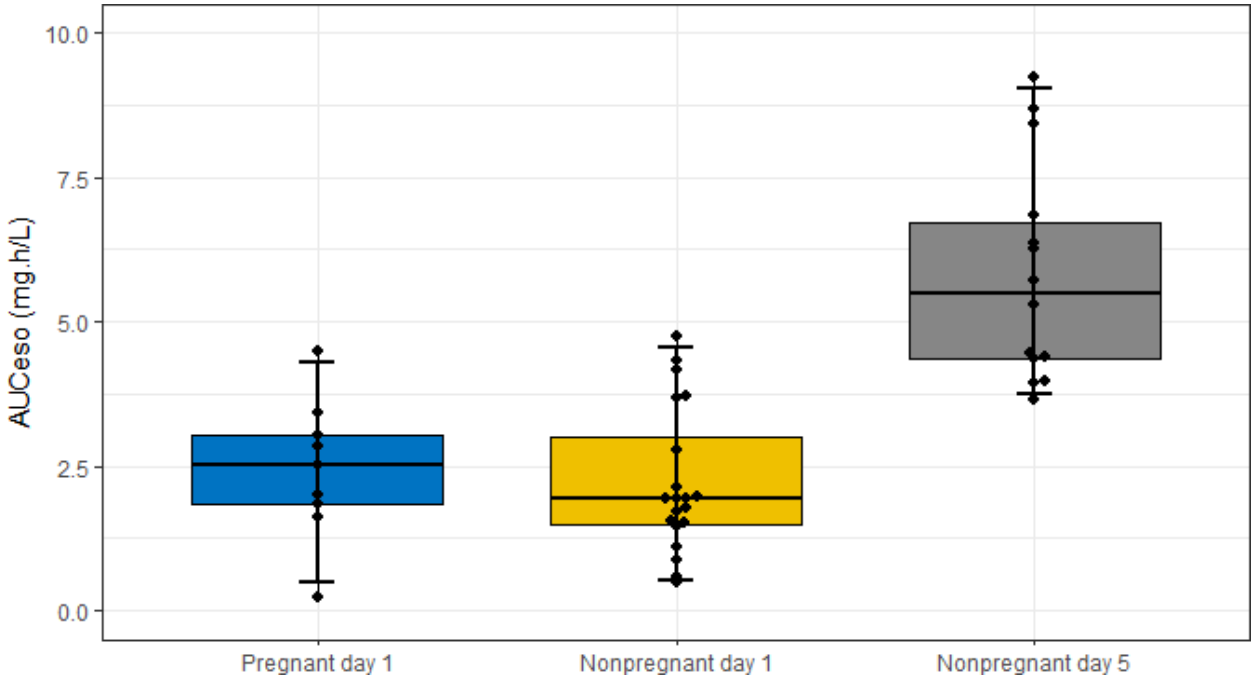


Figure 11. Esomeprazole AUC stratified by pregnant day1 (blue box), non-pregnant day1 (yellow box), and non-pregnant day5 (grey box). The dots represent individual values. Whiskers show the 2.5th and 97.5th percentiles.

Day1 AUC_{hyd}/AUC_{eso} of non-pregnant subjects was significantly larger (p-value < 0.05) than those of both day1 pregnant patients and day5 non-pregnant subjects. Day1 AUC_{hyd}/AUC_{eso} of pregnant subjects was not significantly different (p-value > 0.05) from that of day5 non- pregnant subjects (Figure 12).

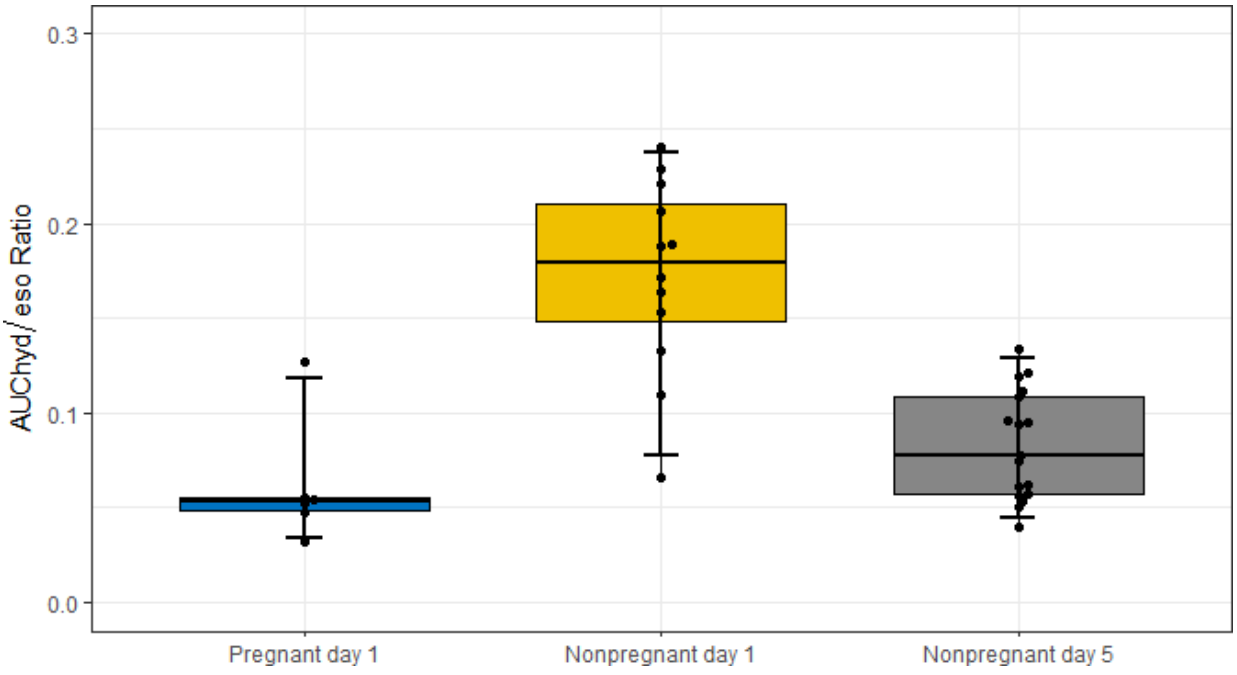


Figure 12. Ratio of 5-hydroxy esomeprazole to esomeprazole AUC (AUC_{hyd}/AUC_{eso}) stratified by pregnant day1 (blue box), non-pregnant day1 (yellow box), and non-pregnant day5 (grey box). The dots represent individual values. Whiskers show the 2.5th and 97.5th percentiles.

