

Fentanyl Pharmacokinetics in Pregnant Sheep after Intravenous and Transdermal Administration to the Ewe

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Abstract: Fentanyl is used for pain treatment during pregnancy in human beings and animals. However, fentanyl pharmacokinetics during pregnancy has not been fully established. The aim of this study was to characterize fentanyl pharmacokinetics in pregnant sheep after intravenous and transdermal dosing during surgical procedure performed to ewe and foetus. Pharmacokinetic parameters reported for non-pregnant sheep and nominal transdermal dose rate were utilized for *a priori* calculation to achieve analgesic fentanyl concentration (0.5–2 ng/ml) in maternal plasma. A total of 20 Aland landrace ewes at 118–127 gestational days were used. In the first protocol, 1 week before surgery, 10 animals received 2 µg/kg fentanyl intravenous bolus, and on the operation day, transdermal fentanyl patches at nominal dose rate of 2 µg/kg/hr were applied to antebrahium, and ewes were then given a 2 µg/kg intravenous bolus followed by an intra-operative 2.5 µg/kg/hr infusion. In the second protocol, 10 animals received fentanyl only as transdermal patches on the operation day and oxycodone for rescue analgesia. The data were analysed with population pharmacokinetic modelling. Intra- and post-operative fentanyl concentrations were similar and slightly lower than the *a priori* predictions, and elimination and distribution clearances appeared slower during than before or after the surgery. Transdermal patches provided sustained fentanyl absorption for up to 5 days, but the absorption rate was slower than the nominal dose rate and showed a high interindividual variability. Further research is warranted to evaluate the clinical relevance of the observations made in sheep.

Opioids are commonly used to treat pain during labour and caesarean section, and also for pregnant women for surgical procedures and other severe pain conditions. Fentanyl, a synthetic opiate derivative, has a rapid onset and short half-life [1]. It is metabolized to inactive metabolites. These properties make fentanyl a feasible compound for labour analgesia. During labour, fentanyl is commonly administered spinally, epidurally, intravenously or intramuscularly. Transdermal delivery of fentanyl has been studied in other populations, but the pharmacokinetics of transdermal fentanyl in pregnant women is not known. Furthermore, possible impact of labour- or caesarean section-induced transient changes in physiology on pharmacokinetics of fentanyl is not established.

In animal studies, intravenous and transdermal fentanyl pharmacokinetics has been studied in several species, including llamas, non-pregnant sheep and horses [2–5]. Pharmacokinetics of intravenously administered fentanyl in pregnant sheep has been studied by Craft *et al.* [6] with radioimmunoassay. However, the results may be confounded by cross-reaction of radioimmunoassay with fentanyl metabolites in plasma as shown in horses [7]. Furthermore, the fentanyl concentrations were monitored only up to 120 min. after administration during

which the fentanyl kinetics are mainly governed by distribution processes, thus not allowing reliable estimation of elimination clearance. Moreover, to our knowledge, no data about transdermal fentanyl exposure in pregnant sheep determined with liquid chromatography–mass spectrometry have been published.

The fentanyl dosing regimen for pregnant sheep undergoing major abdominal surgery was designed based on the reported information on fentanyl pharmacokinetics in non-pregnant sheep. The primary aim of the current study was to determine fentanyl pharmacokinetics in pregnant sheep after intravenous and transdermal dosing during abdominal surgery and, thus, to gain confidence on the adequacy of pain treatment. The dosing regimen with target plasma fentanyl concentration of 0.5–2 ng/ml was calculated *a priori* based on the pharmacokinetics reported in non-pregnant sheep and nominal dose rate of transdermal patches. Subsequent drug concentration measurements and further pharmacokinetic modelling were conducted to evaluate the analgesia protocol during and after the surgery.

Materials and Methods

A priori exposure and dose prediction. A two-compartment model and pharmacokinetic parameters in non-pregnant sheep reported by

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Ahern *et al.* [5] were used for *a priori* prediction of fentanyl pharmacokinetics in pregnant sheep and for designing the intra-operative dosing regimen. The dosing regimen for fentanyl consisted of intravenous loading dose 5 min. before the first incision followed by intra-operative infusion in combination with intra- and post-operative transdermal administration using fentanyl matrix patch (Durogesic®; Janssen Pharmaceutica N. V., Beerse, Belgium) attached 1 hr prior to surgery.

Using the medians of two-compartment model macroconstants (A, alpha, B and beta) in non-pregnant sheep reported by Ahern *et al.* [5] and assuming that transdermal absorption rate is constant equal to nominal dose rate of fentanyl patches, fentanyl exposure after transdermal and intravenous administration at different doses and infusion rates was simulated. In addition, uncertainty in *a priori* predictions was visualized by running simulations with all possible combinations of the extremes of the two-compartment model macroconstants reported by Ahern *et al.* [5]. The intravenous loading dose, infusion rate and transdermal dose rate were defined by targeting simulated median fentanyl concentrations to a range of 0.5–2.0 ng/ml which is needed for analgesia in human beings and is often extrapolated to animals [8]. The simulations were conducted with differential equation solver software Berkeley Madonna (version 8.13.18; R Macey and G F Oster, University of California, Berkeley, CA, USA).

