Pharmaceutical Sciences and Biomedical Analysis Journal

SCIENTIFIC LITERATURE

Rapid Communication

Transfer of Paracetamol and Aniline in the Ex Vivo Human Placental Perfusion Model

Aleksandra Opalinska[1], Dominika Przygodzka[2], Grzegorz Buszewicz[2], Grzegorz Teresinski[2], Line Mathiesen[1] and Lisbeth E. Knudsen[1*]

[1]Department of Public Health, University of Copenhagen, Denmark

[2]Department of Forensic Medicine, Medical University of Lublin, Poland

ARTICLE INFO

Received Date: June 07, 2019
Accepted Date: July 03, 2019
Published Date: July 05, 2019

KEYWORDS

Acetaminophen
Aniline
Metabolism
Over-the-counter medicine
Placental transport
Pregnancy

Citation for this article: Aleksandra Opalinska, Dominika Przygodzka, Grzegorz Buszewicz, Grzegorz Teresinski, Line Mathiesen and Lisbeth E. Knudsen. Transfer of Paracetamol and Aniline in the Ex Vivo Human Placental Perfusion Model. Pharmaceutical Sciences and Biomedical Analysis Journal. 2019; 2(1):114

Corresponding author:
Lisbeth E. Knudsen,
Department of Public Health, University of Copenhagen, Denmark;
Email: liek@sund.ku.dk

ABSTRACT

Introduction: Paracetamol (acetaminophen) is the first choice pain relieving medicine recommended for pregnant women. Recent findings link paracetamol exposure *in utero* with subsequent genital malformation occurrence in children, as well as asthma, ADHD (Attention Deficit Hyperactivity Disorder) and other developmental disorders. The reported presence of paracetamol in the urine samples from individuals never treated with this medicine indicates other sources of exposure. The industrial compound aniline, which is metabolized to paracetamol in the human body, is a possible source. The present study investigates the transfer and metabolism of paracetamol and aniline in the human placenta, using a human ex vivo placental perfusion model.

Materials and methods: The human ex vivo placental perfusion system was used with freshly donated placentae from Caesarean sections from women providing informed consent. Paracetamol was added to final concentrations of 2.5µg/ml (n=5) and 25 µg/ml (n=2) in the maternal circulation, resulting in seven successful perfusions with paracetamol. Aniline was added to final concentrations of 25µg/ml (n=2) and 50 µg/ml (n=1), resulting in one successful perfusion with aniline. Samples from the maternal and fetal circulation were collected at different time intervals and analysed by UHPLC-MS/MS Triple Quadrupole system. Success criteria for a perfusion were the leakage from the fetal reservoir less than 3 ml/h and the fetal/maternal (FM) ratio greaterthan 0.75 for antipyrine. Identification of aniline, antipyrine, paracetamol and its metabolites was carried out on the base of the Multiple Reaction Monitoring (MRM) mode.

Results: Eight cotyledons were successfully perfused (seven with paracetamol and one with aniline). Paracetamol was transferred across the placenta via passive diffusion at rates comparable with antipyrine. The metabolite of paracetamol sulfate was present initially in the maternal circulation and the concentration increased in both circulations during perfusion. The metabolite of paracetamol glucuronide was detected, however no clear time dependent pattern was found. Aniline was studied in a single successful perfusion where transport and metabolism was found.

Conclusion: Comparative studies of the transport and metabolism of paracetamol and aniline are recommended for the hypothesis of paracetamol from aniline sources

also contributing to *in utero* exposures. Our results support information initiatives towards pregnant women in minimising their paracetamol intake.

