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2021-10-01

Hakomäki, H, Kokki, H, Lehtonen, M, Räsänen, J, Voipio, H-M, Ranta, V-P & Kokki, M 2021, 'MATERNAL AND FETAL BUPRENORPHINE PHARMACOKINETICS IN PREGNANT SHEEP DURING TRANSDERMAL PATCH DOSING Buprenorphine pharmacokinetics in pregnant sheep', European Journal of Pharmaceutical Sciences, vol. 165, 105936. https://doi.org/10.1016/j.ejps.2021.105936

http://hdl.handle.net/10138/336572
https://doi.org/10.1016/j.ejps.2021.105936

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MATERNAL AND FETAL BUPRENORPHINE PHARMACOKINETICS IN PREGNANT SHEEP DURING TRANSDERMAL PATCH DOSING

Buprenorphine pharmacokinetics in pregnant sheep

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ARTICLE INFO

Keywords:
Buprenorphine
Pregnancy
Sheep, Transdermal

ABSTRACT

Background: Buprenorphine is used in the opioid maintenance treatment for opioid dependent patients, including pregnant women. Despite the wide use, limited data exists on buprenorphine pharmacokinetics and fetal exposure during pregnancy. The aim of our study was to determine the buprenorphine pharmacokinetics during transdermal patch dosing to pregnant sheep and, to determine the extent of transplacental transfer of buprenorphine to the fetus.

Methods: Pregnant sheep in late gestation (n=50) received 20, 25 or 40 µg/h of buprenorphine as a 7-day extended-release transdermal patch. Plasma samples were collected from the ewe and the fetus on days 1 – 6, and buprenorphine and norbuprenorphine concentrations were determined. During the exposure period the sheep had a surgical procedure on the second day, a recovery phase, and an experimental procedure on the sixth day. In the experiment, hypoxia was induced under anesthesia for 18 sheep to investigate if decreased fetal pH would cause ion-trapping of buprenorphine in the fetus. The fetal/maternal plasma concentration ratio was determined on the second and on the sixth exposure day at baseline and during hypoxia. Maternal pharmacokinetics were modelled with a population pharmacokinetic method using the data from this study and our previous intravenous administration study.

Results: The transdermal patch provided an extended release of buprenorphine throughout the exposure period, but the release rate declined approximately 20 h after patch placement. The median fetal/maternal plasma concentration ratio was 13 – 27 % throughout the exposure period at baseline. A ratio over 100 % was observed for four sheep on the sixth exposure day (102 – 269 %). A minor increase was seen in the median fetal/maternal-ratios during maternal hypoxia. Norbuprenorphine was undetected in all plasma samples.

Conclusions: The low transplacental passage of less than one fourth of the ewe’s exposure supports buprenorphine as an alternative to methadone in opioid maintenance therapy during pregnancy.

1. Introduction

Buprenorphine (BUP) is a semi-synthetic opioid analgesic, used for the treatment of moderate-to-severe pain and opioid use disorder. Its main pharmacological effects arise from partial agonism at the µ-opioid receptor, but its complex pharmacological profile also include antagonism at the δ- and κ-opioid receptors, as well as agonistic interactions with the opioid receptor-like 1 receptor (Huang et al., 2001; Negus et al., 2002). Compared to other (full agonist) opioids, BUP is associated with less analgesic tolerance and drug addiction, making it a feasible drug for the treatment of opioid use disorder. BUP is metabolized in the liver via
due to increased body fat and plasma volume. Based on these changes with significant physiological changes in the pregnant women (Ander...

transdermal patch during pregnancy are increased hepatic and renal clearance, decreased plasma AUC (area under the curve) and at the same time plasma concentrations are reduced. This is an important finding, as the risk of drop-out is high, and is usually caused by dissatisfaction with the treatment as withdrawal symptoms surface with mental wellbeing and overall quality of life (Sordo et al., 2017; Thomas et al., 2016). The pharmacokinetics of BUP and fetal exposure during transdermal patch dosing in pregnancy has not been reported.

In the present study, our aim was to determine the pharmacokinetics of BUP in the ewe during pregnancy during continuous administration of BUP and determine the extent of transplacental transfer to the fetus. The effect of maternal hypoxia on fetal BUP exposure was also evaluated. As this type of study could not be done in humans due to ethical reasons, we utilized a pregnant sheep model. BUP was administered continuously as a transdermal patch throughout six consecutive days to demonstrate subacute exposure in the animal-model.