Animals. The study protocol was reviewed and approved by the National Animal Experiment Board of Finland. The animal care and experimental procedures were conducted according to the national legislation [9,10] and the EU Directive 2010/63/EU [11].

Altogether 20 time-mated, 2- to 11-year-old Aland landrace ewes (Lammastila Sikka Talu, University of Turku, Rymättylä, Finland) were used in this study in two experimental protocols with 10 animals each. At the beginning of the experiments, the sheep were on between 118 and 127 gestational days (median 124) having either one or two fetuses and weighed between 41 and 67 kg (median weight 53 kg). Two weeks before the experiments, the pregnant ewes were transported to the Laboratory Animal Centre.

Exposure study. The exposure study was carried out in two experiments with 10 sheep each (table 1). Surgical procedures were carried out as described by Erkinaro *et al.* [12]. All animals were weighed before fentanyl dosing, and the required doses of intravenous and transdermal fentanyl were then calculated. In each experiment, both external jugular veins were catheterized and separate veins were used for administration of fentanyl and blood sampling.

The first protocol was performed in two parts, the first part 6–11 days before the experimental surgery and the second one during and after the surgery.

The first part of the first protocol was conducted to characterize systemic distribution and elimination of fentanyl in pregnant sheep. The sheep received 2.0 µg/kg intravenous bolus of fentanyl (Fentanyl Hameln 50 µg/ml; Hameln pharmaceuticals GmbH, Hameln, Germany). After the bolus, timed blood samples were collected during the first 24 hr.

The second part of the first protocol was conducted during the intra- and post-operative pain treatment to the ewe and foetus. On the operation day, transdermal fentanyl matrix patches were prepared to obtain a total dose of 2.0 µg/hr/kg (range 1.9–2.1 µg/hr/kg). One hour before surgery, the sheep were sedated with midazolam and ketamine. The skin of the left antebrachium was clipped. The patch application site was first scrubbed with a liquid soap gauze for 30 sec., shaved with a razor blade and then cleansed with 70% ethanol wipes for 30 sec. The skin was allowed to air-dry, and the fentanyl patches were applied onto the skin. One layer of inflexible adhesive tape was wrapped around the antebrachium to hold the patches in place, after which three layers of flexible self-adhesive bandage were added. The bandage was wound firmly to give constant pressure on the patches, yet avoiding too excessive tightness.

Endotracheal anaesthesia was induced with intravenous propofol and maintained with isoflurane end-tidal concentration of 1–2.5% in an oxygen/air mixture. In addition to transdermal fentanyl administration, just before the surgery, animals received 2.0 µg/kg fentanyl intravenous injections, followed by infusion of 2.5 µg/kg/hr during the operation. Approximately 30 min. before the end of surgery, infusion was stopped. Timed blood samples were collected during and after the operation. Pre-operatively and during the first post-operative days, the ewes were given 1 g of ampicillin intravenously.

In the second protocol, 10 sheep received fentanyl as transdermal patch on the operation day 1 hr before surgery. Patches were applied and sheep were anaesthetized as described above. The sheep received additionally 0.5 mg/kg intravenous (n = 5) or epidural (n = 5) oxycodone at the beginning of surgery and 1.0 µg/kg intravenous fentanyl injections as rescue pain medication when necessary based on clinical status of the animals. Timed blood samples were collected during and after the operation.

Sample collection. Four millilitres of blood samples was obtained from jugular vein catheters approximately at the designated time-points, and the actual sampling time was recorded for data analysis. After sampling, the catheters were flushed with 0.9% NaCl, and then,

Table 1.

Fentanyl dosing regimens used in the exposure study.

	Fentanyl administration	
	Transdermal	Intravenous
Protocol 1 n = 10		
6–11 days before surgery	<ul style="list-style-type: none"> No transdermal administration 	<ul style="list-style-type: none"> 2.0 µg/kg bolus
On the operation day	<ul style="list-style-type: none"> 2 µg/kg/hr 36–95 min. before surgery left attached until the end of pharmacokinetic study 	<ul style="list-style-type: none"> Just before surgery 2.0 µg/kg loading dose followed by 2.5 µg/kg/hr infusion infusion stopped 30 min. before end of surgery
Protocol 2 n = 10		
On the operation day	<ul style="list-style-type: none"> 2 µg/kg/hr 22–64 min. before surgery left attached for several post-operative days Samples for fentanyl quantification were collected up 24 hr or until the first rescue fentanyl injection. 	<ul style="list-style-type: none"> No intravenous administration included in the pharmacokinetic study

50 IU/ml heparin solution was injected to maintain patency. In the first part of the first protocol, timed plasma samples were collected at 5, 15, 30, 45 and 60 min. and at 2, 4, 7, 10 and 24 hr after bolus injection of fentanyl.