Day1 AUC_{sulf}/AUC_{eso} of pregnant patients was significantly larger (p-value < 0.05) than those of both day1 non-pregnant and day5 non-pregnant subjects. Day5 AUC_{sulf}/AUC_{eso} of non-pregnant subjects was significantly larger (p-value < 0.05) than those of day1 non-pregnant subjects (Figure 13).

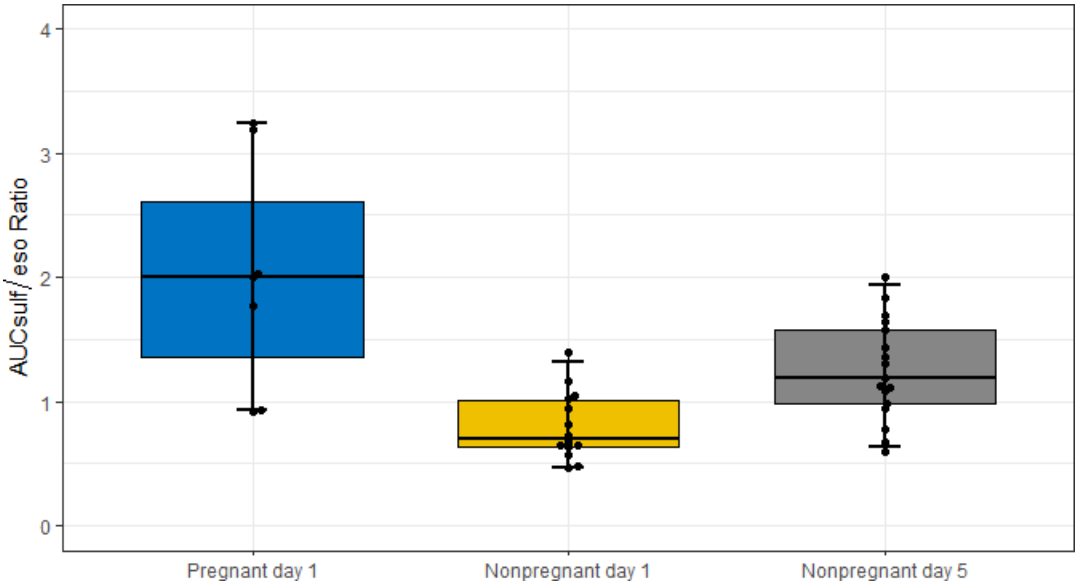


Figure 13. Ratio of esomeprazole sulphone to esomeprazole AUC (AUC_{sulf}/AUC_{eso}) stratified by pregnant day1 (blue box), non-pregnant day1 (yellow box), and non-pregnant day5 (grey box). The dots represent individual values. Whiskers show the 2.5th and 97.5th percentiles.

11.6 C_{max} and AUC comparisons between preclinical *in vitro* and *in vivo* pregnant studies

The lowest preclinical concentration found to be effective for lowering sFlt-1 and sEng was 17.3 mg/L, and this was taken as the preclinical target for this study. When adjusted for protein binding (f_u = ~5.3%), the unbound preclinical effective concentration is 0.917 mg/L. From this, unbound esomeprazole AUC ($fAUC_{0-24}$) in the preclinical study was calculated to be 9.29 mg·h/L.

Median C_{max} on day1 in the pregnant women was 0.695 mg/L. When adjusted for protein binding in pregnancy (f_u = ~ 5.02%), the unbound median C_{max} in the pregnant patients is 0.0349 mg/L. The preclinical unbound concentration of 0.917 mg/L is more than 25-fold higher than the unbound C_{max} in pregnant.

Median AUC (AUC_{0-24}) observed on day1 in the pregnant women study was 2.52 mg·h/L. Assuming the binding to be 5.02%, this gives unbound esomeprazole AUC ($fAUC_{0-24}$) of 0.127 mg·h/L. Preclinical target $fAUC_{0-24}$ (9.29 mg·h/L) is more than 70-fold higher than the $fAUC_{0-24}$ (0.053 mg·h/L) achieved with 40 mg dose of esomeprazole in pregnant patients.

The above median AUC_{0-24} for the pregnant patients was the AUC on day1, but on day5 the value may be larger due to possible auto-inhibition. To compare the expected exposure in pregnancy on day5, or with increasing doses of esomeprazole, simulations were used.

11.7 Simulation results

Simulations were performed for an 86-kg pregnant patient which was considered a representing typical weight for preeclampsia patients.

- Scenario one followed (i) dose proportional increase in AUC with dose escalation and (ii) no change in AUC with repeated dosing.
- For scenario two, values to represent maximum increase of AUC were obtained from oral esomeprazole literature data of non-pregnant subjects given in Table 2 and were put as an effect on bioavailability (Andersson, Hassan-Alin, *et al.*, 2001). The value used to depict (i) maximum increase in AUC with dose escalation was 1.61 and the value used to depict (ii) maximum increase in AUC with repeated dosing was 2.59.
- For scenario three, values to represent increase of AUC were obtained from intravenous esomeprazole literature data of non-pregnant subjects given in Table 2 (Andersson, Hassan-Alin, *et al.*, 2001). These values were put as an effect on bioavailability. The value used to depict (i) AUC increase with dose escalation was 1.3 and the value used to depict (ii) AUC increase with repeated dosing was 1.86.

The simulations results are shown in Table 7 and depicted in Figure 14.

Table 7. Simulation results for dose escalation and repeated dosing of esomeprazole based on model developed for patients with early-onset preeclampsia.

	Day 1	Steady state	
Dose	**AUC_{0-24} ($fAUC_{0-24}$) mg·h/L**	**AUC_{0-24} ($fAUC_{0-24}$) mg·h/L**	**C_{max} (fC_{max}) mg/L**
Scenario one – linear dose effect and no auto-inhibition			
40 mg OD	2.31 (0.116)	2.31 (0.116)	0.625 (0.0314)
40 mg BID	4.62 (0.232)	4.62 (0.232)	0.629 (0.0316)
80 mg OD	4.62 (0.232)	4.62 (0.232)	1.25 (0.0628)
80 mg BID	9.21 (0.462)	9.25 (0.464)	1.26 (0.0633)
120 mg OD	6.94 (0.348)	6.94 (0.348)	1.87 (0.0939)
120 mg BID	13.82 (0.694)	13.87 (0.696)	1.89 (0.0949)
Scenario two – maximum effect on dose and on auto-inhibition			
40 mg OD	2.31 (0.116)	5.99 (0.301)	1.62 (0.0813)
40 mg BID	4.62 (0.232)	11.98 (0.601)	1.63 (0.0818)
80 mg OD	7.44 (0.373)	19.42 (0.975)	5.25 (0.264)
80 mg BID	14.83 (0.744)	38.84 (1.95)	5.28 (0.265)
120 mg OD	11.17 (0.561)	29.13 (1.46)	7.87 (0.395)
120 mg BID	22.24 (1.12)	58.26 (2.92)	7.92 (0.398)
Scenario three – intermediate effect on dose and on auto-inhibition			
40 mg OD	2.31 (0.116)	4.3 (0.216)	1.16 (0.0582)
40 mg BID	4.62 (0.232)	8.59 (0.431)	1.17 (0.0587)
80 mg OD	6.01 (0.302)	11.19 (0.562)	3.02 (0.152)
80 mg BID	11.97 (0.601)	22.38 (1.12)	3.04 (0.153)
120 mg OD	9.02 (0.453)	16.78 (0.842)	4.54 (0.228)
120 mg BID	17.96 (0.902)	33.57 (1.69)	4.56 (0.229)

