Investigation of in vitro transdermal absorption of fentanyl from patches placed on skin samples obtained from various anatomic regions of dogs

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Objective—To investigate in vitro transdermal absorption of fentanyl from patches through skin samples obtained from various anatomic regions of dogs.

Sample Population—Skin samples from 5 Greyhounds.

Procedure—Skin samples from the dogs' thoracic, neck, and groin regions were collected postmortem and frozen. After samples were thawed, circular sections were cut and placed in Franz-type diffusion cells in a water bath (32°C). A commercial fentanyl patch, attached to an acetate strip with a circular hole, was applied to each skin sample. Cellulose strips were used as control membranes. Samples of receptor fluid in the diffusion cells were collected at intervals for 48 hours, and fentanyl concentrations were analyzed by use of high-performance liquid chromatography.

Results—Mean ± SD release rate of fentanyl from the patch, defined by its absorption rate through the non–rate-limiting cellulose membrane, was linear during the first 8 hours (2.01 ± 0.05 μg/cm^2 of cellulose membrane/h) and then decreased. Fentanyl passed through skin from the groin region at a faster rate and with a significantly shorter lag time, compared with findings in neck or thoracic skin samples.

Conclusions and Clinical Relevance—In vitro, fentanyl from a patch was absorbed more quickly and to a greater extent through skin collected from the groin region of dogs, compared with skin samples from the thoracic and neck regions. Placement of fentanyl patches in the groin region of dogs may decrease the lag time to achieve analgesia perioperatively; however, in vivo studies are necessary to confirm these findings.(*Am J Vet Res* 2004;65:1697–1700)

Fentanyl, a derivative of 4-anilinopiperidine, is a potent analgesic drug that is a selective and pure agonist for the μ opioid receptor.[1] Fentanyl has a short duration of action that limits its use in prolonged analgesia regimens[2]; however, its low molecular weight (336.5 d) and high lipophilicity (logP, 3.94), combined with its potency, make fentanyl a useful and appropriate drug for transdermal administration.[1] Advantages of transdermal delivery of drugs, compared with other routes of administration, include relatively constant plasma concentration, decreased adverse effects (particularly with pulsatile delivery modes), high degree of patient compliance, and the ability of the patient to be ambulatory.[3,4]

In several studies, the use of fentanyl patches in dogs and cats was investigated. The minimum effective plasma concentration of fentanyl in humans ranges from 1 to 2 ng/mL,[4,5] and similar concentrations have been achieved by use of fentanyl patches in dogs[6] (1.6 ng/mL) and cats[7] (1.58 ng/mL). The degree of analgesia achieved via use of fentanyl patches is similar to that achieved via use of oxymorphone following ovariohysterectomy in dogs[8] and at least as effective as morphine administered via an epidural injection following major orthopedic surgery in dogs.[9]

One disadvantage of transdermal delivery of fentanyl (other than adverse effects including hypoventilation, nausea, or sedation) is the delay in onset of action of the drug.[1] In humans, transdermally administered fentanyl is first detected in plasma 2.25 hours after application of the patch, whereas peak plasma concentrations occur between 14 and 22 hours.[3] A similar delay in onset of action was reported in dogs in which intervals as long as 24 hours were required to achieve steady-state plasma fentanyl concentrations.[6,10] Marked individual variability in diffusion of fentanyl through skin is reported in humans[1,3] and dogs[6,10] and may be related to structural differences in skin and regional blood flow. Schultheiss et al[11] suggested that variability in epidermal thickness may account for variable diffusion of fentanyl through canine skin. Furthermore, results of several studies[12-14] revealed marked differences in transdermal drug absorption at different anatomic sites. The purpose of the study reported here was to investigate in vitro transdermal absorption of fentanyl from commercially available patches placed on skin samples obtained from various anatomic regions of dogs.

Materials and Methods

Skin samples—Skin samples were obtained from 5 Greyhounds that were brought to the University of Queensland Veterinary School for euthanasia. Informed consent for collection of skin samples was obtained from owners before dogs were euthanatized via IV administration of sodium pentobarbital. Hair in the areas of skin to be sampled was removed by use of clippers. Skin samples from the thoracic (central portion of the thorax, approx midway between the costochondral junction and vertebrae), neck (dorsal portion, just cranial to the scapula), and groin (2 cm lateral to the midline) regions were collected; an area of approximately 5 cm^2 was removed from each region, and care was taken to

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collect from the same area on each dog. Subcutaneous fat was removed, and skin samples were frozen at –20°C until tested.[15] The experimental protocol was approved by the Animal Ethics Committee of the University of Queensland (Approval No. SVS/363/03).

