Assessment of serum concentrations and sedative effects of fentanyl after transdermal administration at three dosages in healthy llamas

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Objective—To determine the serum concentrations and sedative effects of fentanyl after transdermal administration at 3 dosages in llamas.

Animals—9 healthy adult female llamas (mean age, 8 ± 3 years; mean weight, 150 ± 18 kg).

Procedure—Llamas were allocated to 1 of 3 groups (3 llamas/group). Fentanyl patches (each providing transdermal delivery of 75 µg of fentanyl/h) were placed on shaved areas of the antebrachium of all llamas. In group 1, llamas were treated with 1 patch (anticipated fentanyl dosage, 75 µg/h). In group 2, llamas were treated with 2 patches (anticipated fentanyl dosage, 150 µg/h). In group 3, llamas were treated with 4 patches (anticipated fentanyl dosage, 300 µg/h). For each llama, the degree of sedation was assessed by use of a subjective scoring system and a blood sample was collected for determination of serum fentanyl concentration at 12, 24, 36, 48, 60, and 72 hours after patch placement.

Results—Following the placement of 4 patches, mean ± SD serum fentanyl concentration in group 3 llamas reached 0.3 ± 0.08 ng/mL within 12 hours. This concentration was sustained for 72 hours. In group 2, application of 2 patches provided inconsistent results; in group 1, application of 1 patch rarely provided measurable serum fentanyl concentrations. No llamas became sedated at any time.

Conclusions and Clinical Relevance—Results suggest that application of four 75 µg/h fentanyl patches provides consistent, sustained serum fentanyl concentrations without sedation in llamas. However, the serum concentration of fentanyl that provides analgesia in llamas is not known. (*Am J Vet Res* 2005;66:907–909)

Fentanyl is a potent mu- and kappa-agonist opioid analgesic drug that is highly lipid soluble, which makes it suitable for transdermal delivery.[1] Administration of fentanyl via a transdermal route has been used in several animal species[2-14] but not in camelids. Because llamas tend to resent excessive handling and often become stressed when medications must be repeatedly injected and because they are pseudoruminants and orally administered drugs may have limited efficacy,[15] transdermal delivery of drugs could be particularly useful in these animals.

On the basis of data regarding the absorption of fentanyl via transdermal administration in other species, our hypothesis was that transdermal fentanyl patches would provide measurable serum fentanyl concentrations in llamas. Because opioids, including fentanyl, can cause sedation in some species,[16] we also hypothesized that fentanyl could cause sedation in camelids. The purpose of the study reported here was to determine the serum concentrations and sedative effects of fentanyl after transdermal administration at 3 dosages in llamas.

Materials and Methods

The study was approved by the Animal Care and Use Committee at Oregon State University. Nine healthy adult female llamas (mean age, 8 ± 3 years; mean weight, 150 ± 18 kg) were included in the study. A thorough physical examination including assessment of heart rate, respiratory rate, and rectal body temperature was performed prior to (baseline values) and at least once on every day of the study. On the first day of the study, the llamas were weighed (to calculate the fentanyl dosage) and a 14-gauge, 13-cm polytef catheter[a] was placed percutaneously into the right jugular vein in an area that had been clipped free of hair, aseptically prepared, and injected with a local anesthetic agent (2 mL of 2% lidocaine). The llamas were allocated to 1 of 3 groups (3 llamas/group). Group 1 llamas were to receive one 75 µg/h fentanyl patch[b] (equivalent to approx 0.5 mg of fentanyl/kg; anticipated dosage, 75 µg/h). Group 2 llamas were to receive two 75 µg/h patches (equivalent to approx 1 mg of fentanyl/kg; anticipated dosage, 150 µg/h). Group 3 llamas were to receive four 75 µg/h patches (equivalent to approx 2 mg of fentanyl/kg; anticipated dosage, 300 µg/h). Fentanyl dosages were extrapolated from the low-end dosages recommended for dogs and cats.[16] For application of each patch, a 5-cm square area on the medial aspect of the antebrachium was clipped free of hair and wiped clean with water. To ensure adherence, the patches were pressed firmly to the llamas' skin and the corner of each patch was stapled to the skin by use of stainless-steel surgical staples; the area of patch application was covered circumferentially with an adhesive bandage.[c] Gloves were not worn during patch placement. The bandages were checked each day to ensure that patches remained in contact with the skin. In the event that patches became detached, llamas with detached patches were to be removed from the study.

