Fentanyl is a potent opioid agonist that is delivered via a TFP for postoperative analgesia in veterinary medicine. Sheeps commonly serve as surgical research animals, and the care and welfare of animals enrolled in biomedical research are of paramount importance. As a result, postoperative analgesia must be provided. Transdermally administered fentanyl is an attractive option to researchers because it offers a potent analgesic agent in a delivery form that allows prolonged delivery and duration of action. Transdermal fentanyl patch systems have been used during ovine orthopedic research. The pharmacokinetics of fentanyl administered via TFPs in goats and horses have been evaluated. However, to the authors' knowledge, the pharmacokinetics of fentanyl administered via TFPs in sheep have not yet been reported. The objective of the study reported here was to determine the pharmacokinetics of IV administered fentanyl in healthy sheep and the pharmacokinetics of fentanyl administered via TFP in sheep in a surgical trial.

Objective—To investigate the pharmacokinetics of fentanyl administered transdermally and IV in sheep.

Animals—21 adult female sheep.

Procedures—Fentanyl was administered IV to 6 healthy sheep. Transdermal fentanyl patches (TFPs) were applied to 15 sheep 12 hours prior to general anesthesia and surgery. Serial blood samples were collected for 18 hours after IV injection and 84 hours after TFP application. Fentanyl concentrations were quantified via liquid chromatography–mass spectrometry, and pharmacokinetic values were estimated.

Results—All sheep completed the study without complications. Following a dose of 2.5 µg/kg administered IV, the β half-life was 3.08 hours (range, 2.20 to 3.36 hours), volume of distribution at steady state was 8.86 L/kg (range, 5.55 to 15.04 L/kg), and systemic clearance was 3.62 L/kg/h (range, 2.51 to 5.39 L/kg/h). The TFPs were applied at a mean dose of 2.05 µg/kg/h. Time to maximum plasma concentration and maximal concentration were 12 hours (range, 4 to 24 hours) and 1.30 ng/mL (range, 0.62 to 2.73 ng/mL), respectively. Fentanyl concentrations were maintained at >0.5 ng/mL for 40 hours after TFP application.


Materials and Methods

Drug administration and sample collection—This study was approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Twenty-one adult Polypay-crossbreed ewes (mean ± SD age, 3 ± 1 years; mean ± SD weight, 63.0 ± 8.2 kg) were used. Six sheep were administered a single dose (2.5 µg/kg) of fentanyl IV, and 15 sheep used in an orthopedic research study were administered fentanyl via TFP at a targeted dose of 2.0 µg/kg/h. Sheep were housed in groups of 4 in 4 × 4-m hospital stalls for the duration of the study. All sheep were fed a maintenance diet of timothy hay ad libitum and formulated sheep feed once per day. Water was available ad libitum. Feed and water were routinely tested to ensure quality control.

In all sheep, an indwelling venous catheter was placed in the left jugular vein by use of an aseptic technique and maintained for the duration of the study to...
allow for blood sampling. Fentanyl was administered IV in the contralateral vein, which was similarly catheterized. Blood samples (5 mL) were collected at 0, 2, 3, 10, 30, and 45 minutes and 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 hours after fentanyl administration.

Each sheep in the TFP treatment group was weighed 12 hours prior to surgery, the required dose of fentanyl was calculated, and the appropriate number and combination of patches were prepared (2.5, 5, 7.5, or 10 mg). The total quantity of fentanyl in the patches applied to the sheep was 12.8 ± 1.8 mg (mean ± SD) or an estimated dose of 0.20 ± 0.01 mg/kg. The left antebrachium was selected as the site of patch application, as described. In brief, the left antebrachium was clipped circumferentially and shaved on the lateral aspect (site of patch application) with care not to traumatize the skin. After skin preparation, the patches were applied and secured in place. Patches were removed 72 hours after application of blood samples (5 mL) were collected immediately prior to and after application of the patches and at the following time points: 2, 4, 6, 12, 24, 36, 48, 60, 72, and 84 hours. Twelve hours prior to surgery, feed and water were withheld and anesthesia was carried out as described. All TFP-treated sheep underwent a unilateral left tibial osteotomy that was repaired by use of a 7-hole locking compression plate 12 hours after patch application. This meant that the left forelimb with the applied patch was on the so-called down side during anesthesia. The patch was protected with padding during the surgery such that effective air circulation around the patch was maintained. All sheep received perioperative administration of antimicrobials daily for 3 days starting immediately prior to induction and phenylbutazone (2.2 mg/kg, IV) daily for 3 days.