OD = once a day, BID = twice a day, C_{max} = total C_{max}, fC_{max} = unbound C_{max},

AUC_{0-24} = total AUC, $fAUC_{0-24}$ = unbound AUC

Simulated $fAUC_{0-24}$ is compared to preclinical $fAUC_{0-24}$ of 9.29 mg·h/L and simulated fC_{max} to preclinical fC_{max} of 0.917 mg/L

A dose of 120 mg BID is expected to achieve C_{max} (total concentration) at steady-state of 1.89, 7.92, and 4.56 mg/L for scenario one, two, and three, respectively, while the expected steady-state AUC is 13.87, 58.26, and 33.57 mg·h/L for scenario one, two, and three, respectively.

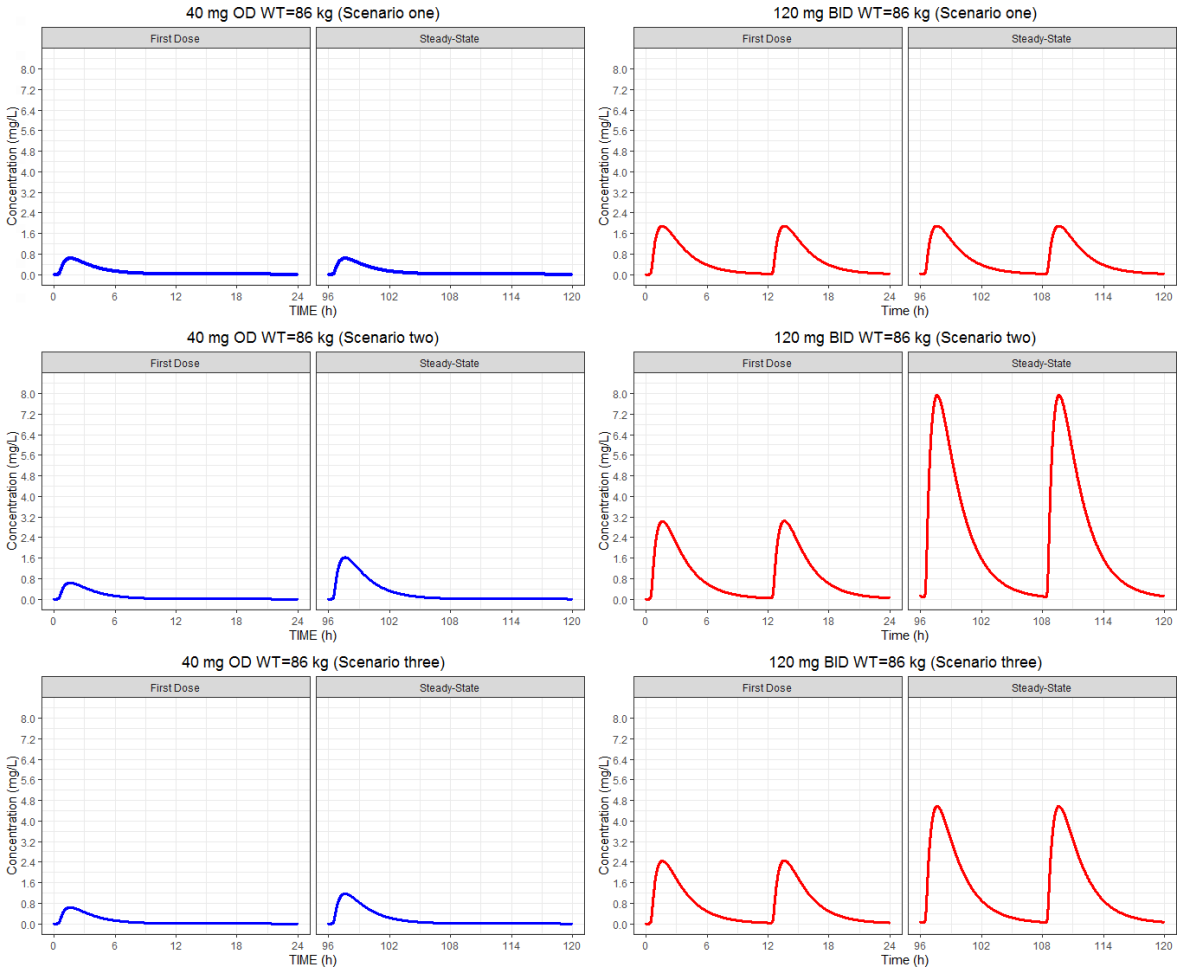


Figure 14. Results for the three scenarios simulated with single and repeated dosing of esomeprazole at 40 mg daily (left, first dose scenario one is based on observed values in patients) and 120 mg twice daily (right, based on simulations) dose. OD = once a day, BID = twice a day.

After adjusting for protein binding in pregnant patients (5.02%), the 120 mg BID simulation values can be compared to the preclinical targets.

If the pharmacokinetic target is C_{max}, which assumes that achieving the *in vitro* concentrations even if for a short time is enough to produce the observed effect, an effective unbound concentration of 0.917 mg/L must be achieved (derived from the preclinical target concentration of 17.3 mg/L or 50 µM and adjusting for protein binding). The predicted steady state C_{max} values at the highest dose were short of the target, i.e. 0.0949, 0.398, 0.229 mg/L for scenario one, two, and three, respectively.

When targeting a $fAUC_{0\text{-}24}$ value of 9.29 mg·h/L in the preclinical study, the $fAUC_{0\text{-}24}$ values predicted at steady state with the simulations were also much lower, i.e. 0.696, 2.92, and 1.69 mg·h/L for scenario one, two, and three, respectively.