In vitro study—Skin samples were thawed, and circular sections (approx 2 cm in diameter) were cut and mounted in Franz-type diffusion cells with the stratum corneum side uppermost. Cellulose membranes were used as controls to indicate maximum absorption rates of fentanyl under the experimental conditions.[16] Approximately 3.5 mL of PBS solution (pH, 7.4) containing 4% bovine serum albumin was used as a receptor fluid and added to the lower reservoir of the diffusion cell with a magnetic flea for stirring. One milliliter of PBS solution was added to the donor reservoir, and the diffusion cell was placed in a water bath containing a magnetic stirring plate and allowed to equilibrate at 35°C for 60 minutes. The temperature of the skin in the diffusion cell was maintained at approximately 32°C. The PBS solution was removed from the donor reservoir before the fentanyl patch[a] was applied to the stratum corneum of the skin sample or cellulose membrane. Prior to application of the patch, the patch was attached to an acetate sheet cut to the same size as the patch but with a circular section removed from its center. Skin or cellulose membrane would therefore only adhere to the exposed area of the patch in the center of the acetate sheet, an area corresponding to the area of the opening to the receptor reservoir in the diffusion cell (diameter of 1.5 cm). This procedure ensured that fentanyl could only exit the patch through a defined area of skin immediately overlying the receptor fluid. The receptor fluid was collected via a side port in each diffusion cell and immediately replaced with an equivalent volume of fresh solution at 2, 4, 6, 8, 12, 18, 24, 30, 36, and 48 hours after patch application. The receptor fluid was immediately frozen and stored at –20°C until analysis. Fentanyl concentrations were reported as the mean of 5 replicates for each data point.

Analysis of fentanyl concentration—Receptor fluid samples were thawed, and 200 µL of fluid was added to 200 µL of acetonitrile that contained midazolam (10 mg/mL) as an internal standard. The samples were centrifuged (1,000 × *g* for 5 minutes), and 25 µL of supernatant was injected into a high-performance liquid chromatography system consisting of a pumping system,[b] autoinjector,[c] and detector.[d] A software system[e] was used to evaluate the data. The mobile phase consisted of 0.14% perchloric acid and 45% acetonitrile and was filtered (pore size, 0.45 µm) and degassed prior to use. A 150 × 3.9-mm steel column[f] with a flow rate of 1.0 mL/min was used. Linearity of the detector set at 200 nm was determined by mixing a series of samples containing fentanyl[g] in the receptor phase fluid (4% bovine serum albumin) to final concentrations of 0.1, 0.5, 1.0, 5.0, 10.0, 20.0, and 50.0 µg/mL. The detector response was linear to 50 µg/mL. The intrarun precision was determined by use of 10 aliquots of 2 concentrations of fentanyl (0.5 and 5.0 µg/mL) in receptor phase fluid, and the coefficients of variation were 1.90% and 0.40%, respectively.

Data and statistical analyses—The concentration of fentanyl in receptor fluid at each collection time was measured and divided by the duration of the collection period to give a rate of fentanyl absorption. The cumulative absorption rates were calculated by adding the rate of fentanyl absorption during a time period to the rates in all previous time periods to determine total fentanyl absorption through skin or cellulose membrane for 48 hours. Differences in the rates of fentanyl absorption at each collection time were compared via 1-way ANOVA by use of commercial software[h] with the Tukey test for pairwise comparisons between means. Fentanyl absorption rates and lag times (time from application of the patch to the appearance of a measurable concentration of fentanyl in receptor fluid) were calculated via linear regression analysis of the cumulative absorption rates between 0 and 8 hours and 8 and 48 hours; a 2-tailed Student *t* test for unpaired data was used to compare means. A value of $P < 0.05$ was considered significant.