All blood samples were collected into evacuated tubes containing lithium heparin† and gently rotated for 30 seconds. Within 1 hour of collection, plasma was obtained via centrifugation at 377 × g for 10 minutes, transferred to an evacuated tube containing lithium heparin, and stored at −80°C pending analysis.

Fentanyl analysis—Liquid chromatography–MS and gas chromatography–MS methods as described in the quantification of fentanyl in plasma of humans and horses were used. Fentanyl standard (0.05 mg/mL) was used, and the internal standard was d-9 clenbuterol (200 µg/mL in methanol†). All reagents used, including water, were of analytic quality. Water, acetone, methyl tert-butyl ether, ammonium hydroxide, and formic acid were purchased and used.

Standards and quality control samples—Working solutions were prepared in 50:50:1 acetonitrile:water:formic acid at 0.1, 2.5, 5.0, 7.5, 10, 50, 1, and 100 ng/mL from reference materials. Calibration curves were prepared daily in drug-free equine plasma containing 1% NaF/mL to inhibit plasma cholinesterase activity. A calibration curve was prepared in 0.9 mL of negative control plasma by use of 0.1 mL of the working solutions. Positive controls were similarly prepared in duplicate at 0.5 and 5 ng/mL. Calibrators and controls were analyzed in duplicate. Negative control plasma samples with and without internal standard were analyzed with each analytic batch. Working solutions were stable throughout the duration of the study.

Sample preparation and extraction—One milliliter of ammonium hydroxide solution (1:1 ammonium hydroxide:water) was added to 1 mL of calibrators, controls, and test samples. Self-suppression of either target analyte or internal standard was avoided by use of a labeled internal standard, d-9 clenbuterol, which has extraction, positive electrospray, and chromatographic properties similar to fentanyl. Extraction efficiency and chromatographic elution of d-9 clenbuterol were similar to fentanyl while avoiding chromatographic elution at the exact retention time of fentanyl. Internal standard (0.1 mL [100 ng/mL]) was added to all calibrators, control samples (except 1/2 negative controls), and test samples and then mixed by use of a vortex for 15 seconds. Methyl tert-butyl ether (5 mL) was added to all tubes, and tubes were capped and mixed by use of a rotating rack for 10 minutes prior to centrifugation at 377 × g for 10 minutes. The organic layer (top layer) was transferred to clean borosilicate tubes and evaporated to dryness at 65°C under a steady stream of nitrogen or air. The residues were reconstituted in 100 µL of 0.1% formic acid in water and transferred to 2-mL autosampler vials fitted with 0.2-mL limited-volume inserts. Analyte recovery from the matrix was 94.5%.

LC-MS-MS electrospray ion analysis—Analysis of the sample extracts was performed by use of a linear ion trap interfaced to a liquid chromatograph and autosampler. Analysis was performed in positive electrospray ion mode. The mobile phase comprised 2.33 mM aqueous formic acid (A; pH, 6) and 0.1% formic acid–acetonitrile (B). The pump was operated in a linear gradient from 13% A to 100% B at 0.2 mL/min at ambient temperature for 4 minutes, with a 1-minute hold time at 100% B. The system was re-equilibrated to initial conditions for 1 minute. Total cycle time per sample was 7.5 minutes. Chromatographic separation of analytes was achieved by use of a fused core carbon 18 column (2.7-µm particle size, 2.1 × 50 mm).† The electrospray ion source was operated with a positive spray voltage of 5.5 kV and sheath gas (high-purity nitrogen) of 18 arbitrary units. The capillary temperature was 350°C. Ion trap settings for MS-MS analyses were as follows: fentanyl protonated molecular ion [M+H]+ (m/z, 337.3; collision energy, 20 eV), norfentanyl protonated molecular ion [M+H]+ (m/z, 353.1, collision energy, 20 eV), hydroxyl fentanyl protonated molecular ion [M+H]+ (m/z, 353.3; collision energy, 26 eV wideband), and d-9 lenbuterol internal standard protonated molecular ion [M+H]+ (m/z, 286.2; collision energy, 23 eV wideband). Product ions monitored for quantitative analysis were fentanyl (m/z, 188 and 216), norfentanyl (m/z, 84), hydroxyfentanyl (m/z, 188 and 204), and internal standard (m/z, 204 and 268).