The predicted exposures at 120 mg BID are much below the putative targets. Assuming C_{max} is the pharmacokinetic target, to achieve preclinical effective unbound concentrations of 0.917 mg/L (derived from the target concentration of 17.3 mg/L or 50 µM), a dose of approximately 500 mg BID would be required. If AUC is assumed to be the pharmacokinetic target, doses higher than 500 mg BID would be needed to achieve the target preclinical $fAUC_{0-24}$. Such high doses have not been used clinically before and may be unsafe.

12 DISCUSSION

12.1 Population pharmacokinetic model

The population pharmacokinetic characteristics of esomeprazole were described in patients with early-onset preeclampsia. The population pharmacokinetic model reported CL/F and V_d/F of 19.2 L/h and 44.2 L, respectively. These values are greater than values from other population pharmacokinetic models of single dose 40 mg oral esomeprazole in non-pregnant: 8.66 L/h and 18.7 L in patients with gastroesophageal reflux disease (J. Li, T. Lind, 2005), ~7.5 L/h and ~16 L in healthy subjects (Nagase *et al.*, 2020), and 9.61 L/h and 9.42 L in healthy subjects (Hunfeld *et al.*, 2010). This increase in both CL/F and V_d/F could be due to lower bioavailability in pregnant patients. The larger V_d/F in our model could also be due to lower plasma protein binding since albumin levels decrease by 31% at the final trimester of pregnancy (Ke, Rostami-Hodjegan, *et al.*, 2014). This could possibly increase distribution to tissues. Higher V_d, ~27 L, has been previously reported for intensive care patients with hypoalbuminemia after single 40 mg intravenous esomeprazole, compared to ~16 L in healthy subjects (Wilder-Smith *et al.*, 2005), which further supports this assumption (Tian *et al.*, 2018).

Our model shows large interindividual variability during absorption, which is consistent with another report (Nagase *et al.*, 2020). This could be due to the interindividual differences in gastric pH. Most of the day1 non-pregnant profiles showed multiple peaks in the absorption phase, but not all subjects did. The double peaks could be due to mealtimes for these subjects, which were given five minutes post dose, or due to the MUPS formulation used. The label of the 40 mg esomeprazole MUPS formulation claims that it can be taken with or without food, and indeed, once the drug was absorbed, concentrations in line with that in the PIE study were achieved. However, the onset of absorption seems to be very erratic when the drug is given shortly before a meal. This is physiologically plausible, since there could be an initial fast release in the stomach and absorption from the small intestine of a fraction of the dose, followed by a slower release of the remaining fraction due to the presence of food. Some of the pellets might be coated with food contents and

transit to the small intestine more slowly. Day5 non- pregnant profiles showed no double peaks during the absorption phase despite the same mealtimes on day5 as on day1. This could be because esomeprazole increases pH in the gastric environment, and this might facilitate faster release in the stomach and thereby dissolution and absorption from the small intestine.

12.2 Comparison of AUC_m/AUC_p between pregnant and non-pregnant

The model developed describes the pharmacokinetics of esomeprazole in pregnant patients with preeclampsia on the first day of dosing, and the expected effect of auto-inhibition on clearance needs to be accounted for to predict steady-state exposures. Data on steady-state exposures in pregnancy was not available, but it is known from literature that the changes in esomeprazole pharmacokinetics with repeated dosing are due to changes in metabolism (Andersson, Hassan-Alin, *et al.*, 2001). Therefore, metabolite to parent AUC ratios, for which we had results both in pregnant and non-pregnant subjects, were compared to infer what happens to the metabolism of esomeprazole in pregnancy and with repeated dosing.

In non-pregnant subjects, CYP2C19 activity decreases from day1 to day5 due to autoinhibition (Andersson, Hassan-Alin, *et al.*, 2001), therefore the similarities between day1 pregnant and day5 non-pregnant AUC_{hyd}/AUC_{eso} ratios could be indicating a downregulation of CYP2C19 in pregnancy. Day1 AUC_{sulf}/AUC_{eso} ratios of pregnant patients were significantly higher than both day1 and day5 ratios of non-pregnant subjects which could be explained by an upregulation of CYP3A4 as well as a downregulation of CYP2C19 in pregnancy. This is in line with what is known in literature about upregulation of CYP3A4 in pregnancy (Isoherranen and Thummel, 2013; Papageorgiou, Grepper and Unadkat, 2013) and could be indicative that metabolism in pregnancy shifts dominantly to the CYP3A4 pathway. This shift in the metabolic pathway of esomeprazole to CYP3A4 in pregnancy might not significantly affect clearance because, even if CYP3A4 increases, CYP2C19, which at lower doses is responsible for most of the clearance, is downregulated. The upregulation of CYP3A4 in pregnancy may also decrease bioavailability since CYP3A4 is dominantly present in the gut. This could explain the model-reported higher values for CL/F and V_d/F in the pregnant patients. The complex interplay between changes in bioavailability and metabolism in pregnancy could be the reason why clearance appears higher in pregnancy while the AUC between pregnant and non-pregnant subjects on single dosing is similar.

12.3 Dose escalation and repeated dosing in pregnant patients

Based on the results of the AUC_m/AUC_p comparisons, one can speculate about the pharmacokinetics of esomeprazole in pregnancy. In non-pregnant, there is more than dose proportional increase in AUC with increasing doses, indicating a saturation of clearance and/or first-pass metabolism. Since the K_m of esomeprazole for CYP2C19 is much lower compared to that of CYP3A4, it can be surmised that CYP2C19 is the metabolic pathway that gets saturated (Andersson, Hassan-Alin, *et al*., 2001). In pregnancy, CYP2C19 is downregulated and is expected to play a smaller role in metabolism of esomeprazole (Ke, Nallani, *et al*., 2014). Therefore, the nonlinear increase in AUC with increasing doses in pregnant is expected to be less pronounced than in non-pregnant since the nonlinear increase is due to saturation of CYP2C19.

The increase in AUC with repeated dosing in pregnancy is not expected to be as significant as in non-pregnant since the increase is due to autoinhibition of CYP2C19.

12.4 Simulations

Out of the three scenarios, scenario three best describes the changes with dose escalation and with repeated dosing in pregnancy. The similarity of AUC between 40 mg IV and 40 mg oral formulations on repeated dosing could be indicating that with oral capsules at 40 mg repeated dosing saturation of first-pass metabolism is nearly achieved. This indicates that the values used to depict exposure increase with dose escalation and to depict exposure increase with repeated dosing could be overestimating the exposures when used for doses higher than 40 mg. However, even with this overestimation, simulations for the highest dose used clinically, i.e. 120 mg BID, showed that the preclinical target concentration would not be achieved with this dose in pregnant patients and quite high doses would be required to reach the preclinical $fAUC_{0-24}$ or C_{max}.