2. Materials and methods

2.1. Animals

The animal transport, husbandry and experimental procedures were carried out according to the Finnish national legislation (Act on the Protection of Animals Used for Scientific or Educational Purposes (497/2013), Decree on the Protection of Animals Used for Scientific or Educational Purposes (564/2013)) and the EU directive 2010/63/EU (Parliament of Finland, 2013; The European Parliament and the Council of the European Union, 2010; Government of Finland, 2013). The study protocol was approved by the National Animal Experiment Board of Finland (reference no. ESAVI/7840/04.10.07/2017).

The study was conducted with 50 time-mated Åland landrace sheep (Lammastila Sikka Talu, University of Turku, Rymättylä, Finland). The sheep were 1 - 8 -years-old (median 3 years) and weighted 45 – 79 kg (median 59 kg). All sheep were near term pregnancies at the beginning of the study at 119 - 126 gestational days (median 124 days), term pregnancy being 145 gestational days. Sheep had 1 – 3 fetuses (median 2 each). The animal husbandry and over-all care was carried out as explained previously (Hakomäki et al., 2021).

2.2. Buprenorphine administration and sample collection

One week prior to the BUP transdermal study, 14 out of the 50 sheep went through an IV pharmacokinetic study, where 10 µg/kg of BUP was administered through a jugular vein catheter, and the plasma concentrations were followed for 48 h, as described previously (Hakomäki et al., 2021). During the transdermal study, BUP was administered as a 7 day extended-release transdermal patch (Norspan®, Mundipharma DC B.V., Utrecht, Netherlands) continuously throughout six days. The exposure studies were done in five sessions, with 10 sheep in each assembly. Sheep were administered 20 µg/h (1st and 2nd assembly, sheep ID 1 - 10 and ID 41 - 50), 25 µg/h (3rd assembly, sheep ID 31 - 40) or 40 µg/h (3rd and 4th assembly, sheep ID 11 - 30) of BUP through a transdermal patch. The transdermal patch was placed onto the left ante-brachium 24 h prior to a surgical procedure. When two patches were applied (40 µg/h exposure), the sheep had one 20 µg/h releasing patch in each antebrachium. Prior to the patch placement, the forearm area was shaved and washed with soap. The area was disinfected with ethanol solution and was left to dry before the patch was applied. The patch was kept in place with adhesive tape and bandage throughout the experiment. The proper placement of the patch was confirmed daily, and measures were taken if the patch had moved or wrinkled.

During the transdermal patch dosing, the sheep went through a surgical procedure and an experimental procedure under anesthesia. On the second exposure day sheep (n=50) had a surgical procedure where laparotomy and hysterotomy were performed to the ewe, and thoracotomy to one of the fetuses, to place an arterial catheter to the fetus for fetal monitoring and sample collection. After surgery, the sheep had a 5-day recovery period. On the sixth exposure day 18 sheep had an

| Nonstandard abbreviations | Definition |
|--------------------------|------------|
| BUP | Buprenorphine |
| CL | Plasma clearance |
| F/M-ratio | Fetal/maternal plasma concentration ratio |
| F_Rnorb | Fraction of the transdermal patch nominal release rate |
| IV | Intravenous |
| kdep | Rate constant of the transdermal patch depletion |
| LC/MS/MS | Liquid chromatography - tandem mass spectrometry |
| LLOQ | Lower limit of quantification |
| NBUP | Norbuprenorphine |
| Q | Intercompartmental clearance |
| V1 | Apparent volume of distribution in the central compartment |
| V2 | Apparent volume of distribution in the peripheral compartment |

CYP3A4 by N-dealkylation into the main metabolite norbuprenorphine (NBUP).

During pregnancy BUP is used for the opioid maintenance treatment among opioid addicted women, as well as illicitly as a recreational drug. The ongoing opioid crisis has increased the number of pregnant women addicted to opioids (Haight et al., 2018; Maeda et al., 2014). This has raised a concern, and actions have been taken to find an appropriate treatment protocol for these women. Recent research seems to advocate BUP maintenance treatment as one of the best options for the mother-neonate dyad, as methadone treatment is associated with higher incidence and more severe cases of neonatal abstinence syndrome, longer hospital stays, and higher prevalence of pregnancy complications (Gaalma et al., 2012; Jones, Arria, et al., 2012; Jones, Finnegan, et al., 2012). Enrolling the opioid addicted pregnant women into BUP maintenance treatment is associated with positive outcomes for both the pregnant women and their fetus, as the women are directed from the path of opioid abuse to a medically controlled environment, where pharmacological treatment is accompanied by psychological aid, cognitive behavioral approaches, and counselling. For the women, this usually means reduced illicit opioid use, lower rate of overdose deaths and overall mortality, reduced criminal activities and risky-behavior, reduction in sexually transmitted diseases, improved physical and mental wellbeing and overall quality of life (Sordo et al., 2017; Thomas et al., 2014). These outcomes have direct positive effects on the pregnancy and on the wellbeing of the fetus and the newborn.