Quantitative analysis—Analytes were quantified by use of LC acquisition software. Calibrations and determinations of unknown samples were the summed ion currents of the product ions. A linear unweighted calibration curve with forced zero intercept was used
for fentanyl quantification. Limit of quantification was 10 pg/mL.

Intra-assay precision and accuracy were evaluated by analyzing the prerun and postrun calibrations for all study batches (n = 9). Precision is expressed as coefficient of variation (%), and accuracy is expressed as percentage of the target concentration. Ranges are provided for intra-assay data (Table 1).

The between-run precision ranged from 0.1 to 10 ng/mL, and accuracy of the concentration was 9.2% and 92.1%, respectively. Recovery was determined by comparison of neat working stocks and fortified negative plasma following preparation via the extraction method. Method recovery was approximately 95%. No method or instrument carryover was detected within the reporting range of results. Standard operating procedures for the quantification of analytes by this laboratory meet requirements for accreditation by the American Association for Laboratory Accreditation and ISO 17025 International Guidelines.15

Pharmacokinetic analysis—The AUC following IV or TFP administration of fentanyl was analyzed by use of nonlinear least squares regression analysis.16-19 A 2-compartment model and a 3-compartment mammary model were fitted to the AUC from each sheep. The number of compartments required to best describe the IV data were based on the appearance of the curve, the reduction in the sums of squares, and the minimization of FSD of each compartmental parameter. The software allows translation between the compartmental models and equivalent exponential forms. The fractional rate constants for the IV 2-compartment model with elimination from the central compartment (k10) and the distribution to the peripheral compartment (k12 and k13) were converted to the macro-constant exponents α and β, as described.18,19

A 1-compartment model with transfer from the patch (transfer rate constant) into and elimination from the central compartment (elimination rate constant) was fitted to TFP data from each sheep. A number of weighting schemes [W(K)] were used for data during the fitting process. The FSD is in the form of W(K) = 1/C*QO(K)**, where QO(K) is the k0 observed datum and C is the FSD. The SD weighting scheme is in the form of W(K) = 1/C**. The FSD weighting process favors the terminal phase of the decay curve, whereas SD favors the larger and intermediate data points. The fitting process (iterations) ceased when the improvement in the sums of squares of the last iteration was < 1%.18,19

The A and B coefficients (ng/mL) for IV administration were calculated from the dose, volume of central compartment (Vc), and the relevant compartmental rate constants.20 Half-lives were calculated as the Ln2 divided by the exponential constants. The total AUC from 0 to last data point (AUC∞) was calculated by use of the trapezoid.

The volume of the central compartment was calculated as follows:

\[ Vc = \frac{D_n}{C_0} \]

where Dn is the dose administered IV and C0 is the plasma concentration at time zero, calculated as the sum of the coefficients A and B. Ratios of the intercompartmental rate constants k1/k2, Vc were used to calculate the volumes of compartments Vc, and the volume of distribution at steady state (Vss) following IV administration was calculated as the sum of all compartments as follows:21

\[ Vss = \left(\frac{k_{12} + k_{21}}{k_{21}}\right) Vc \]

Clearance (Cl) following the IV administration was calculated as follows:

\[ Cl = \frac{D_n}{AUC_{ss}} \]

Pharmacokinetic parameter estimates of fentanyl are expressed as median and range values. The plasma concentrations of fentanyl are expressed as mean ± SD values.

Results

IV administration—Decrease of the AUC of fentanyl administered IV was best described by use of a 2-compartment model (Figure 1). Plasma concentrations were 1,647.7 ± 479.2 pg/mL and 11.0 ± 2.9 pg/mL at 2 minutes and 10 hours, respectively. Pharmacokinetic