12.5 Limitations

Our study suffers from a number of limitations. It was not possible to jointly model the pregnant and non-pregnant data, as initially planned, due to the erratic absorption in the non-pregnant study, likely caused by food intake shortly after esomeprazole dose. The large variability in absorption and double peaks, which are quite complicated to model, would have made it difficult to then separate the effect of food from that of pregnancy on bioavailability.

Still, the AUC from the non-pregnant studies can be used for comparisons with pregnant since exposures and concentrations achieved are similar on day1. Therefore, metabolite to parent AUC ratios were compared to investigate and confirm the potential changes in activities of CYP2C19 and CYP3A4 during pregnancy. The Mann-Whitney-Wilcoxon test was used for all AUC_m/AUC_p comparisons, although day1 and day5 non-pregnant data had some common subjects. This test has less power than a paired statistical test, but significant differences between day1 and day5 non-pregnant esomeprazole AUCs were detected nonetheless, indicating this did not negatively affect the analysis. The sampling schedule for the non-pregnant studies was up to 8 hours which was not optimal, but a 24-hour sample was included into the datasets with pre-dose concentrations since it is known that esomeprazole concentrations decrease and are undetectable by 24 hours. During the AUC calculations, pharmacokinetic profiles for which the terminal phase could not be calculated were excluded by the software. This could have introduced bias in the AUC analysis; however, these AUC_m/AUC_p comparisons were not meant to be rigorously quantitative but were rather used to understand trends in the effect of pregnancy and inhibition with repeated dosing in the metabolic pathways.

The amount of data in pregnant patients was small and only day1 data was available. To predict the nonlinear changes in exposure with repeated dosing, AUC increases with repeated dosing had to be extrapolated from literature data. Additionally, only low dose, i.e. 40 mg, data was available for the pregnant patients, due to which exposure changes for higher doses had to be extrapolated from literature data. Although this is not ideal, for the purposes of this analysis, we expect that these extrapolations have enabled us to do a rough comparison of pregnant exposures with those of the preclinical study by Onda *et al*.

In non-pregnant subjects, at higher oral doses of esomeprazole, there is saturation of clearance and increase in bioavailability. However, for simplicity, the expected increase in exposure with dose escalation and with repeated dosing for all the simulated scenarios was put as an effect only on bioavailability. This could mean that the shape of the pharmacokinetic profile has not been adequately captured and could possibly underestimate the increase in AUC simulated for scenario two and scenario three if auto-inhibition effect on clearance is significant. On the other hand, it was also observed that for oral 40 mg esomeprazole, oral AUC is similar to IV AUC at steady state, indicating that saturation of first-pass metabolism is nearly achieved with 40 mg at steady state (Table 2).

This shows that our simulated scenarios are rather optimistically overestimating the increase in exposure.

In vitro-in vivo extrapolation is a difficult task, and it is inevitable that a number of unknown factors that cause differences between the *in vitro* experiment and the human body cannot be correctly adjusted for. In this analysis, in comparing preclinical exposure with clinical exposure we attempted to adjust for protein binding and estimated the effect of esomeprazole degradation. This was done based on retrospective information obtained from different sources and was not an exact extrapolation. Additionally, it is unknown which pharmacokinetic parameter, i.e. C_{max} or AUC, is related to the efficacy of PPIs in preeclampsia. The simulations show that the preclinical target is more achievable if the pharmacokinetic metric which is linked to effect is C_{max}. In the preclinical study, they incubated tissues with esomeprazole at a concentration of 17.3 mg/L, then performed measurements of efficacy after 24 hours. Since it is unclear whether incubation time is an important component of efficacy, we considered both the AUC over 24 hours of incubation and the instantaneous concentration as $C_{max.}$

These limitations indicate that further research is required to make decisions on dosage regimen of esomeprazole for preeclampsia. Further research is needed both in the form of preclinical studies and pharmacokinetic-pharmacodynamic modelling to identify the target pharmacokinetic metric. Preclinical studies with different duration could investigate how efficacy changes with different experimental conditions, i.e. by exposing placental tissue to esomeprazole over varying incubation periods and observing the effect over longer time. Identifying the type of binding esomeprazole has with the preeclampsia target will be useful to make dosing decisions. If binding with target is covalent, a once-off high concentration could be enough to result in effect for days. In such a scenario, the intravenous route of administration would be preferred because higher peak concentrations could be achieved, and less frequent dosing would be required. Further investigation is also needed to investigate whether any of the metabolites of esomeprazole show efficacy in preeclampsia.

Another alternative also needs to be explored going forward, which is that perhaps a PPI other than esomeprazole could be investigated. Although esomeprazole was chosen from the *in vitro* experiment because of its high potency, there might be some merit in looking at the other PPIs that were shown to have efficacy in the preclinical study.

13 CONCLUSIONS

A population pharmacokinetic model of esomeprazole after single 40 mg dose was developed for patients with early-onset preeclampsia from the preeclampsia intervention with esomeprazole (PIE) trial. V_d/F and CL/F derived from this model were higher than those previously reported for healthy subjects.

Metabolite-to-parent ratio comparisons between pregnant patients and non-pregnant healthy subjects were performed. These showed higher AUC_{sulf}/AUC_{eso} in pregnant patients compared to day 1 and day 5 in non-pregnant subjects. AUC_{hyd}/AUC_{eso} was lower in pregnant patients than in non-pregnant on day one while it was similar to that in non-pregnant on day five. These ratios indicate that in pregnancy, metabolism has been shifted to the CYP3A4 pathway which means the nonlinear pharmacokinetic changes with repeated dosing are not expected to be present in the pregnant patients to the same extent as in non-pregnant subjects.

C_{max} and AUC_{0-24} obtained in pregnant women were compared with those from the *in vitro* preclinical studies where esomeprazole showed efficacy for preeclampsia after adjusting for protein binding. C_{max} and AUC_{0-24} obtained from the *in vitro* study were 25-fold and 70-fold higher than those in the pregnant patients.

Furthermore, simulations were performed with the model for higher doses of esomeprazole and these showed that that the preclinical target concentration and exposure could not be achieved with the highest dose that has been clinically used for esomeprazole.

Further studies are required to investigate the pharmacokinetic marker that best describes the relation between concentration and effect.