Abbreviations:

ADHD: Attention Deficit Hyperactivity Disorder; CV: coefficients of variation; FM: fetal/maternal; IP30, indicative permeability coefficient; LOD: limit of detection; LOQ: limit of quantitation; MRM: multiple reaction monitoring; NA4AP: N-acetyl-4-aminophenol; NAPQUI/NAPQI: N-acetyl-p-benzoquinoneimine; pO2: capillary; UHPLC-MS/MS: ultra high performance liquid chromatography – tandem mass spectrometer

INTRODUCTION

Paracetamol (acetaminophen, NA4AP, APAP, N-acetyl-4-aminophenol) is one of the most common analgesic and antipyretic over-the-counter medicine. It is the first choice of medicine recommended to pregnant women in pain. In Denmark more than 50% of women take paracetamol at least once during a pregnancy [1]. The ubiquitous presence of paracetamol in the urine samples from individuals not consuming this medicine indicates other sources of exposure [2,3]. One of the exposure sources is industrial compound aniline, which is metabolized to paracetamol in the human body. Aniline is used in the production of rubber, food, cosmetic colorants and pesticides. Recent findings, both from epidemiological and animal studies, raise the concern that paracetamol exposure *in utero* may be the cause of a range of currently ubiquitous medical concerns in children, such as compromised reproductive capacity [4,5] asthma [6], ADHD, problems with concentration and other neurological disorders[7-9]. Coningset al., (2017) and Weigandet al., (1984) used the isolated perfused human placenta tissue and showed in their articles that paracetamol can freely pass the placental barrier [10,11]. After maternal use before delivery, paracetamol metabolites can be detected in newborns urine and blood [12,13].

The main organ responsible for metabolism of paracetamol after oral intake is the liver [14]. The major metabolites of paracetamol are: paracetamol glucuronide (~40%), paracetamol sulfate (~35%) and a reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI/NAPQUI; ~5%) [2]. It has been shown, that paracetamol metabolism varies among humans, which may result in decreased capability of producing the nonreactive metabolites [15]. The most abundant metabolites (~85%) after aniline exposure is free paracetamol and its glucuronide and sulfate conjugates [2]. The present study investigates the transfer and metabolism of paracetamol and aniline in the human placenta. Using term human placentae, obtained immediately after Caesarean sections, a human ex vivo placental perfusion model was applied.

MATERIALS AND METHODS

Recruitment of donors and sampling of placentae

After obtaining written consent of women delivering by Caesarean section, the placentae were handed to our study personnel immediately after birth, weighed and infused with heparin in physiological saline to prevent coagulation. The placenta was transported to the laboratory and cannulations and transport studies performed. The recruitment procedure has been approved by the Regional Ethics Committee (No H-1602 1492).

Perfusion studies

The placental dual closed circuit perfusion studies have been described in detail by Mathiesen et al., (2014) [16]. A chorionic artery-vein pair was cannulated. The cotyledon was placed in the perfusion chamber. Three cannulae, representing maternal arteries, were placed in the intervillous space through the basal plate and flow was established with a recycling system. The pre-perfusion (60 minutes) ensured intact perfusion of the cannulated cotyledon by flow measurements. The test compound was added to the maternal circulation and samples from maternal and fetal perfusates were taken at consecutive intervals. As control compound antipyrine was added (2.5mg/ml). The experiment duration was three hours and it was kept under physiological conditions. The values of pH, pO2 and glucose were corrected during perfusion when outside of certain values (pH: 7.45-7.55, pO2: maternal circuit value range 6.9- 15.4 kPa, pO2fetal< pO2maternal, glucose: >3mmol/l).Success criteria for each perfusion were the leakage from the fetal reservoir less than 3 ml/h and the fetal/maternal ratio greaterthan 0.75 for antipyrine. From five perfusions with paracetamol concentration of 2.5 µg/ml, two perfusions with paracetamol concentration of 25 µg/ml, two perfusions with aniline concentration of 25 µg/ml and one perfusion with aniline concentration of 50 µg/ml, eight cotyledons were successfully perfused.

Transfer of Paracetamol and Aniline in the Ex Vivo Human Placental Perfusion Model. Pharmaceutical Sciences and Biomedical Analysis Journal. 2019; 2(1):114.