As BUP is becoming increasingly popular in the opioid addiction treatment during pregnancy, more studies are needed to determine safe, yet efficacious treatment protocol. Currently, the doses and dosing regimens used during pregnancy are based on the recommendations given to non-pregnant patients, even though pregnancy is associated with significant physiological changes in the pregnant women (Anderson, 2005; Loebstein et al., 1997; Zhang et al., 2018). The main changes in BUP pharmacokinetics during pregnancy are increased hepatic and renal clearance, decreased plasma AUC (area under the plasma-concentration curve), as well as increased volume of distribution due to increased body fat and plasma volume. Based on these changes Caritis and colleagues have suggested increased dosing regimen for pregnant women, as the clearance of BUP seems to be enhanced during pregnancy (Bastian et al., 2017; Caritis et al., 2017). Finding the optimal dose for the pregnant woman entering BUP maintenance treatment is crucial, as the risk of drop-out is high, and is usually caused by dissatisfaction with the treatment as withdrawal symptoms surface with insufficient dosing (Muruganandam et al., 2019).

Transdermal BUP pharmacokinetics have been studied in men and non-pregnant women (Andresen et al., 2011; Kapil et al., 2013; Wang et al., 2016), in non-pregnant animals using pigs (Thiede et al., 2014), dogs (Andaluz et al., 2009; Pieper et al., 2011), and cats (Murrell et al., 2007). The pharmacokinetics of intravenous (IV) BUP have been reported for pregnant sheep (Hakomäki et al., 2021), but to the best of our knowledge, the pharmacokinetics of BUP and fetal exposure during transdermal patch dosing in pregnancy has not been reported.
experimental procedure, where hypoxemia was induced to the ewe for a 2 h period under anesthesia determined as partial pressure of oxygen (pO2) in maternal arterial blood of 7 kPa (the fraction of oxyhemoglobin between 70 % and 75 %). The surgical procedure and the induction of hypoxemia has been described previously (Bhide et al., 2017). Prior to the procedures, sheep were premedicated with intramuscular 5-ketamine 50 mg and midazolam 10 mg. The anesthesia was induced with IV propofol 300 – 400 mg and maintained with sevoflurane end tidal concentration 1.5 – 2.5 % administered via endotracheal tube. In addition to BUP transdermal patch dosing, oxycodeone 5 mg/h was administered during the procedures to maintain sufficient pain relief. During the recovery period, oxycodeone 0.0125 mg/kg/h was administered for three days via epidural pump. IV or intramuscular fentanyl 50 µg was used as a rescue medication if the sheep showed signs of pain during the procedures or the recovery period.

Plasma samples were collected from the ewe and the fetus throughout the exposure period. On the second exposure day a plasma sample was drawn from the ewe before the surgery, and paired plasma samples from the ewe and the fetus after the fetal catheter was placed, and at the end of surgery. During the recovery period one plasma sample was collected from the ewe daily. On the sixth exposure day several paired plasma samples were taken from the ewe and the fetus on sequential process, where BUP was initially released from the patch at a rate of 0.315 µg/cm²/day. A second peak plasma concentration was observed on the second exposure day after insertion of the fetal arterial catheter, and on the sixth exposure day at the beginning of the experimental procedure, where hypoxemia was induced to the ewe for a 2 h period under anesthesia determined as partial pressure of oxygen (pO2) in maternal arterial blood of 7 kPa (the fraction of oxyhemoglobin between 70 % and 75 %). The surgical procedure and the induction of hypoxemia has been described previously (Bhide et al., 2017). Prior to the procedures, sheep were premedicated with intramuscular 5-ketamine 50 mg and midazolam 10 mg. The anesthesia was induced with IV propofol 300 – 400 mg and maintained with sevoflurane end tidal concentration 1.5 – 2.5 % administered via endotracheal tube. In addition to BUP transdermal patch dosing, oxycodeone 5 mg/h was administered during the procedures to maintain sufficient pain relief. During the recovery period, oxycodeone 0.0125 mg/kg/h was administered for three days via epidural pump. IV or intramuscular fentanyl 50 µg was used as a rescue medication if the sheep showed signs of pain during the procedures or the recovery period.