In the second part of the first protocol, on the operation day, the baseline blood sample was collected before transdermal fentanyl patch application and the second sample just prior to the intravenous bolus administration. During surgery, samples were collected at 5 min. and 1 hr after the beginning of infusion and just before the end of infusion. After surgery, samples were collected at 30 min. after the end of infusion and at 10, 24, 48, 72 and 96 hr after patch application.

In the second protocol, plasma samples for fentanyl quantification were collected at 2, 10, 30 and 60 min. and at 2, 4, 7, 10 and 24 hr after the beginning of surgery or until the first rescue fentanyl dose.

Blood samples were collected in heparinized plasma tubes, which were turned 10 times and then allowed to stand at room temperature for 30 min. The blood was centrifuged at 985 g for 10 min. Plasma was divided to cryotubes which were stored first at -35°C and within a week moved to -70°C .

Sample analysis. Plasma was analysed with liquid chromatography–mass spectrometry. Two hundred microlitres of plasma was mixed with 50 μl of internal standard solution (50 ng/ml propranolol in water). Supernatants were extracted following precipitation in 96-plate (Waters Sirocco; Waters Corp., Milford, CT, USA). Three hundred microlitres of acetonitrile and 150 μl of plasma sample (after internal standard addition) were added to the wells of the plate. The plate was shaken for 5 min. and centrifuged for 20 min. The quality control (1, 10 and 50 ng/ml in MeOH) and standard samples (dynamic range 0.004–200 ng/ml) were prepared by mixing 270 μl of plasma with 30 μl of standard solution, and analysed in triplicate in each batch.

An ultra-performance liquid chromatographic system (Waters Acquity UPLC liquid chromatograph; Waters Corp.) with an autosampler and column oven with $2.1 \times 50 \text{ mm } 1.7 \mu\text{m}$ particle size column (Water BEHC18; Waters Corp.) and pre-column filter was used. The aqueous eluent phase was 5 mM ammonium bicarbonate (pH 9.8), and the organic phase was methanol. A gradient elution 1-1-90-90% methanol in 0-1-2.5-3.5 min. was performed, followed by 1-min. equilibration. The data were acquired using a triple quadrupole MS (Water XEVO-TQ-S; Waters Corp.) equipped with a z-spray electrospray source, using multiple reaction monitoring. Positive electrospray ionization with 500 V capillary voltage was used. Argon was used as a collision gas and its flow was 0.18 ml/min. The desolvation temperature was 650°C and the source temperature was 150°C . Nitrogen was used as a desolvation gas at 1200 l/hr and as a nebulizer gas at full flow rate. The multiple reaction monitoring transition reactions were m/z 337 >188 (collision energy 21 eV, cone voltage 40 V) for fentanyl and m/z 260 >116 (collision energy 18 eV, cone voltage 28 V) for internal standard propranolol. Dwell time of 0.010 sec. was used for MRM reactions. Quantitation was performed with peak area ratios of the analyte and the internal standard. The mass spectrometer and ultra-performance liquid chromatography system were operated with Masslynx 4.1 software (Waters Corp.).

At the lower limit of quantification (0.004 ng/ml), the accuracy of assay was 127% and the coefficient of variation was 6.6%. The calibration range was 0.004–200 ng/ml.

Data analysis. The pharmacokinetic data were analysed with NONMEM (version 7.2.0; ICON Development Solutions, Ellicott City, MD, USA) with Intel[®] Compiler XE v13.0. The first-order conditional estimation method with interaction (FOCE-I) was used. Additional software employed were Pirana, Perl-speaks-NONMEM and Xpose [13]. Model selection criteria were (1) mechanistic plausibility of the model, (2) convergence of the model fit, (3)

reasonable parameter estimate precision (<50% RSE), (4) visual inspection of the goodness-of-fit plots' (fig 4) and (5) objective function value.

To describe the data, a compartmental model was applied (fig. 1). A two-compartment model with intravenous dosing to, elimination from and the observed plasma fentanyl concentrations corresponding to the central compartment was preliminarily selected for systemic kinetics based on fitting the model to plasma fentanyl concentrations after IV bolus. Volumes of central (V1) and peripheral (V2) compartments, elimination clearance (CL) and intercompartmental clearance (Q) were expressed per kg of body-weight without formal covariate modelling. Thereafter, the data from both protocols were used simultaneously for subsequent model fitting. Transdermal absorption was modelled as constant dose rate to transdermal depot compartment from which the drug absorbs to central compartment with first-order rate coefficient. Also models without transdermal depot compartment and models with decay of transdermal absorption rate due to depletion of fentanyl in the batch were attempted but discarded according to model selection criteria outlined above.

The model without surgery effects or interoccasion variability consistently under-predicted the fentanyl concentrations in plasma during the surgery for all individuals (not shown) suggesting that the error is due to impact of surgery or co-medication administered during surgery on fentanyl disposition rather than random interoccasion variability. Consequently, the effect of surgery on V1, V2, CL and Q was evaluated systematically. As the timing of transdermal dosing in relation to surgery was similar for each individual, the data did not allow reasonable testing whether transdermal absorption kinetics is affected by the surgery.