<table>
<thead>
<tr>
<th>Fentanyl (ng/mL)</th>
<th>Min CV (%)</th>
<th>Max CV (%)</th>
<th>Min Acc (%)</th>
<th>Max Acc (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>2.26</td>
<td>24.58</td>
<td>78.95</td>
<td>182.18</td>
</tr>
<tr>
<td>0.25</td>
<td>0.51</td>
<td>29.35</td>
<td>96.81</td>
<td>127.68</td>
</tr>
<tr>
<td>0.5</td>
<td>0.50</td>
<td>8.62</td>
<td>98.02</td>
<td>124.08</td>
</tr>
<tr>
<td>0.75</td>
<td>1.48</td>
<td>10.04</td>
<td>91.86</td>
<td>122.74</td>
</tr>
<tr>
<td>1</td>
<td>0.91</td>
<td>9.19</td>
<td>98.65</td>
<td>115.92</td>
</tr>
<tr>
<td>5</td>
<td>0.40</td>
<td>2.77</td>
<td>94.94</td>
<td>101.80</td>
</tr>
<tr>
<td>10</td>
<td>0.17</td>
<td>2.08</td>
<td>99.15</td>
<td>100.79</td>
</tr>
</tbody>
</table>

Figure 1—Plasma concentration (mean ± SD) of fentanyl following IV administration of 2.5 µg/kg in 6 sheep. Solid line is the line of best fit.
Table 2—Pharmacokinetic parameter estimates (median and range) following a single IV administration of fentanyl (2.5 µg/kg) in 6 sheep.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (µg/mL)</td>
<td>1,225.4</td>
<td>469.5–1,887.4</td>
</tr>
<tr>
<td>α (h)</td>
<td>0.23</td>
<td>0.14–0.25</td>
</tr>
<tr>
<td>λ1,2 (h)</td>
<td>3.08</td>
<td>2.20–3.36</td>
</tr>
<tr>
<td>B (µg/mL)</td>
<td>95.7</td>
<td>67.6–180.8</td>
</tr>
<tr>
<td>β (h)</td>
<td>0.22</td>
<td>0.21–0.31</td>
</tr>
<tr>
<td>λ3 (h)</td>
<td>3.29</td>
<td>2.76–4.90</td>
</tr>
<tr>
<td>Cl (L/h/kg)</td>
<td>2.24</td>
<td>1.44–3.81</td>
</tr>
<tr>
<td>V1 (L/kg)</td>
<td>6.84</td>
<td>4.73–9.18</td>
</tr>
<tr>
<td>V2 (L/kg)</td>
<td>8.86</td>
<td>5.55–15.04</td>
</tr>
<tr>
<td>VSS (L/kg)</td>
<td>2.24</td>
<td>1.44–3.81</td>
</tr>
<tr>
<td>AUC∞ (µg·h/mL)</td>
<td>709.5</td>
<td>450.9–955.5</td>
</tr>
<tr>
<td>α (µg·h·kg⁻¹)</td>
<td>1,302.0</td>
<td>616.5–2,731.2</td>
</tr>
<tr>
<td>λ1,2 (µg·h·kg⁻¹)</td>
<td>15.6</td>
<td>10.8–27.2</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>4.5</td>
<td>1.4–6.9</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>12.0</td>
<td>4–24</td>
</tr>
<tr>
<td>Cmax (µg/mL)</td>
<td>3,362</td>
<td>1,302.0–5,392.1</td>
</tr>
<tr>
<td>AUC∞ (µg·h/mL)</td>
<td>44,277.0</td>
<td>28,736.2–63,835.1</td>
</tr>
</tbody>
</table>

Parameter estimates were determined (Table 2). The half-life α and β values were 0.21 and 3.08 hours, respectively. Mean ± SD of the FSD for all compartmental parameter estimates used in defining the IV model was 0.026 ± 0.010.

TFP administration—A mean of 12.8 ± 1.8 mg of fentanyl was transdermally administered to the sheep 12 hours before surgery. Increases and decreases in the plasma concentrations following TFP administration were best described by use of a 2-compartment model with first-order transfer and elimination from the central compartment (Figure 2). Pharmacokinetic parameter estimates were determined (Table 3). The transfer half-life and elimination half-life were 4.5 and 15.6 hours, respectively. Fentanyl was quantifiable in all sheep at 84 hours, with a plasma concentration of 84.6 ± 45.7 µg/mL. Mean FSD for all compartmental parameter estimates used in defining the TFP model was 0.077 ± 0.12. Total quantity of fentanyl administered IV versus TFP was 130.8 ± 18.9 µg versus 12,800 ± 1,800 µg, respectively; the IV dose represented only 1.02% of the TFP dose. All sheep completed the study without complications or adverse effects of either the drug or surgery.