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The blood samples were collected in heparinized plasma tubes and centrifuged at 2000 g for 10 min. Plasma was divided into two cryotubes and stored first at -35 °C and then moved to -85 °C until analysis.

2.3. Buprenorphine quantification

The concentrations of BUP and its main metabolite NBUP were quantified as free bases from all plasma samples. Plasma samples were analyzed with quantitative liquid chromatography with triple quadruple mass spectrometric detection (LC/MS/MS), with liquid-liquid extraction, as described previously (Hakomäki et al., 2021). The lower limit of quantification (LLOQ) in plasma samples for BUP was 0.01 µg/L and 0.04 µg/L for NBUP with upper limit of linearity of 25 µg/L.

2.4. Data analysis and pharmacokinetic modeling

Paired maternal and fetal plasma BUP concentrations were determined on the second exposure day after insertion of the fetal arterial catheter, and on the sixth exposure day at the beginning of the experiment. Plasma concentration measurements below LLOQ were excluded from the data analysis. The differences in fetal/maternal (F/M) plasma concentration ratios and pH values between the sampling times were tested with Kruskal-Wallis - test and further pairwise comparison with Dunn’s method if a statistically significant difference was observed (SigmaPlot version 13, Systat software, San Jose, California, USA).

The maternal BUP concentrations from this study and our previous IV administration study (Hakomäki et al., 2021), the latter with 14 sheep of which all were also included in the current transdermal patch study after a 7-day wash-out period, were simultaneously analyzed with population pharmacokinetic modeling, using stochastic approximation expectation maximization algorithm (SAEM) for non-linear mixed effects models in Monolix software version 2019R2 (Lixoft, Antony, France). Additional software used in the model building from Lixoft were Mixplore and Mixeditor. GraphPad Prism version 8 was used as an imaging software (GraphPad Software, La Jolla, California, USA).

Outliers were excluded from the dataset used for the modeling. These included sheep that experienced malfunctions in the patch placement (wrinkling or detachment) and required a patch change during the exposure period (n=7). Additionally, excluded from the modeling dataset were abnormally high BUP plasma measurements during the surgical (n=2) or experimental (n=3) procedures, determined as follows: the median plasma concentrations during surgery and experimentation were calculated for each dose group and the measurement was excluded as an outlier if it was 3-fold or higher compared to the median concentration. The data for the modeling included 43 sheep from the transdermal study, of which 11 had plasma measurements only from the surgical procedure, 14 that had measurements from the surgical procedure and the recovery period, and 18 who had measurements from all three phases including the experimental procedure.

A two-compartment model, with a central compartment and a peripheral compartment was built with apparent volumes of the compartments V1 and V2, elimination clearance CL, and intercompartmental clearance Q (Fig. 1). The absorption from the transdermal patch could not be explained with a pure first order, or zero order process. Thus, the transdermal administration was modelled as a sequential process, where BUP was initially released from the patch at a constant, zero-order rate for the first 20 h, expressed as the nominal release rate of the transdermal BUP patch multiplied with the fraction of the nominal release rate (FRnom). FRnom was introduced to the model to explain the potential difference between the nominal and the observed rate of BUP release from the transdermal patch. After the first 20 h the release rate (nominal * FRnom) from the transdermal patch declined exponentially over time, defined by the estimated (first order) rate constant of depletion (kdep).

The between-subject variability in parameter estimates was modelled with random effect variables (etas). These were assumed to be centered around zero, and have model estimated variance, omega². The residual error was explained with the proportional error model. Model selection criteria were visual inspection of the goodness-of-fit plots, mechanistic plausibility of the model, parameter precision < 50 % relative standard error (R.S.E.), and the objective function value (-2 log-likelihood). Models with different number of systemic compartments and different zero- and first-order transdermal absorption rates were tested. A base model was used for covariate modeling, where weight, age and the number of fetuses were introduced as continuous covariates. The covariates were screened using a statistical method, the Wald test in Monolix.

The covariates were tested in the model as:

\[ P_i = P_{pop} \times (cov/mean(cov))^\beta_{cov} \times exp^n \]

where \( P_i \) is the predicted parameter value for the individual, \( P_{pop} \) is the typical population value, \( cov \) is the individual value for the continuous covariate, \( mean(cov) \) is the mean covariate value for the population, \( \beta_{cov} \) is the value describing the effect covariate has on the predicted value, and \( n \) is used to describe between-subject variability assuming log-normal distribution.