Results

A priori intra- and post-operative exposure and dose prediction.

Based on the pharmacokinetic parameters reported for non-pregnant sheep and the nominal transdermal dose rate, it was predicted that the targeted fentanyl concentration in ewe plasma (0.5–2.0 ng/ml) would be achieved with transdermal patch dosing at nominal dose rate of 2 $\mu\text{g}/\text{kg}/\text{hr}$ starting at 1 hr before the surgery combined with a 2.0 $\mu\text{g}/\text{kg}$ intravenous loading dose at the start of the surgery followed by

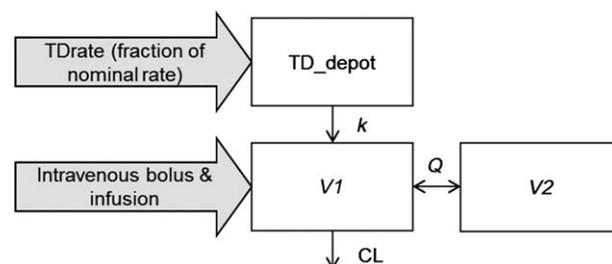


Fig. 1. A schematic representation of the pharmacokinetic model. Systemic pharmacokinetics was described with a two-compartmental model with central (V1) and peripheral (V2) apparent volumes and intercompartmental (Q) and elimination clearances (CL). Transdermal dosing was modelled with a constant dose rate (TDrate) into a TD_depot compartment from which the drug was absorbed to the central compartment with an absorption rate coefficient k . TD_depot compartment enabled description of delay in transdermal absorption and gradual increase in absorption rate to the central compartment up to absorption rate equal to TDrate.

2.5 µg/kg/hr intravenous infusion until the end of the surgery. During the surgery, the *a priori* predicted median concentration was approximately 1 ng/ml, and after the surgery, it was 0.5 ng/ml (fig. 2).

Observed fentanyl concentrations in ewe plasma during the surgery were mostly within both the targeted and *a priori* predicted ranges (fig. 2). In contrast, the observed concentrations after surgery were below the *a priori* predicted median concentration and thus partially below the targeted concentration range.

Pharmacokinetic analysis.

The transdermal depot compartment applied in the population pharmacokinetic model accounts for gradual increase in transdermal absorption rate up to constant absorption rate but does not account for decrease in absorption rate due to depletion of fentanyl from the patches. However, the model is able to adequately capture the behaviour observed in the current study (figs 3 and 4).

In the final model, typical values in population were estimated for apparent volumes of distribution of central (V1) and peripheral (V2) compartments, intercompartmental clearance (Q), elimination clearance (CL), constant transdermal absorption rate (TDRate) and absorption rate coefficient (*k*). Additionally, log-normally distributed between-subject variability (BSV) was estimated for CL, TDRate and *k* and residual error was described with a proportional error model [13]. During surgery, elimination clearance, intercompartmental clearance and central compartment volume were multiplied by factors

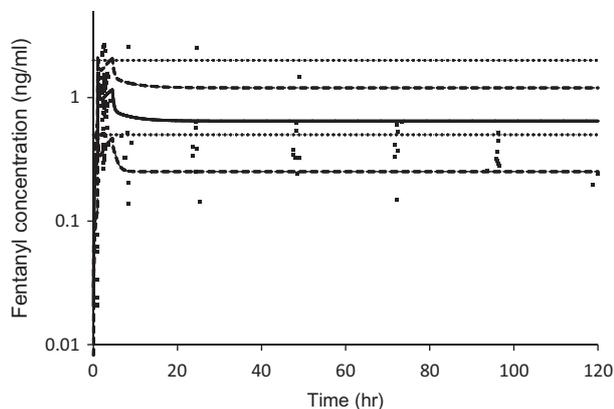


Fig. 2. *A priori* predicted fentanyl exposure and observed concentrations after intravenous and transdermal delivery. Transdermal delivery at 2.0 µg/kg/hr dose rate was initiated at time 0. Approximately 1 hr later, shortly before the surgery, an intravenous bolus dose of 2.0 µg/kg and 2.5 µg/kg/hr intravenous infusion was administered. Ahern *et al.*'s [5] macroconstants were utilized to calculate systemic clearances, clearances between central and peripheral compartment and the volumes of compartments. The solid line represents the median curve and dashed lines the uncertainty caused by variability in pharmacokinetic input parameters. Intravenous bolus dose and infusion rate were determined by targeting the median concentration between 0.5 and 2.0 ng/ml (dotted lines). The observations (black squares) lie mainly between target concentrations, although concentrations after surgery are lower than *a priori* predicted median concentration.