14 REFERENCES

Acharya, C. *et al.* (2016) 'A diagnostic tool for population models using non-compartmental analysis: The ncappc package for R', *Computer Methods and Programs in Biomedicine*. Elsevier Ireland Ltd, 127, pp. 83–93. doi: 10.1016/j.cmpb.2016.01.013.

Ahmed, A. *et al.* (2000) 'Induction of placental heme oxygenase-1 is protective against TNFalpha-induced cytotoxicity and promotes vessel relaxation.', *Molecular medicine (Cambridge, Mass.)*, 6(5), pp. 391–409. doi: 10.1007/BF03401783.

Anderson, B. J. and Holford, N. H. G. (2008) 'Mechanism-based concepts of size and maturity in pharmacokinetics', *Annual Review of Pharmacology and Toxicology*, 48, pp. 303–332. doi: 10.1146/annurev.pharmtox.48.113006.094708.

Anderson, B. J. and Holford, N. H. G. (2009) 'Mechanistic basis of using body size and maturation to predict clearance in humans', *Drug Metabolism and Pharmacokinetics*, 24(1), pp. 25–36. doi: 10.2133/dmpk.24.25.

Andersson, T., Hassan-Alin, M., *et al.* (2001) 'Pharmacokinetic studies with esomeprazole, the (S)-isomer of omeprazole', *Clinical Pharmacokinetics*, 40(6), pp. 411–426. doi: 10.2165/00003088-200140060-00003.

Andersson, T., Röhss, K., *et al.* (2001) 'Pharmacokinetics and pharmacodynamics of esomeprazole, the S-isomerof omeprazole', *Alimentary Pharmacology and Therapeutics*, 15(10), pp. 1563–1569. doi: 10.1046/j.1365-2036.2001.01087.x.

Armaly, Z. *et al.* (2018) 'Preeclampsia: Novel mechanisms and potential therapeutic approaches', *Frontiers in Physiology*. Frontiers Media S.A., 9(JUL), pp. 1–15. doi: 10.3389/fphys.2018.00973.

BARBER, A. *et al.* (2001) 'Heme oxygenase expression in human placenta and placental bed: reduced expressionof placenta endothelial HO-2 in preeclampsia and fetal growth restriction', *The FASEB Journal*, 15(7), pp. 1158–1168. doi: 10.1096/fj.00-0376com.

Barnett, H. Y. *et al.* (2020) 'Methods for Non-Compartmental Pharmacokinetic Analysis With Observations Below the Limit of Quantification', *Statistics in Biopharmaceutical Research*. Taylor & Francis, 0(0), pp. 1–18. doi: 10.1080/19466315.2019.1701546.

Beal, S. L. (2001) 'Ways to fit a PK model with some data below the quantification limit', *Journal of Pharmacokinetics and Pharmacodynamics*, 28(5), pp. 481–504. doi: 10.1023/A:1012299115260.

Benoit, J. and Rey, É. (2011) 'Preeclampsia: Should Plasma Albumin Level Be a Criterion for Severity?', *Journalof Obstetrics and Gynecology Canada*. Elsevier Masson SAS, 33(9), pp. 922–926. doi: 10.1016/S1701-2163(16)35017-4.

Bulitta, J. B. and G. Holford, N. H. (2014) 'Non-Compartmental Analysis', in *Wiley StatsRef: Statistics Reference Online*. Chichester, UK: John Wiley & Sons, Ltd. doi: 10.1002/9781118445112.stat06889.

Chaiworapongsa, T. *et al.* (2014) 'Pre-eclampsia part 1: Current understanding of its pathophysiology', *Nature Reviews Nephrology*, 10(8), pp. 466–480. doi: 10.1038/nrneph.2014.102.

Cluver, C. A. *et al.* (2015) 'Double blind, randomized, placebo controlled trial to evaluate the efficacy of esomeprazole to treat early onset pre-eclampsia (PIE Trial): A study protocol', *BMJ Open*, 5(10), pp. 1–8. doi: 10.1136/bmjopen-2015-008211.

Cluver, C. A. *et al.* (2018) 'Esomeprazole to treat women with preterm preeclampsia: a randomized placebo-controlled trial', *American Journal of Obstetrics and Gynecology*. Elsevier Inc., 219(4), pp. 388.e1-388.e17. doi: 10.1016/j.ajog.2018.07.019.

Costantine, M. M. (2014) 'Physiologic and pharmacokinetic changes in pregnancy', *Frontiers in Pharmacology*. Frontiers Media SA, 5, p. 65. doi: 10.3389/fphar.2014.00065.

Cudmore, M. *et al.* (2007) 'Negative regulation of soluble Flt-1 and soluble endoglin release by heme oxygenase-1', *Circulation*, 115(13), pp. 1789–1797. doi: 10.1161/CIRCULATIONAHA.106.660134.

Dai, D. M. *et al.* (2017) 'Hematocrit and plasma albumin levels difference may be a potential biomarker to discriminate preeclampsia and eclampsia in patients with hypertensive disorders of pregnancy', *Clinica Chimica Acta*, 464(January 2013), pp. 218–222. doi: 10.1016/j.cca.2016.12.001.

Dean, L. (2019) 'Esomeprazole Therapy and CYP2C19 Genotype Drug class : Proton Pump Inhibitors', (Md), pp. 1–7.

Deepa, K. N., Kabir, S. and Amran, M. S. (2016) 'Fluorescence Spectroscopic Analysis of the Interaction Between Omeprazole and Bovine Serum', *World journal of pharmacy and pharmaceutical sciences*, 5(9), pp. 16–25. doi: 10.20959/wjpps20169-7533.

Dressman, J. B. and Thelen, K. (2009) 'Cytochrome P450-mediated metabolism in the human gut wall', *Journalof Pharmacy and Pharmacology*, 61(5), pp. 541–558. doi: 10.1211/jpp/61.05.0002.

English, F. A., Kenny, L. C. and McCarthy, F. P. (2015) 'Risk factors and effective management of preeclampsia', *Integrated Blood Pressure Control*, 8, pp. 7–12. doi: 10.2147/IBPC.S50641.

George, E. M. and Granger, J. P. (2013) 'Heme oxygenase in pregnancy and preeclampsia', *Current Opinion in Nephrology and Hypertension*, 22(2), pp. 156–162. doi: 10.1097/MNH.0b013e32835d19f7.

Ghulmiyyah, L. and Sibai, B. (2012) 'Maternal Mortality From Preeclampsia/Eclampsia', *Seminars in Perinatology*. Elsevier Inc., 36(1), pp. 56–59. doi: 10.1053/j.semperi.2011.09.011.