3. Results

In the majority of the pregnant sheep, the peak maternal plasma concentration \( C_{max} \) was measured 20 – 30 h after the patch application that was the first measurement for most sheep (n=33), after which a gradual decline in the plasma concentrations was observed (Fig. 2). Seven sheep required a patch change during the exposure period due to patch wrinkling or detachment and show a second peak plasma
concentrations after 30 h. Some abnormally high plasma concentrations were observed during the surgical procedure (n = 2), as well as during the experimentation procedure (n = 3).

NBUP was undetected in all samples.

3.1. Paired maternal and fetal plasma samples at baseline

The plasma BUP concentrations and F/M ratios are presented in Table 1. On the sixth exposure day the fetal plasma BUP concentration was below LLOQ for 3 out of 6 measurements in the dose group 20 µg/h and for 2 out of 7 in the dose group 40 µg/h. The median fetal plasma BUP concentration was 13 – 27 % of the ewe’s plasma concentration on the second exposure day and 13 – 18 % on the sixth exposure day. However, high between-subject variability was observed. On the second exposure day the highest F/M-ratios were measured for ID 49 in the dose group 20 µg/h (65 %), for ID 31 in the dose group 25 µg/h (39 %) and for ID 19 in the dose group 40 µg/h (35 %). On the sixth exposure day the highest F/M-ratios were measured for ID 6 (269 %) and for ID 11 (75 %) in the dose groups 20 µg/h and 40 µg/h, respectively.

3.2. Paired maternal and fetal samples during experimentation

Eighteen out of the 50 sheep had an experimentation procedure, where hypoxia was induced to the ewe under anesthesia. In 14 out of the 18 sheep, paired plasma samples were drawn, and pH was measured (Table 2). In four out of the 18 sheep the indwelling fetal arterial catheter did not work, and only maternal samples were taken.

At the beginning of the experimentation procedure, the median fetal arterial blood pH was 7.29 and the maternal arterial blood pH 7.37. During hypoxia the median maternal pH remained steady (P-value 0.157), whereas the median fetal pH decreased with the increased duration of hypoxia (P-value 0.020). A statistically significant decrease

Fig. 1. Schematic representation of the two-compartment base model, where V1 and V2: the apparent volumes of distribution in the central and peripheral compartments, Q: the intercompartmental clearance, CL: the elimination clearance. The release rate of buprenorphine from the transdermal patch was the nominal rate multiplied by the estimated fraction of the nominal rate (FRnom) for 20 h. Thereafter, the nominal rate * FRnom, declined exponentially with a rate constant of depletion (kdep).

Fig. 2. Individual maternal plasma buprenorphine concentrations from 50 pregnant sheep after transdermal buprenorphine patch dosing of 20, 25 or 40 µg/h presented on a semilogarithmic plot.
in fetal pH was seen 30 minutes after hypoxia was discontinued (normoxia 30 min, P-value 0.044) compared with the baseline. The median plasma BUP F/M-ratio increased with the increased duration of hypoxia and decreased fetal pH and was highest 30 minutes into normoxia. High plasma BUP F/M-ratio increased with the increased duration of hypoxia changing it between 10 h and 30 h, in 1 h intervals, but these changes did not improve the model compared to 20 h value (-2 log likelihood difference between rival models below 2.2 %) and complicated the model so that volumes of distribution and intercompartmental clearances needed to be fixed. A proportional error model was the best for the residual errors.

3.3. Population pharmacokinetic modeling

A two-compartment base model was built to analyze time-concentration profiles of BUP in pregnant sheep. The population estimates for the final model are presented in Table 3 and the fit of the model for the transdermal data in Fig. 3. The FR_{nom} was fixed to 1 after estimated values between 0.9 – 1.05 were obtained with different initial values. The time period for the zero-order release rate was fixed to 20 h, as this was the time after which a decline in the release rate was observed for most sheep. We tested the effect of the fixed time period by changing it between 10 h and 30 h, in 1 h intervals, but these changes did not improve the model compared to 20 h value (-2 log likelihood increased and the maximal change seen in CL and k_{dep} was ±11.2 %). A three-compartment model was tested but did not improve the fit of the model or have an impact on the parameter estimates (CL and k_{dep} difference between rival models below 2.2 %) and complicated the model so that volumes of distribution and intercompartmental clearances needed to be fixed. A proportional error model was the best for the residual errors.