(fCL, fQ and fV1, respectively) to account for apparent changes in fentanyl disposition during surgery. Estimated pharmacokinetic parameter values were mainly in the same range with parameter values published by Ahern *et al.* [5] (table 2), with the exception of systemic clearance which was slightly lower than the range estimated by Ahern *et al.* However, as plasma fentanyl concentrations in non-pregnant sheep were not available for the current population pharmacokinetic analysis, the data do not allow robust evaluation of statistical significance of the apparent impact of pregnancy on fentanyl pharmacokinetics in sheep. The estimated transdermal absorption rate was approximately one-third of the stated nominal rate. The highest between-subject variabilities were observed in estimated absorption rate and absorption rate coefficient. Factors in intra-operative systemic clearance, apparent volume of central compartment and intercompartmental clearance (fCL, fV1 and fQ) were below one, which indicates that during surgery, both distribution and elimination clearances as well as apparent volume of the central compartment are lower than pre- and post-operatively.

In the first part of the first protocol, one sheep showed atypical time–concentration curve with low initial peak concentration after intravenous bolus administration (fig. 5A). The reason for atypical behaviour is not known but may be due to unintended partial subcutaneous or intramuscular dosing and, thus, these data were excluded from the pharmacokinetic analysis.

Additionally, in the second part of the first protocol, one sheep showed high intra- and post-operative fentanyl concentrations followed by decline to similar levels with the other sheep after 2–3 days (fig. 5B). This behaviour might be explained by substantially faster transdermal absorption than nominal and a decline in absorption rate at 25 hr due to depletion of the drug from the patch. The population pharmacokinetic model used was not able to capture such behaviour and, thus, these data were excluded from the population analysis.

In most of the animals, post-operative concentrations ranged between 0.1 and 1.0 ng/ml after patch application, although a slight increase followed by a decline could be seen in most of the animals (figs 5B and 3C). Fentanyl concentrations in ewe plasma were above 0.1 ng/ml for 5 days (figs 4 and 3B). One of the animals appeared inactive and lost its appetite on the second day after surgery. Consequently, it was soon noticed that the bandage had loosened, detaching the patch from the skin. The patch was reattached with a new bandage, after which the condition of the sheep improved. The patch detachment and reattachment coincided with a decline and a rise in plasma fentanyl concentrations (fig. 3C, ID 39, 50 hr).

Discussion

Fentanyl is an effective opioid analgesic which is used to treat pain during pregnancy in various species. However, its pharmacokinetics during pregnancy is not established. In the present study, fentanyl exposure was evaluated after intravenous and transdermal administration in pregnant sheep and the exposure was predicted *a priori* based on the information