Chemicals and materials

Ammonium carbonate (J.T. Baker, USA)

Formic acid (Fluka, Germany)

Ammonia solution (25%, Sigma-Aldrich, Germany)

Ethyl acetate (Honeywell, Riedel-de-Haën, Germany)

Water (Honeywell, Riedel-de-Haën, Germany)

Methanol (Honeywell, Riedel-de-Haën, Israel)

Acetonitrile (Honeywell, Riedel-de-Haën, Germany)

N-acetylbenzoquinoneimine (Sigma-Aldrich, Canada)

Paracetamol sulfate potassium salt (Sigma-Aldrich, India)

Paracetamol-β-D-glucuronide (Sigma-Aldrich, Germany)

Aniline (Merck, Germany)

Paracetamol (Sigma-Aldrich, analytical grade, Germany)

Antipyrine (Aldrich-Chemie, Steinheim, Germany)

Diazepam-D5 (Cerilliant, Round Rock)

Sample preparations

For the UHPLC-MS/MS analysis samples were prepared by liquid-liquid extraction. To 200 µl of sample 10µl of internal standard Diazepam-D5 (Cerilliant, Round Rock) and 200 µl of ammonium carbonate buffer (pH = 9) was added. Extraction was carried out with 1.2 ml of ethyl acetate on reciprocating shaker for 20 min at 280 rpm. Samples were centrifuged at 17968 x g for 10 min, 1 ml of supernatant was transferred and evaporated under stream of nitrogen at 40°C. The dried extracts were diluted in 50 µl of methanol and 10 µl of sample was used for injection.

UHPLC-MS/MS procedure

Analysis was performed using ultra high performance liquid chromatography (1260 Agilent Technologies, Germany). Separation was carried out on Poroshell 120 EC-C180 column 3.0 x 50 mm with a 2.7 µm particle size (Agilent Technologies, USA) at 40°C. As a mobile phase was used high-purity water with 0.1% formic acid (A) and acetonitrile (B). The gradient program started with 5% of B, follow by linear gradient up to 90% B in 5 min, and finishing with 90% B constant for 2 min. After this 7 min run time, 3 min of post-time followed using the initial 5% of B. The follow rate was set constant at 0.5 ml/min. Detection was achieved using triple quadrupole (QqQ 6460, Agilent Technologies, USA) equipped with an electros pray ionization (ESI) Jet Stream source. Source parameters were optimized as follows: collision gas temperature 300°C, collision gas flow 10 l/min, nebulizer 40 psi, sheath gas temperature 400°C, sheath gas flow 12 l/min. Identification of aniline, antipyrine, paracetamol and its metabolites was carried out on the base of the multiple reaction monitoring (MRM) mode. The assay was linear from 100 ng/ml to 50000 ng/ml for antipyrine, from 10 ng/ml to 5000 ng/ml for aniline and paracetamol, from 2 ng/ml to 5000 ng/ml for paracetamol glucuronide, paracetamol sulfate and NAPQI. All r2 values were 0.995 or greater. Limit of detection (LOD) for all compounds was 1 ng/ml. Limit of quantitation (LOQ) for aniline, antipyrine and paracetamol was 10ng/ml and 2 ng/ml for metabolites. All precision values determined by Coefficients of Variation (CV) were below 5%.