Table 1
Maternal and fetal plasma buprenorphine concentrations and fetal/maternal plasma concentration ratios (F/M-ratio) expressed as median (minimum-maximum) after a transdermal patch administration of 20, 25 or 40 µg/h of buprenorphine. The data are from the second exposure day (median time after a patch placement 26 h) during surgery and from the sixth exposure day (median time after a patch placement 145 h) at the beginning of experiment.

| Exposure day | Measurement | Dose group | 20 µg/h | 25 µg/h | 40 µg/h |
|--------------|-------------|------------|---------|---------|---------|
|              |             | Fetal      | Maternal| Fetal   | Maternal|
|              |             |            |         |         |         |
| 2nd day      | C_{plasma} (µg/L) | Median     | (0.03-0.55) | 0.19 | 0.03 | 0.10 |
|              |             | (min-max)  | (0.01-0.29) | (0.04-0.15) | 0.02 | (0.01-0.04) |
|              |             | n          | 20       | 13      | 10     | 6     | 20     | 18     |
| 6th day      | C_{plasma} (µg/L) | Median     | (7.65)   | 15      | 27     | 13    |
|              |             | (min-max)  | (21-39)  | (3.53)  |
|              |             | n          | 13       | 6       | 18     |

Table 2
Maternal and fetal median (minimum-maximum) pH, fetal/maternal (F/M) plasma buprenorphine concentration ratio, and number of measurements (n) from 14 pregnant sheep during experimentation, where hypoxia was induced to the ewe. Experimentation was done on the sixth exposure day of transdermal buprenorphine patch dosing of 20 – 40 µg/h. Measurements were taken before hypoxia (baseline), 30, 60 and 120 minutes after induction of hypoxia, 30 minutes after ending hypoxia (normoxia 30 min), and at the end of the experiment (end).

| Maternal pH | Baseline | Fetal | Hypoxia 30 min | Hypoxia 60 min | Hypoxia 120 min | Normoxia 30 min | End |
|-------------|----------|-------|----------------|----------------|-------------------|-----------------|-----|
| Median (min-max) | 7.37 | (7.34-7.45) | 7.40 | (7.36-7.46) | 7.39 | (7.34-7.48) | 7.36 | (7.28-7.45) | 7.34 | (7.26-7.41) |
| n           | 11       | 11    | 11             | 11             | 11                | 9               | 7   |
| F/M-ratio (%) | 7.29 | (7.20-7.34) | 7.24 | (7.21-7.38) | 7.20 | (6.96-7.37) | 7.16 | (6.77-7.29) | 7.13 | (6.75-7.29) | 7.24 | (7.11-7.33) |
| Median (min-max) | 18 | (9-269) | 23 | (11-220) | 24 | (6-87) | 29 | (7-102) | 37 | (19-188) | 32 | (1-239) |
| n           | 7        | 8     | 8              | 8              | 8                 | 8               | 7   |

Table 3
Population pharmacokinetic parameter estimates for the two-compartmental base model for pregnant sheep receiving buprenorphine as a transdermal patch.

| Structural model parameter | Estimated value | R.S.E. (%) |
|----------------------------|-----------------|------------|
| CL (L/h)                   | 134             | 10.5       |
| FR_{nom} fixed             | 1               |            |
| k_{dep} (h^{-1})           | 0.0146          | 11.4       |
| V1 (L)                     | 118             | 20.5       |
| V2 (L)                     | 475             | 35.3       |
| Q (L/h)                    | 155             | 26.1       |
| Standard deviation of the random effects |                   |            |
| Omega CL                   | 0.593           | 12.3       |
| Omega k_{dep}              | 0.494           | 21.6       |
| Omega V1                   | 0.635           | 24.1       |
| Omega V2                   | 1.12            | 25.3       |
| Omega Q                    | 0.837           | 21.8       |
| Standard deviation of proportional error b | 0.340 | 4.5 |

1 The calculated half-life of the depletion = 47.5 h (obtained as ln2/k_{dep}).
The base model was used for covariate modeling, where weight, age and number of fetuses were introduced to the model. The addition of covariates did not improve the objective function value, nor were they statistically significant (P-value > 0.05).

Abbreviations: R.S.E., Relative standard error; Omega, standard deviation of the random effects; CL, plasma clearance; FR_{nom}, fraction of the transdermal patch nominal release rate; k_{dep}, rate constant of the transdermal patch depletion; V1 and V2, apparent volumes of distribution; Q, intercompartmental clearance; b, proportional residual error.