Gojnic M, Petkovic S, Papic M, M. T. (no date) 'Plasma albumin level as an indicator of severity of preeclampsia', *Clin Exp Obstet Gynecol*.

Hall, D. R. (2016) 'Understanding expectant management of pre-eclampsia', *Obstetrics and Gynecology Forum* 26(3), pp. 22–27.

Hassan-Alin, M. *et al.* (2000) 'Pharmacokinetics of esomeprazole after oral and intravenous administration of single and repeated doses to healthy subjects', *European Journal of Clinical Pharmacology*, 56(9–10), pp. 665– 670. doi: 10.1007/s002280000206.

Hastie, R. *et al.* (2019) 'Proton pump inhibitors and preeclampsia risk among 157 720 women: A Swedish population register-based cohort study', *Hypertension*, 73(5), pp. 1097–1103. doi: 10.1161/HYPERTENSIONAHA.118.12547.

Holt, S. and Howden, C. W. (1991) 'Omeprazole - Overview and opinion', *Digestive Diseases and Sciences*, 36(4), pp. 385–393. doi: 10.1007/BF01298864.

Hunfeld, N. G. *et al.* (2010) 'A comparison of the acid-inhibitory effects of esomeprazole and pantoprazole in relation to pharmacokinetics and CYP2C19 polymorphism', *Alimentary Pharmacology and Therapeutics*, 31(1),pp. 150–159. doi: 10.1111/j.1365-2036.2009.04150.x.

Hunfeld, N. G. *et al.* (2012) 'A comparison of the acid-inhibitory effects of esomeprazole and rabeprazole in relation to pharmacokinetics and CYP2C19 polymorphism', *Alimentary Pharmacology and Therapeutics*, 35(7),pp. 810–818. doi: 10.1111/j.1365-2036.2012.05014.x.

Huppertz, B. (2008) 'Placental origins of preeclampsia: Challenging the current hypothesis', *Hypertension*, 51(4 PART 2 SUPPL.), pp. 970–975. doi: 10.1161/HYPERTENSIONAHA.107.107607.

Ichikawa, H. *et al.* (2004) 'Lansoprazole ameliorates intestinal mucosal damage induced by ischemia-reperfusionin rats', *World Journal of Gastroenterology*, 10(19), pp. 2814–2817. doi: 10.3748/wjg.v10.i19.2814.

Isoherranen, N. and Thummel, K. E. (2013) 'Drug metabolism and transport during pregnancy: How does drug disposition change during pregnancy and what are the mechanisms that cause such changes?', *Drug Metabolismand Disposition*, 41(2), pp. 256–262. doi: 10.1124/dmd.112.050245.

J. Li, T. Lind, T. T. (2005) 'Population pharmacokinetics of esomeprazole in adult patients with gastroesophageal reflux disease', *European Journal of Pharmacology*, 50(2), pp. 157–158. doi: 10.1016/0014-2999(78)90013-4.

Jeong, H. (2010) 'Altered drug metabolism during pregnancy: Hormonal regulation of drug-metabolizing enzymes', *Expert Opinion on Drug Metabolism and Toxicology*, 6(6), pp. 689–699. doi: 10.1517/17425251003677755.

Ke, A. B., Nallani, S. C., *et al.* (2014) 'Expansion of a PBPK model to predict disposition in pregnant women of drugs cleared via multiple CYP enzymes, including CYP2B6, CYP2C9 and CYP2C19', *British Journal of Clinical Pharmacology*, 77(3), pp. 554–570. doi: 10.1111/bcp.12207.

Ke, A. B., Rostami-Hodjegan, A., *et al.* (2014) 'Pharmacometrics in Pregnancy: An Unmet Need', *Annual Reviewof Pharmacology and Toxicology*, 54(1), pp. 53–69. doi: 10.1146/annurev-pharmtox-011613-140009.

Leaños-Miranda, A. *et al.* (2017) 'Circulating angiogenic factors are related to the severity of gestational hypertension and preeclampsia, and their adverse outcomes', *Medicine (United States)*, 96(4). doi: 10.1097/MD.0000000000006005.

Manktelow, B. N. *et al.* (2013) 'Population-based estimates of in-unit survival for very preterm infants', *Pediatrics*, 131(2). doi: 10.1542/peds.2012-2189.

Marshall, S. A. *et al.* (2019) 'Targeting the vascular dysfunction: Potential treatments for preeclampsia', *Microcirculation*, 26(4), pp. 1–18. doi: 10.1111/micc.12522.

Matok, I. *et al.* (2012) 'The safety of fetal exposure to proton-pump inhibitors during pregnancy', *Digestive Diseases and Sciences*, 57(3), pp. 699–705. doi: 10.1007/s10620-011-1940-3.

Maynard, S. E. *et al.* (2003) 'Excess Placental Soluble fms-like Hypertension , and Proteinuria in', *Journal of Clinical Investigation*, 111(5), pp. 649–58. doi: 10.1172/JCI200317189.Introduction.

McKeage, K. *et al.* (2008) 'Esomeprazole', *Drugs*, 68(11), pp. 1571–1607. doi: 10.2165/00003495-200868110-00009.

Mould, D. R. and Upton, R. N. (2013) 'Basic concepts in population modeling, simulation, and model-based drug development - Part 2: Introduction to pharmacokinetic modeling methods', *CPT: Pharmacometrics and Systems Pharmacology*, 2(4). doi: 10.1038/psp.2013.14.

Müller, A. *et al.* (2019) 'The soluble fms-like tyrosine kinase-1 (sFLT-1) to placental growth factor (PIGF) ratio as a possible indicator for the severity of preeclampsia - single institution experience', *Medicinski Glasnik*, 16(1),pp. 53–59. doi: 10.17392/994-19.

Nagase, M. *et al.* (2020) 'Population pharmacokinetic analysis of esomeprazole in Japanese subjects with various CYP2C19 phenotypes', *Journal of Clinical Pharmacy and Therapeutics*, (January), pp. 1–9. doi: 10.1111/jcpt.13129.

Omotayo, M. O. *et al.* (2016) 'Calcium supplementation to prevent preeclampsia: Translating guidelines into practice in low-income countries', *Advances in Nutrition*, 7(2), pp. 275–278. doi: 10.3945/an.115.010736.

Onda, K. *et al.* (2015) '[6-or]', *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health*. International Society for the Study of Hypertension in Pregnancy, 5(1), p. 3. doi: 10.1016/j.preghy.2014.10.010.