Discussion

This study evaluated the pharmacokinetic properties of IV and transdermally administered fentanyl in sheep. The transdermal application protocol resulted in more consistent fentanyl absorption with less variability in plasma concentrations, compared with previous studies. Variability observed in total absorption between previous studies and the present study may have been attributable to species differences, but was more likely attributable to variability in patch application protocols. Other investigators have described rates of absorption and results within each study as extremely variable. The relatively consistent plasma concentrations of fentanyl obtained in the present study were the result of careful attention to patch application, leading to reproducible and effective skin-to-patch contact.

Fentanyl concentrations obtained after transdermal application reached a plateau after reaching peak concentration, prior to a linear decline. The plateau response corresponded to surgery and the immediate postoperative period. Reports of previous studies postulated absorption variability to be attributable to patch placement, ineffective adherence, skin thickness, blood flow at the site, and core body temperature. In the present study, it was hypothesized that anesthesia most likely caused vasodilatation and increased perfusion of the skin at the site of application, which may have led to increased absorption during surgery and possibly contributed to the postoperative plateau of fentanyl concentration in plasma. Additionally, recumbency during surgery and the postoperative period may have caused increased temperature of the patch-skin region, resulting in increased absorption. Results indicated that plasma fentanyl concentrations could be influenced by anesthesia and surgery. Thus, as is recommended on the drug warnings supplied with the transdermal system, care should be taken to avoid heating of the patch-skin interface to avoid variable fentanyl absorption.

The plasma fentanyl concentration required for optimal analgesia in sheep has not been determined; however, the concentration (0.5 to 2.0 ng/mL) needed for analgesia in human is commonly extrapolated to animal species. In cats subjected to onychectomy, fentanyl patches delivering 25 µg/h have been com...
pared with IM administration of butorphanol. In those cats, on the basis of evaluation of clinical variables and subjective evaluation of the response to noociceptive stimulus, the investigators concluded that serum concentrations of 0.5 to 7.0 ng/mL were associated with analgesia. In dogs undergoing abdominal surgery, fentanyl patches delivering 25 and 50 µg/h induced serum concentrations of 0.11 to 1.1 ng/mL, from which the authors concluded that an analgesic level was achieved in all animals. In the present study, TFP application resulted in plasma fentanyl concentration that rapidly increased to > 0.5 ng/mL, which was maintained for 40 hours. Repeated application of fentanyl patches as hypothesized in horses should be readily applicable to sheep, allowing for prolonged analgesic effects if a longer duration of action than was observed in the present study is required. However, further study is needed to determine whether the reported observation in horses is applicable to sheep.

The use of LC-MS to quantify fentanyl concentrations in plasma was reported and validated in humans. The technique used in the present study allowed for effective evaluation of the pharmacokinetic properties of fentanyl in sheep. This is the first study, to the authors’ knowledge, that determined concentrations of fentanyl in plasma after IV and transdermal administrations in sheep. The elimination β half-life of fentanyl following IV administration was 3.08 hours, compared with 1.2 hours and 1.0 hour for goats and horses, respectively. The rapid decrease in plasma concentrations following IV administration of fentanyl suggested that the rate of decline in TFP application was related to absorption. For transdermally applied fentanyl, the plasma concentrations were consistent with a so-called flip-flop model that was detected in another fentanyl pharmacokinetic study. A previous application study in human patients with patches containing 2.5 and 10 mg revealed that following 3 days of continuous use, 28% to 84.4% of the fentanyl remained in the patch. Sufficient quantity was available in the patch for misuse and abuse in humans; therefore, an adequate disposal policy is urged following their use in animals. In the study reported here, the residual volume of fentanyl in the applied patches was not evaluated.

The TFP application induced stable blood fentanyl concentrations in sheep in the present study, and effects were considered superior to buprenorphine in a double-blind pain evaluation study. It should be emphasized that careful patch application is the key to consistent results, and the data from the present study may be applicable only to the specific patch used. The information reported in this study can be used to more effectively plan intra- and postoperative analgesia in clinical cases and sheep used in research.

References

32. Marquardt KA, Thrarrt RS, Musallam NA. Fentanyl remaining in a transdermal system following three days of continuous use. Ann Pharmacother 1995;29:969–971.