4. Discussion

To the best of our knowledge, this is the first study to present BUP pharmacokinetics after transdermal administration in pregnant sheep. This is also the first study to evaluate transdermal BUP pharmacokinetics in pregnant participants and the first study to evaluate the transplacental transfer of BUP during the prenatal period. In the present study the median fetal exposure was relatively low. The plasma BUP concentration was 13 – 27 % of the ewe’s plasma concentration and remained steady throughout the exposure period of six days in pregnant sheep. The highest F/M-ratio was observed for ID 6 on the sixth exposure day (269 %) at baseline, with 20 µg/h 7-day extended-release BUP.

Fig. 3. Individual fits (dashed line), population fits (solid line) and maternal observations (dots) of the population pharmacokinetic model from 43 pregnant sheep that received 20, 25 or 40 µg/h buprenorphine through a 7-day transdermal patch dosing.
patch. For others the F/M-ratios were below 100 % at normoxia. A novel finding was that during maternal hypoxia, and as fetal pH decreased, the F/M-ratio increased. F/M-ratios above 100 % were measured for three sheep during hypoxia (102 – 239 %). This could be caused by ion-trapping of BUP on the fetal side, as more BUP (chemically a base) is ionized in the lower pH environment. The increase in F/M-ratio during hypoxia is clinically important, as in humans short term episodes of acute hypoxia are associated with labor and delivery, as a result of uterine contractions and/or compressions of the umbilical cord. The increase observed in the current study can help predict F/M ratios and fetal exposure in humans under normal conditions, as well as during maternal hypoxia. Additionally, the observed increase is clinically relevant, as the higher the fetal BUP exposure the less sensitive cardiotocogram is in fetal monitoring during delivery, and the higher the risk for neonatal respiratory depression and decreased neonatal alertness (Jansson et al., 2017). However, the increase seen in F/M-ratio was not statistically significant. The low transplacental transfer of BUP in the present study supports the use of BUP as an alternative to methadone treatment in opioid maintenance treatment during pregnancy.

High between-subject variability was seen in the present study, but generally the F/M-ratios are lower (13 – 27 %) than the cord/maternal-ratios of BUP seen in human studies. In human studies, BUP concentrations have been previously measured from the umbilical cord blood samples at delivery from women treated with BUP during pregnancy (Bartu et al., 2012; Gordon et al., 2010). Gordon et al. reported a median F/M plasma concentration ratio of 35 % in nine neonate-mother pairs studied. Bartu and associates measured serum BUP concentrations from 10 neonate-mother pairs and reported a mean F/M-ratio of 43 %. In an experimental human placental perfusion model Nanovskaya et al. reported that less than 10 % of the BUP dose given to the maternal side was transferred to the fetal circulation (Nanovskaya et al., 2002). This difference in fetal exposure could be due to the differences in the collected samples and study setting, species differences in metabolism and placenta, or due to the fact that most of our sheep had twin-pregnancies (and thus had more placental and liver tissue and therefore possibly more metabolism capacity), whereas the above-mentioned human studies were done with singleton pregnancies. However, the three sheep in the present study with singleton pregnancies showed similar F/M-ratios as sheep with multiple pregnancies. The F/M-ratios for BUP in the present study are lower than the F/M-ratios (cord/maternal-ratios) reported for methadone (41 %) in human participants, which supports the use of BUP as an alternative to methadone during pregnancy (Gordon et al., 2010).

The transdermal patch provided extended release of BUP, however, a decline was seen in the release rate of BUP 20 h after patch application. Depletion of the transdermal patch was observed for most and was modelled in the population pharmacokinetic model. Similar depletion of the transdermal patch has been observed for human subjects (Kapil et al., 2013; Wang et al., 2016). However, our data suggests more rapid onset of depletion than previously reported. In human studies BUP plasma C_{max} is reached within 48 – 72 h, after which a decline can be observed. In the present study, C_{max} was reached within the first 24 h after patch application for most, after which plasma concentrations declined. Our model estimates that the initial release rate of the patch is 100 % of the nominal release rate. After 20 h of steady release, the release rate begins to decline exponentially with a typical rate constant of 0.0146 h^{-1}, leading to 16 % of the nominal release rate at 144 h (sixth exposure day). The reason for the more rapid depletion is unknown but we hypothesize it is the result of several factors. These include a higher core temperature of the animal (human 37 ºC vs. sheep 39 ºC), excessive number of hair follicles on the application site, use of soap and alcohol for cleansing the area before application, shaving the application area,

| Dose 40 μg/h | Time (h) |
|--------------|----------|
| ID 12        |          |
| ID 14        |          |
| ID 17        |          |
| ID 18        |          |
| ID 19        |          |
| ID 20        |          |
| ID 21        |          |
| ID 22        |          |
| ID 23        |          |
| ID 24        |          |
| ID 25        |          |
| ID 26        |          |
| ID 27        |          |
| ID 28        |          |
| ID 29        |          |
| ID 30        |          |

Fig. 3. (continued).
and keeping the patch in place with adhesive tape and a tight bandage that caused pressure to the patch. For these reasons, the drug could be initially released faster from the patch. One reason for the depletion could also be the fast hair growth on the application site, that could detach the patch from the skin during the exposure period.