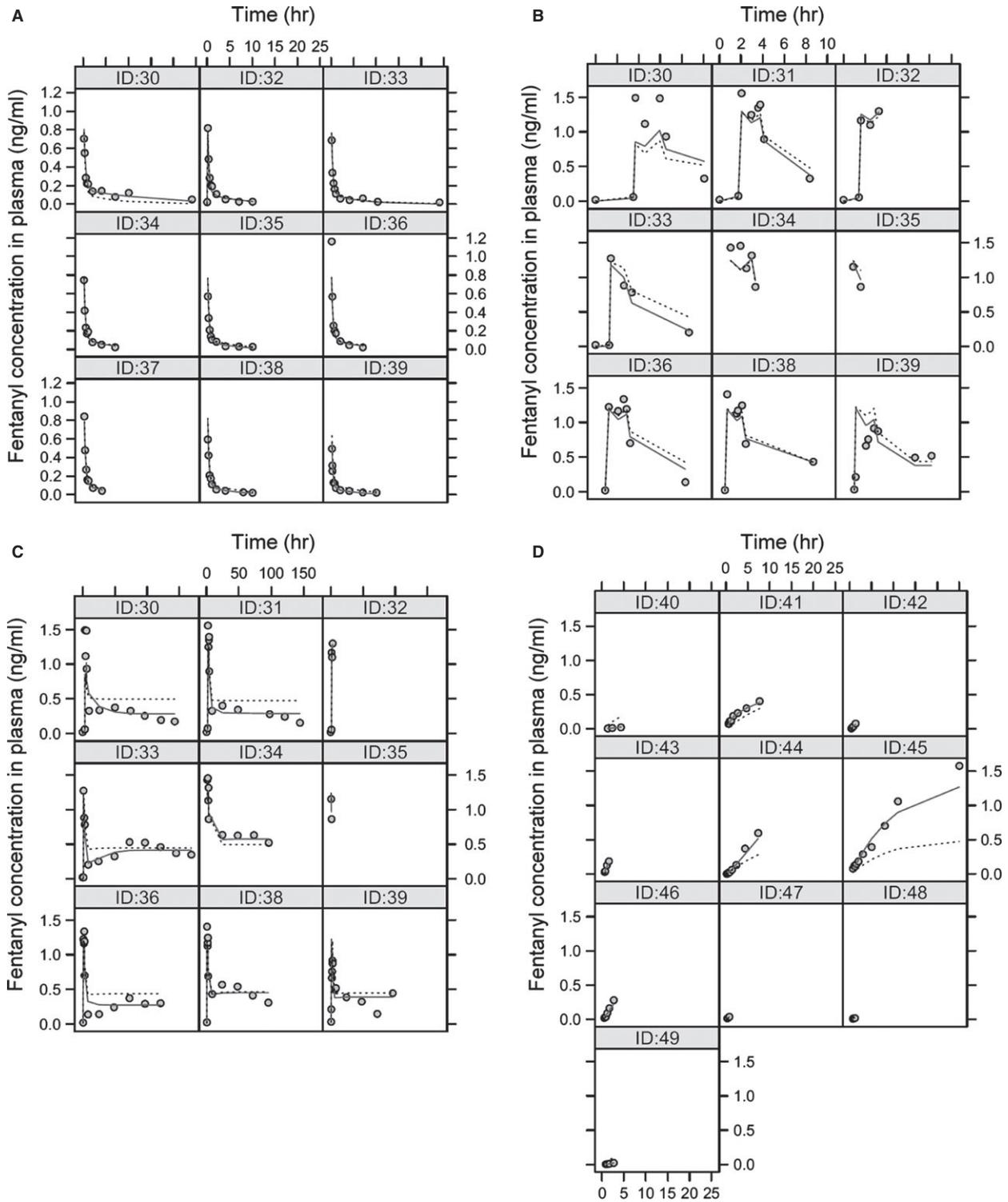


Fig. 3. Observed and predicted fentanyl concentrations in ewe plasma after fentanyl administration. Open circles represent observations, solid lines individual predictions and dash lines population predictions. (A) Pre-operative intravenous bolus (protocol 1). ID 31 was excluded from the analysis. (B) (0–10 hr) and (C) (the whole protocol) Intra-operative transdermal patch, intravenous loading bolus and intravenous infusion (protocol 1). ID 37 was excluded from the analysis. (D) Intra-operative transdermal patch (protocol 2).

available on fentanyl pharmacokinetics in non-pregnant sheep and on nominal transdermal absorption rate of the patches used. The fentanyl concentrations in sheep plasma were

mostly within the *a priori* predicted range, but the population pharmacokinetic analysis suggested that both elimination clearance and transdermal absorption rate were lower than the