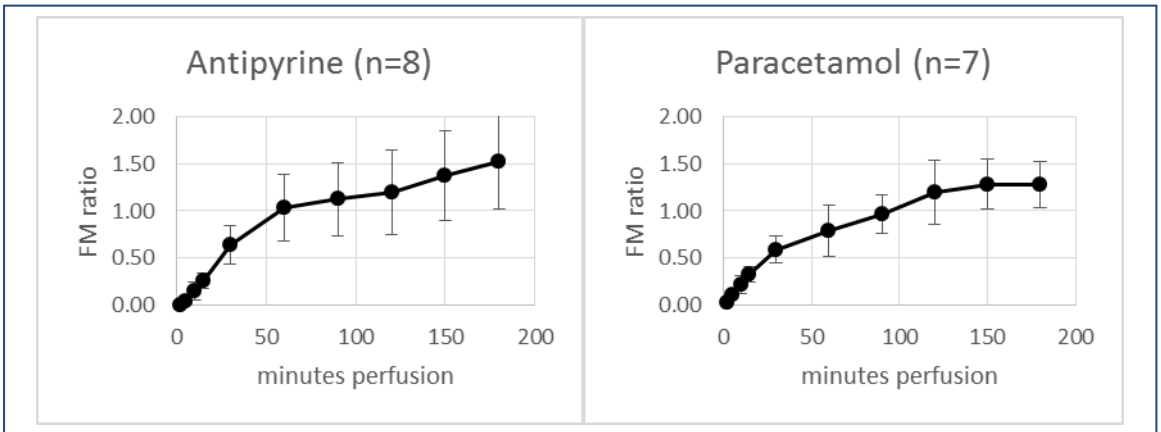

Figure 1: Fetal/maternal concentration ratios in placental perfusion studies with paracetamol (2.5µg/ml and 25 µg/ml) transported from the maternal circulation to the fetal circulation. Antipyrine is the control substance (2.5µg/ml) added to all perfusions (mean ±SD).

Transfer of Paracetamol and Aniline in the Ex Vivo Human Placental Perfusion Model. Pharmaceutical Sciences and Biomedical Analysis Journal. 2019; 2(1):114.

RESULTS

Our perfusions show both in the high and low concentration of paracetamol (2.5 µg/ml and 25 µg/ml respectively), a transfer in a rate similar to antipyrine (including the SD) reaching equilibrium at 60-100 minutes perfusion (Figure 1). The FM ratio is the ratio of concentrations measured in the fetal circulation divided by the concentrations measured in the maternal circulation. The permeability rate can be determined using the indicative permeability coefficient (IP30). It is defined as the slope of the FM ratio curve between time 0 and 30 min, assuming a linear regression. The transfer rates (IP30 as described in Mose et al., (2012) [17]) of paracetamol (0.0186) and antipyrine (0.0211) are very similar (and the transfer rate of paracetamol is within the SD of antipyrine transfer rate (0.0141-0.0281)). No paracetamol was detected in a blank perfusion (results not shown). Paracetamol sulfate was detected in all perfusions (Figure 2). Increased levels of this metabolite were detected in the perfusions with high concentrations of paracetamol. Perfusion with initial paracetamol concentration of 25 µg/ml resulted in the final concentration of paracetamol sulfate in the fetal reservoir of 115 ng/ml. No difference was seen in paracetamol glucuronide, and NAPQI did not show any reliable data (data not shown).

Only one perfusion with aniline (initial concentration 25 µg/ml) hadreliable data and transport as well as metabolism was demonstrated (data not shown). Aniline was detected in the fetal circulation in all of the timepoints, resulting in the aniline concentration of 2130ng/ml in the fetal reservoir after 3h of perfusion. Paracetamol sulfate and paracetamol glucuronide was detected in fetal reservoir during the experiment. At the end of experiments duration paracetamol sulfate concentration was 1.85 ng/ml and paracetamol glucuronide was 37.51 ng/ml in the fetal reservoir. More investigation is needed to support this preliminary finding.