Results

The mean ± SD release rate of fentanyl from the patch, defined by its absorption rate through the non–rate-limiting cellulose membrane, was 2.01 ± 0.05 µg/cm² of cellulose membrane/h during the first 8 hours. The release (absorption) rate decreased to 0.34 ± 0.03 µg/cm² of cellulose membrane/h during the 8- to 48-hour period (**Table 1**; **Figure 1**). Fentanyl absorption rates through canine skin were significantly lower than absorption rates through the cellulose

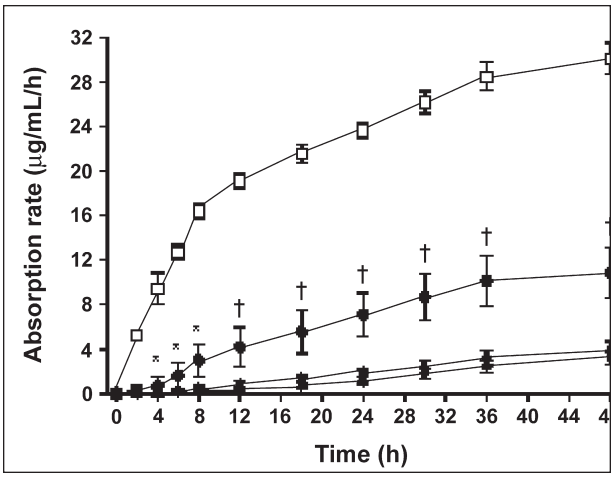

Figure 1—Mean ± SD cumulative absorption rate (µg/mL/h in receptor fluid) of fentanyl from a commercially available patch placed on skin samples obtained from the groin (solid circles), thoracic (solid triangles) and neck (solid diamonds) regions of 5 Greyhounds and a cellulose membrane (control; open squares) at various time points. *Value significantly ($P < 0.05$) greater in skin harvested from the groin region than in skin harvested from the thoracic and neck regions at corresponding time points. †Value significantly ($P < 0.001$) greater in skin harvested from the groin region than in skin harvested from the thoracic and neck regions at corresponding time points. Values for the cellulose membrane were significantly ($P < 0.001$) greater than values for skin harvested from the groin, thoracic, and neck regions at corresponding time points for all time points.

Table 1—Mean ± SD absorption rates (µg/cm²/h) of fentanyl from a commercially available patch placed on canine skin samples obtained from the thoracic, neck, and groin regions of 5 Greyhounds and a cellulose membrane (control) during 2 time periods.

	Fentanyl absorption rate	
Region or membrane	**0 to 8 hours**	**8 to 48 hours**
Thoracic	0.05 ± 0.02	0.09 ± 0.02
Neck	0.02 ± 0.01	0.08 ± 0.02
Groin	0.37 ± 0.18[a]	0.20 ± 0.03[b]
Cellulose	2.01 ± 0.05[c]	0.34 ± 0.03[c]

[a]Significantly ($P < 0.001$) greater than in skin harvested from the thoracic and neck regions for the corresponding period. [b]Significantly ($P = 0.002$) greater than in skin harvested from the thoracic and neck regions for the corresponding period. [c]Significantly ($P < 0.001$) greater than in skin harvested from thoracic, neck, and groin regions for the corresponding period.

membranes during the corresponding 0- to 8-hour and 8- to 48-hour periods. Absorption rates of fentanyl through skin collected from the thoracic and neck regions were not significantly different. Passage of fentanyl through skin from the groin region was significantly faster than that through skin from the thoracic and neck regions during the 0- to 8-hour and 8- to 48-hour periods. Similarly, the cumulative absorption rate of fentanyl through skin obtained from the groin region was significantly higher than through skin from the thoracic and neck regions in the 4- to 8-hour ($P < 0.05$) and 8- to 48-hour ($P < 0.001$) periods (Figure 1). Lag times (y intercept; time), calculated from 0- to 8-hour curves, were significantly ($P = 0.004$) shorter for skin harvested from the groin region (–0.37; < 2 hours) than for skin harvested from the thoracic (–0.09; ≥ 4 hours) and neck (–0.03; ≥ 4 hours) regions.