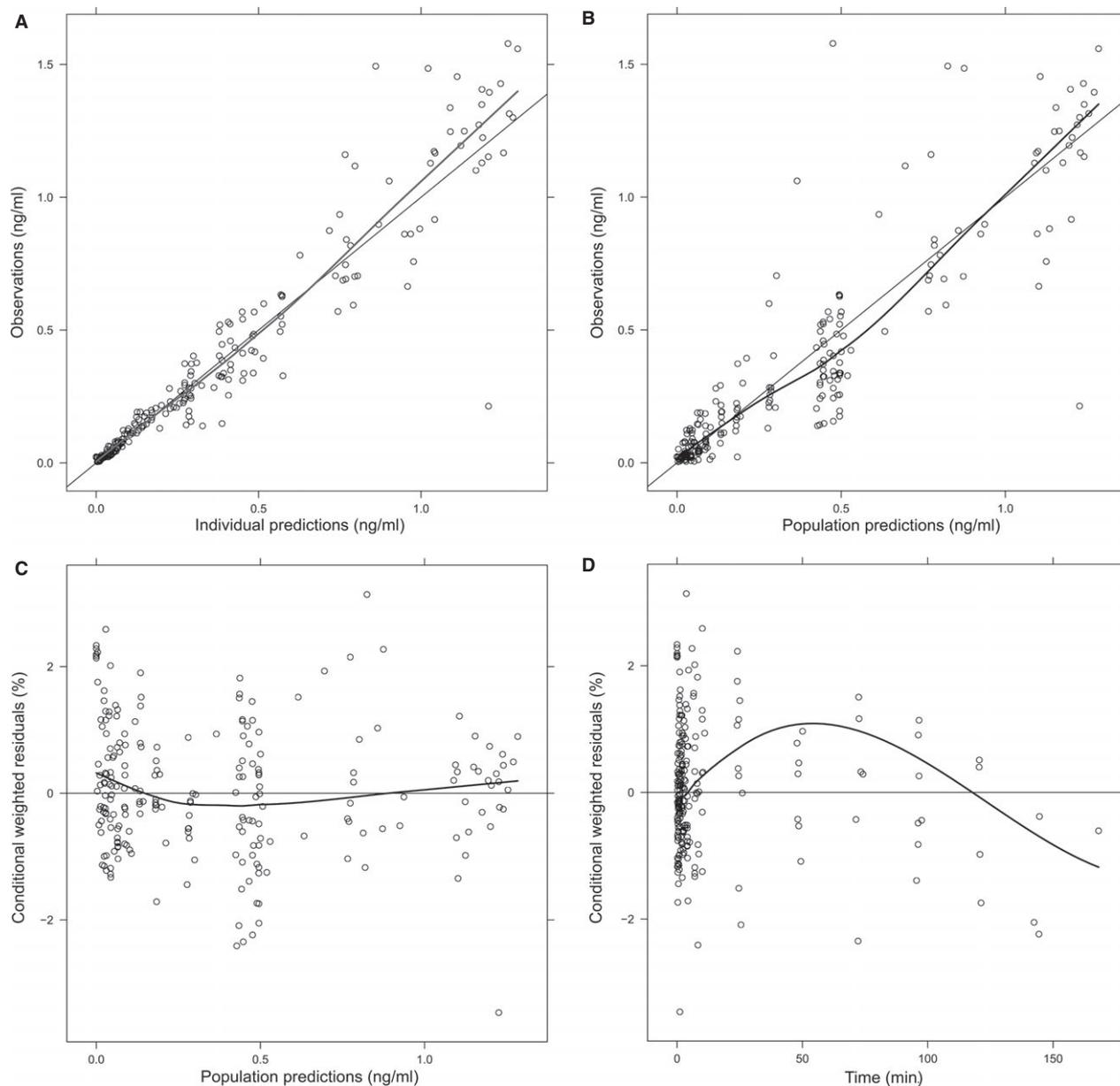


Fig. 4. Performance of the population model. (A) Observed concentrations *versus* individually predicted concentrations. (B) Observed concentrations *versus* population-predicted concentrations. (C) Conditional weighted residuals *versus* population-predicted concentrations. (D) Conditional weighted residuals *versus* time.

prior estimates. Furthermore, transdermal absorption rate showed a high variability between ewes. Intra-operative fentanyl concentrations were slightly higher than expected in view of the pre-operative distribution and elimination kinetics, and transdermal fentanyl was absorbed for several days.

The transdermal absorption rate observed in this study was approximately one-third of the nominal absorption rate of fentanyl matrix patch, and high interindividual variability was seen in both transdermal absorption rate and the time required to reach a steady transdermal absorption rate. These findings are similar to data in some other species. In cats and dogs, the transdermal fentanyl absorption rate is lower than the nominal rate: in cats, the absorption rate is 36% [14] and in dogs 71%

[15] of the nominal dose rate. As it was found in pregnant sheep, high interindividual variability in absorption rates has been observed among human beings, cats, dogs and horses [2,14–19]. Both interspecies and interindividual variabilities in transdermal absorption characteristics of fentanyl have been explained with variation in stratum corneum thickness, epidermal thickness, amount of subcutaneous fat, local blood flow and skin fat content [14,16].

Ahern *et al.* [5] have studied intravenously and transdermally administered fentanyl pharmacokinetics in non-pregnant sheep via liquid chromatography–mass spectrometry. In a study by Ahern *et al.*, the peak concentration (mean 1.3 ng/ml) was observed at 12 hr after fentanyl patch application.

Table 2.

Pharmacokinetic parameters after transdermal fentanyl administration obtained from population pharmacokinetic modelling.

Parameter	Estimate	RSE %	Ahern <i>et al.</i> (range)
CL (l/hr/kg)	1.83	21.7	3.6 (2.5–5.4)
BSV_CL (%)	44.9	38.4	NA
fCL	0.52	40.3	NA
V1 (l/kg)	2.06	10.5	2.24 (1.4–3.8)
fV1	0.78	12.8	NA
V2 (l/kg)	8.2	10.5	6.9 (4.7–9.2)
Q (l/hr/kg)	3.97	9.6	2.7 ¹
fQ	0.51	46.2	NA
TDmax (fraction of nominal)	0.320	32.5	NA
BSV_TDmax (%)	70.1	23.2	NA
K (1/hr)	0.382	37	NA
BSV_K (%)	109.5	49.2	NA

CL, pre- and post-operative elimination clearance; fCL, intra-operative elimination clearance as a fraction of CL; V1, pre- and post-operative apparent volume of central compartment; fV1, intra-operative apparent volume of central compartment as a fraction of V1; V2, apparent volume of peripheral compartment; Q, pre- and post-operative intercompartmental clearance; fQ, intra-operative intercompartmental clearance as a fraction of Q; TDmax, transdermal absorption rate; K, absorption rate constant; RSE, relative standard error; BSV, between-subject variability.

¹Calculated from medians of two-compartment model macroconstants (A, alpha, B and beta) because median and range of Q were not reported by Ahern *et al.*

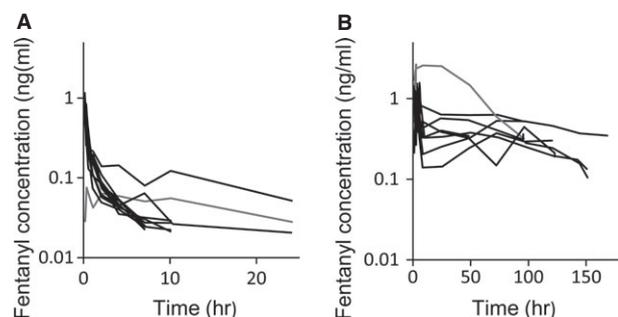


Fig. 5. The concentration–time data obtained from exposure study protocol 1. (A) Pre-operative 2.0 µg/kg intravenous bolus administration to 10 individuals. One individual showed atypical concentration curve (marked with grey) and was excluded from the population pharmacokinetic analysis. (B) Intra-operative 2.0 µg/kg intravenous bolus, 2.5 µg/kg/hr infusion and 2.0 µg/kg/hr transdermal patch to 10 individuals. One individual (marked with grey) showed high fentanyl concentrations and was excluded from the population pharmacokinetic analysis.