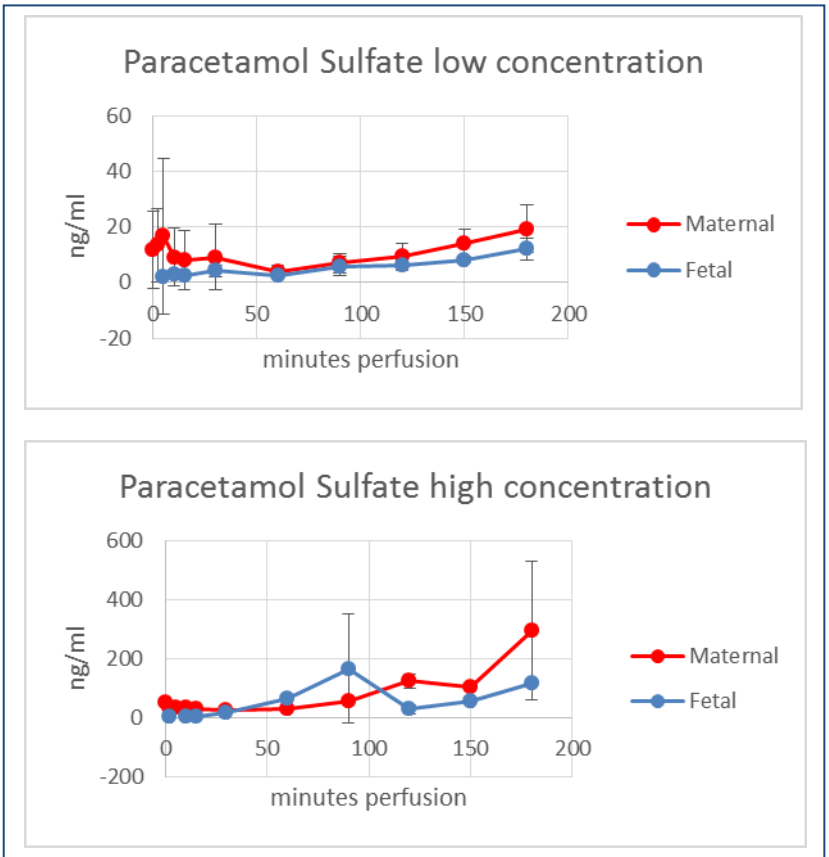

Figure 2: The metabolite paracetamol sulfate was found in maternal and fetal circulation fluids at low (2.5µg/ml) as well as high concentrations (25µg/ml). Increased levels of this metabolite were detected when high concentration of paracetamol was used (mean±SD).

Transfer of Paracetamol and Aniline in the Ex Vivo Human Placental Perfusion Model. Pharmaceutical Sciences and Biomedical Analysis Journal. 2019; 2(1):114.

DISCUSSION

Our results confirm human placental transport of paracetamol and its metabolites at the concentrations within therapeutic range. Paracetamol in human blood samples has effective antipyretic properties at plasma concentrations of 10-20 mg/l [18]. Studies regarding paracetamol metabolism and pharmacokinetics in pregnant and non-pregnant women showed, that administration of a standard dose (1 g) resulted in a serum concentration peaking at 20.8 μg/ml (1.37 x 10-4 M) 48 minutes after intake [19]. The initial study concentration was 2.5 μg/ml, which is lower than paracetamol therapeutic dose. Our studies confirm that paracetamol crosses the placenta via passive diffusion.

The present study shows that the placenta can metabolize both paracetamol and aniline to nontoxic paracetamol sulfate. Identifying different paracetamol metabolites abundance after placental metabolism may suggest a distinctive detoxifying process, when compared with the liver. The results from the present study are in contrary to Weigandet al., (1984) [10], where no paracetamol metabolites were detected in the perfusates. The difference in the findings can be explained with variation in perfusion settings and more sensitive methods for the metabolite detection available, and a longer duration of our experiment. Currently the data on placental metabolism of paracetamol are sparse, though it recently has been shown that paracetamol sulfate and glucuronide cross the placenta [11] and that a single oral dose of paracetamol administrated to the mother prior caesarean delivery results in paracetamol detection in fetal cord blood [13]. More studies related to the transport and metabolisms of aniline in comparison with paracetamol are recommended for the source identification of paracetamol exposures in non- medicated populations. The diversity in adverse outcomes related to prenatal exposures to paracetamol and preliminary findings from the present study supports further efforts to reconsider the safety of paracetamol use during pregnancy.

Funding

The perfusions were financed by University of Copenhagen, Department of Public Health, Section of Environmental Health, Denmark. The analyses for paracetamol and aniline were financed by Medical University of Lublin, Poland.