Venous blood samples for serum fentanyl concentration quantification were collected from the jugular catheter of

Received June 9, 2004.
Accepted August 11, 2004.
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Funded in full by Agricultural Experiment Station grants from Oregon State University.
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each camelid at 12, 24, 36, 48, 60, and 72 hours after placement of the fentanyl patches (patches were applied at time 0). Prior to collection of a blood sample, 5 mL of blood was withdrawn from the catheter and discarded. Following sample collection, catheters were flushed with saline (0.9% NaCl) solution containing heparin. Serum was collected from whole blood samples via centrifugation, and serum samples were analyzed within 30 minutes of collection. Serum fentanyl was measured by use of an ELISA kit.[d] Briefly, the ELISA is based on competition for a limited number of antibody sites between the drug or its metabolite in the serum sample and the drug-enzyme conjugate. The serum sample to be evaluated and the diluted drug-enzyme conjugate were added to a microplate and incubated at room temperature (approx 17°C). The drug-enzyme conjugate became bound to the antibody immobilized by the microplate wells. After incubation, the microplate was washed to remove unbound sample and conjugate. The bound drug-enzyme conjugate was determined by a color substrate, and the extent of color development was inversely proportional to the amount of drug in the serum sample; a microplate reader equipped with a 650-nm filter was used to determine concentration in absorbance units. Standards and samples were assayed in triplicate, and a 5-point standard curve was used. By use of this ELISA, the minimum detectable serum concentration of fentanyl was 0.14 ng/mL.

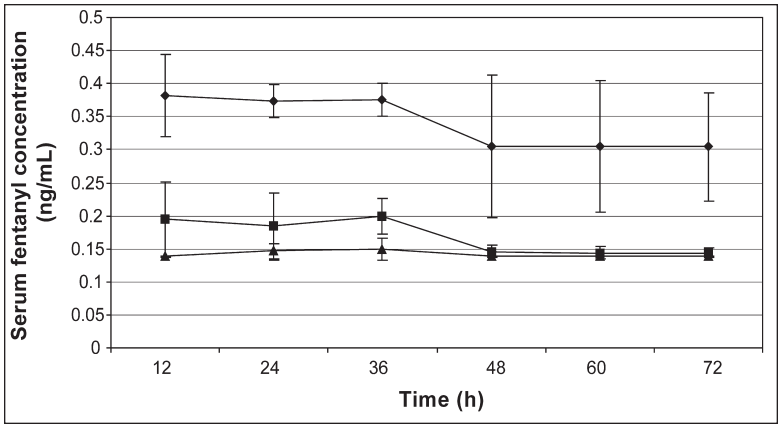

Figure 1—Mean ± SD serum fentanyl concentrations at 12, 24, 36, 48, 60, and 72 hours after transdermal fentanyl patch placement (time 0) in 3 groups of llamas (3 llamas/group). In group 1 (triangles), llamas were treated with one 75 µg/h patch. In group 2 (squares), llamas were treated with two 75 µg/h patches. In group 3 (diamonds), llamas were treated with four 75 µg/h patches. Via an ELISA, the minimum detectable serum concentration of fentanyl was 0.14 ng/mL.

Body temperature was measured once daily for the duration of the study. Heart and respiratory rates and sedation scores were recorded every 4 hours for the first 24 hours following patch placement and then once every 24 hours for the duration of the study. Sedation was graded subjectively on a scale of 1 to 4, according to a previously described method.[17] The sedation scale used for assessment of each llama was as follows: grade 1, mild sedation indicated by slight lowering of head carriage or ear position or by protrusion of the lower lip; grade 2, obvious sedation indicated by signs such as those described for grade 1, plus prolapse of the third eyelid and decreased awareness without recumbency or with normal cushing; grade 3, obvious signs of sedation and recumbency (other than normal cushing), although standing could be easily achieved with minor stimulation; and grade 4, obvious signs of sedation and recumbency with no or minimal arousal following stimulation. If at any time llamas were classified as having grade 4 sedation or bradycardia (ie, heart rate < 40 beats/min) or bradypnea (ie, respiratory rate < 10 breaths/min) for > 1 hour of continuous observation, fentanyl patches were to be removed and the effects of fentanyl reversed via administration of an appropriate narcotic reversal agent such as naloxone.[16]