Discussion

In dogs and cats, fentanyl patches are usually applied to the neck region between the scapulae to minimize the risk of removal or ingestion of the patch. Our in vitro study revealed that application of a patch to skin samples obtained from the groin region of dogs resulted in significantly greater transdermal absorption of fentanyl, compared with skin samples collected from the thoracic and neck regions. Of equal importance was the shorter lag time before fentanyl passed through skin harvested from the groin region (< 2 hours), compared with lag time for skin harvested from the thoracic and neck regions (≥ 4 hours). One of the disadvantages of using fentanyl patches to treat postoperative pain is the need to apply patches at least 12 hours prior to surgery to ensure efficacy.[1] This difficulty is usually addressed via parenteral administration of concurrent titrated doses of fentanyl or other opioids until adequate analgesia is achieved. On the basis of the results of our study, we hypothesize that application of a fentanyl patch in the groin region, potentially resulting in earlier onset and more pronounced analgesia, may provide sufficient pain control at a time when close monitoring of the dog (during anesthesia and the surgical procedure) should rapidly detect the onset of adverse effects (ie, hypoventilation); also, it may be prudent to replace the fentanyl patch in the groin region with a patch applied to the neck region as pain is controlled and the dog recovers. In vivo studies, however, are required to confirm these in vitro findings.

The reason for variable regional kinetics of transdermally administered fentanyl is not certain. Schultheiss et al[11] suggested that variable epidermal thickness may account for the substantially different periods of time required for fentanyl to be first detected in plasma in individual dogs[6,10]; however, there were no significant differences in the total drug (area under the plasma concentration-time curve) or maximum plasma concentration of fentanyl between individual animals in those studies. Variable cutaneous blood flow also affects transdermal drug absorption,[12,14] but the release rate of fentanyl from commercial patches is unlikely to be affected by cutaneous vascularity unless extreme variations in blood flow are present.[1] Alternative explanations for the variability of drug transport among cutaneous anatomic sites have concerned appendageal transport. The results of several studies suggest that transdermal absorption of lipophilic drugs, such as methyl nicotinate,[17] progesterone, and testosterone,[18] may rely, in part, on movement of drug through skin appendages (hair follicles and sweat and sebaceous glands). There are obvious differences in the number and density of hair follicles between the groin and thoracic or neck regions; however, it is not certain whether these factors affected transdermal fentanyl absorption because lower absorption through skin samples obtained from the groin region than through skin samples obtained from the thoracic and neck regions would have been predicted.

It was beyond the scope of our study to investigate pharmacodynamic effects of fentanyl. Plasma fentanyl concentrations generally correlate with patch size, on the basis of the area of the patch that contacts the skin[1,10]; however, in dogs, a patch administering 100 µg of fentanyl/h did not provide significantly greater plasma concentrations of fentanyl, compared with a smaller patch administering 50 µg of fentanyl/h (1.2 ± 0.5 vs 0.7 ± 0.2 ng/mL, respectively).[10] The stratum corneum is the major barrier to drug and water passage through skin,[19] which suggests that application of fentanyl patches to skin in which the integrity of the stratum corneum is compromised (ie, abrasions or dermatologic lesions) should be avoided unless the animal is carefully monitored for signs of fentanyl overdose.

In vitro, fentanyl is absorbed more quickly and to a greater extent from a patch applied to skin samples obtained from the groin region than to skin samples obtained from the thoracic and neck regions. Application of a patch in the groin region may be preferred to reduce lag time and enhance perioperative analgesia in dogs; however, in vivo studies are necessary to confirm these in vitro findings.

[a]Durogesic 25 µg/h, Janssen-Cilag, North Ryde, Australia.
[b]Shimadzu 10A pumping system, Shimadzu Scientific Instruments, Rydalmere, Australia.
[c]Shimadzu 9A auto-injector, Shimadzu Scientific Instruments, Rydalmere, Australia.
[d]Shimadzu 10AXL UV/VIS detector, Shimadzu Scientific Instruments, Rydalmere, Australia.
[e]Shimadzu VP Software, Shimadzu Scientific Instruments, Rydalmere, Australia.
[f]Waters Symmetry Shield C_8, Waters Australia, Rydalmere, Australia.
[g]Sublimaze 50 µg/mL, Janssen-Cilag, North Ryde, Australia.
[h]Microsoft Office Excel 2003, Microsoft Corp, Redmond, Wash.

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Correction: Effect of a commercial anion dietary supplement on acid-base balance, urine volume, and urinary ion excretion in male goats fed oat or grass hay diets

In the article, "Effect of a commercial anion dietary supplement on acid-base balance, urine volume, and urinary ion excretion in male goats fed oat or grass hay diet", (October 2004;65:1391–1397) The equation on page 1392 should appear as below.

$$\text{DCA difference} = \frac{(\text{mEqNa}+\text{mEqK}) - (\text{mEqCl}+\text{mEqS})}{100\text{g of DM}}$$