REFERENCES

1. Rebordosa C, Kogevinas M, Bech BH, Sorensen HT, Olsen J. (2009). Use of acetaminophen during pregnancy and risk of adverse pregnancy outcomes. Int J Epidemiol. 38: 706-714.
2. Modick H, Weiss T, Dierkes G, Bruning T, Koch HM. (2014). Ubiquitous presence of paracetamol in human urine: sources and implications. Reproduction . 147: R105-17.
3. Nielsen JK, Modick H, Morck TA, Jensen JF, Nielsen F, et al. (2015). N-acetyl-4-aminophenol (paracetamol) in urine samples of 6-11-year-old Danish school children and their mothers. Int J Hyg Environ Health . 218: 28-33.
4. Arendrup FS, Mazaud-Guittot S, Jegou B, Kristensen DM. (2018). EDC IMPACT: Is exposure during pregnancy to acetaminophen/paracetamol disrupting female reproductive development? Endocrine Connections. 7: 149-158.
5. Hay-Schmidt A, Finkielman OTE, Jensen BAH, Høgsbro CF, Holm JB, et al. (2017). Prenatal exposure to paracetamol/acetaminophen and precursor aniline impairs masculinisation of male brain and behaviour. Reproduction, BioScientifica. 154: 145-152.
6. Fan G, Wang B, Liu C, Li D. (2017). Prenatal paracetamol use and asthma in childhood: A systematic review and meta-analysis. Allergollmmunopthol. 45: 528-533.
7. Bauer A, Kriebel D, Herbert MR, Bornehag CG, Swan SH. (2018). Prenatal paracetamol exposure and child neurodevelopment: A review. Hormones and Behaviour. 101: 125-147.
8. Brandlistuen RE, Ystrom E, Nulman I, Koren G, Nordeng H. (2013). Prenatal paracetamol exposure and child neurodevelopment: a sibling-controlled cohort study. Int J Epidemiol . 42: 1702-1713.
9. Liew Z, Ritz B, Rebordosa C, Lee PC, Olsen J. (2014). Acetaminophen use during pregnancy, behavioral problems and hyperkinetic disorders. JAMA Pediatr. 168: 313-320.
10. Weigand UW, Chou RC, Maulik D, Levy G. (1984). Assessment of biotransformation during transfer of

propoxyphene and acetaminophen across the isolated perfused human placenta. Pediatric Pharmacology. 4: 145-153.

11. Conings, Tseke, Paulus, Qi, Amant, et al. (2017). O-17 Transplacental transport of paracetamol and its metabolites using the ex-vivo human placenta perfusion model. Arch Dis Child. 102: A7-A8.
12. Levy G, Garrettson LK, Soda DM. (1975). Letter: Evidence of placental transfer of acetaminophen. Pediatrics. 55: 895.
13. Nitsche JF, Patil AS, Langman LJ, Penn HJ, Derleth D, et al. (2017). Brost BC.Transplacental Passage of Acetaminophen in Term Pregnancy. Am J Perinatol. 34: 541-543.
14. Allegaert K, Peeters MY, Beleyn B, Smits A, Kulo A, et al. (2015). Paracetamol pharmacokinetics and metabolism in young women. BMC Anesthesiol. 15: 163.
15. Zhao L, Pickering G. (2011). Paracetamol metabolism and related genetic differences. Drug Metab Rev. 43: 41-52.
16. Mathiesen L, Mørck TA, Zuri G, Andersen MH, Pehrson C, Frederiksen M, et al. (2014). Modelling of human transplacental transport as performed in Copenhagen, Denmark. Basic Clin Pharmacol Toxicol. 115: 93-100.
17. Mose T, Mathiesen L, Karttunen V, Nielsen JKS, Sieppi E, et al. (2012). Meta-analysis of data from human ex-vivo placental perfusion studies on genotoxic and immunotoxic agents within the integrated European project NewGeneris. Placenta. 33: 433-439.
18. Rumack BH. (1978). Aspirin versus acetaminophen: a comparative view. Pediatrics. 62: 943-946.
19. Rayburn W, Shukla U, Stetson P, Piehl E. (1986). Acetaminophen kinetics: comparison between pregnant and nonpregnant women. Am J Obstet Gynecol. 155: 1353-1356.