Concentrations were above 1 ng/ml for several hours and then declined in a linear fashion. In the current study, maximum fentanyl concentrations were achieved later than reported by Ahern *et al.*, but after that fentanyl concentrations were rather stable for several postoperative days, although a slight declining trend and interindividual variability in concentrations were found. In the current study, plasma fentanyl concentrations and transdermal absorption rate were lower than the ones

observed in a study by Ahern *et al.* It can be speculated that the apparent differences in transdermal absorption rate between the current and the study by Ahern *et al.* may also be due to the differences in patch formulation. Durogesic® reservoir patches were phased out and replaced by matrix patches in the late 2000s [17,20] and, although published in 2010, the study by Ahern *et al.* does not specify which one of the fentanyl patch formulations was used.

The animal position during and after the surgery may affect transdermal fentanyl pharmacokinetics, if the temperature of the patch skin area is raised, increasing the transdermal absorption [5]. In the present study, transdermal fentanyl patch was applied to the antebrachium of the ewe, identically to the study by Ahern *et al.* [5]. However, in Ahern's study, the left forelimb with fentanyl patch was placed under the animal during surgery, whereas in the present study, ewes lay in dorsal recumbency during laparotomy and the patch site was free of pressure. This may contribute to the observed differences in transdermal absorption rate and fentanyl concentrations between these two studies. Furthermore, impact of pregnancy on transdermal absorption of fentanyl cannot be ruled out.

To obtain reproducible transdermal absorption, steady fentanyl concentrations in plasma and adequate analgesia, patch application should be performed carefully. Firm patch adherence to the animal skin is crucial for analgesic effect and steady fentanyl concentrations in plasma. It has been discussed that both species differences and interindividual variability in transdermal absorption of fentanyl are likely to be attributable to variability in patch application protocol [5]. However, consistent fentanyl concentrations can be achieved by careful patch application [5]. In the current study, the skin preparation for patch application was standardized and was similar to application in the study by Ahern *et al.* Unlike in the study by Ahern *et al.*, in the current study, the application site was washed with soap and prepared with ethanol to remove excess grease from the animal skin. Nevertheless, in the present study, transdermal absorption of fentanyl continued for 5 days after patch application.

The *a priori* simulation was a practical way to predict appropriate doses for obtaining 0.5–2.0 ng/ml fentanyl concentration in ewe plasma. However, compared to the simulation with the published pharmacokinetic parameters [5], the observed concentrations after surgery were lower. Population pharmacokinetic modelling suggested that this was primarily due to slower than nominal transdermal absorption rate which was not taken into consideration in the simulations.

During surgery, the observed fentanyl concentrations were higher than expected based on distribution and elimination kinetics observed before surgery. Surgery and anaesthesia have an impact on liver perfusion which may affect the distribution and elimination kinetics of fentanyl resulting in unexpectedly high fentanyl concentrations. In support of such speculation, in sheep anaesthetized with inhalation anaesthetics, hepatic blood flow decreases by 46%, hepatic clearance of pethidine decreases to 60% of control, and renal clearance is abolished [21]. Consequently, plasma pethidine concentration in

halothane-anaesthetized sheep was twice as high as in the control group of awake unrestrained sheep treated with pethidine infusion. In addition to potential impact of changes in haemodynamics on fentanyl pharmacokinetics, possible surgery- and anaesthesia-induced increases in skin temperature and perfusion, which increase transdermal absorption, may have affected the transdermal absorption of fentanyl.

Several conclusions can be drawn from the population pharmacokinetic analysis. Distribution kinetics of fentanyl in pregnant sheep is similar to that reported in non-pregnant sheep [5]. Plasma fentanyl concentrations in pregnant ewes during major abdominal surgery were higher than expected based on pharmacokinetics approximately 1 week prior to surgery. This may be due to transient changes in liver perfusion during anaesthesia and surgery and consequent decrease in apparent distribution and elimination clearances and central volume of distribution. There is a high interindividual variability in fentanyl absorption rate from transdermal patches, and the absorption rate in pregnant sheep appears to generally be approximately 30% of the nominal dose rate. Furthermore, comparison with data reported for non-pregnant sheep [5] did not indicate major pregnancy-induced changes in fentanyl pharmacokinetics in sheep, although pregnancy-induced changes in transdermal absorption and fentanyl clearance could not be ruled out. Further research is warranted to evaluate whether the above-mentioned observations in the sheep apply to pregnant women.

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