Onda, K. *et al.* (2017) 'Proton pump inhibitors decrease soluble fms-like tyrosine kinase-1 and soluble endoglin secretion, decrease hypertension, and rescue endothelial dysfunction', *Hypertension*, 69(3), pp. 457–468. doi: 10.1161/HYPERTENSIONAHA.116.08408.

Papageorgiou, I., Grepper, S. and Unadkat, J. D. (2013) 'Induction of hepatic CYP3A enzymes by pregnancy-related hormones: Studies in human hepatocytes and hepatic cell lines', *Drug Metabolism and Disposition*, 41(2) pp. 281–290. doi: 10.1124/dmd.112.049015.

Pasternak, B. and Hviid, A. (2010) 'Use of proton-pump inhibitors in early pregnancy and the risk of birth defects', *New England Journal of Medicine*, 363(22), pp. 2114–2123. doi: 10.1056/NEJMoa1002689.

Pawar, S. K. *et al.* (2017) 'Spectroscopic and molecular modeling approaches to investigate the binding of proton pump inhibitors to human serum albumin', *Journal of Biomolecular Structure and Dynamics*. Taylor & Francis, 35(15), pp. 3205–3220. doi: 10.1080/07391102.2016.1251337.

Pkanalix-NCA (2019) 'Data format for NCA and CA analysis - PKANALIX'.

Rabbani, G. and Ahn, S. N. (2019) 'Structure, enzymatic activities, glycation and therapeutic potential of human serum albumin: A natural cargo', *International Journal of Biological Macromolecules*, 123, pp. 979–990. doi: 10.1016/j.ijbiomac.2018.11.053.

Ramma, W. and Ahmed, A. (2014) 'Therapeutic potential of statins and the induction of heme oxygenase-1 in preeclampsia', *Journal of Reproductive Immunology*, 101–102(1), pp. 153–160. doi: 10.1016/j.jri.2013.12.120.

Raymond, D. and Peterson, E. (2011) 'A critical review of early-onset and late-onset preeclampsia', *Obstetrical and Gynecological Survey*, 66(8), pp. 497–506. doi: 10.1097/OGX.0b013e3182331028.

El Rouby, N., Lima, J. J. and Johnson, J. A. (2018) 'Proton pump inhibitors: from CYP2C19 pharmacogenetics to precision medicine', *Expert Opinion on Drug Metabolism and Toxicology*. Taylor & Francis, 14(4), pp. 447– 460. doi: 10.1080/17425255.2018.1461835.

Saleh, L. *et al.* (2017) 'Low Soluble Fms-Like Tyrosine Kinase-1, Endoglin, and Endothelin-1 Levels in Womenwith Confirmed or Suspected Preeclampsia Using Proton Pump Inhibitors', *Hypertension*, 70(3), pp. 594–600. doi: 10.1161/HYPERTENSIONAHA.117.09741.

Savic, Radojka M *et al.* (2007) 'Implementation of a transit compartment model for describing drug absorption in pharmacokinetic studies', *J Pharmacokinet Pharmacodyn*, 34, pp. 711–726. doi: 10.1007/s10928-007-9066-0.

Schalkwijk, S., Greupink, R. and Burger, D. (2017) 'Free dug concentrations in pregnancy: Bound to measure unbound?', *British Journal of Clinical Pharmacology*, 83(12), pp. 2595–2598. doi: 10.1111/bcp.13432.

Shibata, E. *et al.* (2005) 'Soluble fms-like tyrosine kinase 1 is increased in preeclampsia but not in normotensive pregnancies with small-for-gestational-age neonates: Relationship to circulating placental growth factor', *Journalof Clinical Endocrinology and Metabolism*, 90(8), pp. 4895–4903. doi: 10.1210/jc.2004-1955.

Steegers, E. A. P. *et al.* (2010) 'Pre-eclampsia', *The Lancet*, 376(9741), pp. 631–644. doi: 10.1016/S0140-6736(10)60279-6.

Strand, D. S., Kim, D. and Peura, D. A. (2017) '25 years of proton pump inhibitors: A comprehensive review', *Gut and Liver*, 11(1), pp. 27–37. doi: 10.5009/gnl15502.

Takagi, T. *et al.* (2009) 'Lansoprazole, a proton pump inhibitor, mediates anti-inflammatory effect in gastric mucosal cells through the induction of heme oxygenase-1 via activation of NF-E2-related factor 2 and oxidation of Kelch-like ECH-associating protein 1', *Journal of Pharmacology and Experimental Therapeutics*, 331(1), pp.255–264. doi: 10.1124/jpet.109.152702.

Tian, H. *et al.* (2018) 'Effects of Plasma Albumin on the Pharmacokinetics of Esomeprazole in ICU Patients', *BioMed Research International*. Hindawi, 2018. doi: 10.1155/2018/6374374.

Tong, S. *et al.* (2015) 'Heme oxygenase-1 is not decreased in preeclamptic placenta and does not negatively regulate placental soluble fms-like tyrosine kinase-1 or soluble endoglin secretion', *Hypertension*, 66(5), pp. 1073–1081. doi: 10.1161/HYPERTENSIONAHA.115.05847.

U.S. Preventive Services Task Force (2015) 'Low-Dose Aspirin Use for the Prevention of Morbidity and Mortality from Preeclampsia : Recommendation', *Am Fam Physician*, 91(5), pp. 315D-315G.

Venkatesha, S. *et al.* (2006) 'Soluble endoglin contributes to the pathogenesis of preeclampsia', *Nature Medicine*, 12(6), pp. 642–649. doi: 10.1038/nm1429.

Wandall, J. H. (1992) 'Effects of omeprazole on neutrophil chemotaxis, super oxide production, degranulation, and translocation of cytochrome b-245', *Gut*, 33(5), pp. 617–621. doi: 10.1136/gut.33.5.617.

Wilder-Smith, C. H. *et al.* (2005) 'Intravenous esomeprazole (40 mg and 20 mg) inhibits gastric acid secretion as effectively as oral esomeprazole: Results of two randomized clinical studies', *European Journal of Gastroenterology and Hepatology*, 17(2), pp. 191–197. doi: 10.1097/00042737-200502000-00010.

Yoshida, N. *et al.* (2000) 'A new mechanism for anti-inflammatory actions of proton pump inhibitors - Inhibitory effects on neutrophil-endothelial cell interactions', *Alimentary Pharmacology and Therapeutics, Supplement*, 14(1), pp. 74–81. doi: 10.1046/j.1365-2036.2000.014s1074.x.

Zeisler, H. *et al.* (2016) 'Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia', *New England Journal of Medicine*, 374(1), pp. 13–22. doi: 10.1056/NEJMoa1414838.