The population estimate for CL was higher in the present study (134 L/h) than previously reported for non-pregnant humans in an IV bolus study (mean 50 L/h) (Huestis et al., 2013). Similar differences between pregnant sheep and human have been previously reported by our research team for oxycodone and BUP IV studies (Hakomäki et al., 2021; Kimminen et al., 2018), and could be explained by a higher liver blood flow seen in sheep compared to humans, or by the physiological changes caused by pregnancy (Upton, 2008). Higher CL correlates to lower plasma concentrations. Thus, the initial steady-state plasma concentration during zero-order release process estimated by the population model is approximately 0.15 µg/L for the 20 µg/h releasing patch, which is slightly lower than that observed for non-pregnant humans (0.20–0.30 µg/L) after 20 µg/h BUP dosing (Andresen et al., 2011; Wang et al., 2016). The model estimated volume of distribution was similar in the present study (593 L; total V at steady-state = V1 + V2) to those seen in human studies (mean 743 L) (Huestis et al., 2013).

This experimental study has limitations. The first samples were collected 20–30 h after the patch application on the second exposure day, when the highest plasma concentrations were measured. Thus, we did not collect samples from the absorption phase of the administration and cannot say for sure if higher concentrations would have been measured during the first 20 h of the exposure. However, due to the patch formulation and data from prior human (Kapil et al., 2013) and animal studies, we assume that plasma concentrations much higher than captured by our model are not to be observed for sheep either. Another limitation of our study was that some of the patches wrinkled or moved during the study and needed to be changed. In most of the cases this was noticed during the second exposure day. The changing of the patch resulted in a new peak plasma concentration that was not captured by our model, thus the data from these individuals were excluded from the model dataset. Another limitation of the study is that the model does not allow us to inspect if pregnancy is a significant covariate, since prior data is limited from non-pregnant sheep. Additionally, using a sheep model to predict pharmacokinetics of BUP in humans have limitations, such as species differences in metabolism and placenta, as well as the absorption of BUP from sheep skin, that should be considered.

There are some strengths to this study. The number of animals used in the study increased the reliability of the results and offered us the opportunity to inspect if sheep characteristics (weight, age, and the number or fetuses) explained the variability seen in the pharmacokinetic parameters. Access to the fetus during pregnancy gave us important opportunity to inspect if sheep characteristics (weight, age, and the number or fetuses) explained the variability seen in the pharmacokinetic parameters. Access to the fetus during pregnancy gave us important opportunity to inspect if sheep characteristics (weight, age, and the number or fetuses) explained the variability seen in the pharmacokinetic parameters.

In conclusion, our study was able to show that F/M-ratio of plasma BUP concentrations remain steady under normal conditions after transdermal patch application of BUP in sheep, and that the median fetal exposure is generally less than one fourth of the ewe’s exposure. The low transplacental transfer supports the use of BUP as an alternative to methadone treatment in opioid maintenance treatment during pregnancy. However, a minor increase was observed on the F/M-ratio after induction of maternal hypoxia indicating some ion-trapping of BUP to fetus.

Authorship contributions
Conception & planning: HH, HK, ML, VPR, JR, HMV, MK.
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Data collection: HH, HK, HMM, MK.
Formal analysis: HH, ML, VPR.
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Review & editing: HH, HK, ML, VPR, JR, HMM, MK.
Supervision: HK, ML, VPR.

Funding
This work was supported by the Olvi Foundation, Iisalmi, Finland and The Finnish Concordia Fund, Helsinki, Finland.

Acknowledgments
We appreciate Biocentre Finland and Biocentre Kuopio for supporting LC-MS laboratory facility.
We like to thank the personnel of Oulu Laboratory Animal Centre, University of Oulu for the help to conduct the animal research.
We appreciate the help of Ms. Erica Armstrong in laboratory assistance and revision of grammar.
This is a part of the KuBiCo study for HK.

Supplementary materials
Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2021.105936.

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