In all groups, patches were removed 72 hours after their initial placement. The areas of skin under each patch were carefully examined for signs of dermatologic reaction. The camelids remained under observation for an additional 48 hours prior to being returned to the pastured teaching herd.

Data were analyzed by use of a repeated-measures ANOVA and a Fisher least significant difference test. Values of $P < 0.05$ were considered significant.

Results

No detectable sedation (including no grade 1 sedation) was evident in any llama at any time during the study. Heart rate, respiratory rate, and body temperature were within normal limits for adult llamas at all times during the study and did not significantly change from baseline values at any time. All patches remained firmly in place against the skin throughout the study. After removal of patches, no gross skin reactions were detected; however, no biopsy specimens were obtained for histologic confirmation of a lack of reaction.

For all llamas, serum fentanyl concentrations were measured by use of the ELISA at 12, 24, 36, 48, 60, and 72 hours after patch placement (**Figure 1**). In group 3 llamas, serum fentanyl concentrations were significantly ($P < 0.05$) greater at all time points than serum fentanyl concentrations achieved in llamas in the other 2 groups. In group 3 llamas, mean ± SD serum fentanyl concentrations had reached peak values (0.38 ± 0.06 ng/mL) by 12 hours after patch placement and remained fairly steady until patch removal 72 hours after application (0.30 ± 0.08 ng/mL).

Serum fentanyl concentrations in group 2 llamas were inconsistent and did not reach measurable concentrations at all time points in all 3 camelids. Despite high variability within this group, mean serum fentanyl concentration had reached peak value by 12 hours after patch placement (0.19 ± 0.06 ng/mL); the serum concentration remained at steady state until 36 hours after patch application (0.2 ± 0.03 ng/mL), after which time the value decreased to below the detectable limit (0.14 ng/mL). Only at 36 hours after patch placement was the mean serum fentanyl concentration in group 2 llamas significantly ($P < 0.05$) different from that of group 1 llamas. In group 1, serum fentanyl concentrations were measurable only in 1 llama at 2 time points (24 hours, 0.16 ng/mL; 36 hours, 0.168 ng/mL) and were not measurable at all other time points in the other 2 llamas (serum fentanyl concentrations ≤ 0.14 ng/mL).

Discussion

In the present study, four 75 µg/h transdermal fentanyl patches provided measurable and sustainable serum fentanyl concentrations within 12 hours and for at least 72 hours following patch placement in llamas. This is similar to results reported[1] for transdermal

administration of fentanyl in other species. Factors that contribute to transdermal absorption of fentanyl and possibly contribute to the high variability among fentanyl serum concentrations following patch placement include adequate adherence of the patch to the skin, thickness of the skin at the site of patch application, blood flow to the skin at the site of the patch, and body temperature.[1,18] In the teaching hospital at Oregon State University, fentanyl patches are commonly stapled to the skin of patients; this is done to ensure adherence of the patch and decrease the chance that a patient could remove a patch from the skin via scraping or rubbing. However, we take extreme care to staple only the edges of the patch and not the gel reservoir because puncturing the gel reservoir could alter delivery and subsequent absorption of fentanyl. In the llamas of the study reported here, the antebrachium was chosen as the site of patch placement because the patch could tightly adhere to the flat surface of the medial aspect of the antebrachium and be protected with a bandage; furthermore, in most species, the skin over this area is thin and has a good blood supply. The body temperature for all treated llamas was within reference limits throughout the duration of the study; thus, this was not a factor affecting absorption of fentanyl. One final critique of our study is that all llamas included in the investigation were female, and gender-specific sensitivity to the effects of drugs, including opioids, has been identified in humans.[19] However, to our knowledge, gender-specific sensitivity to drugs has not been reported in camelids.

Fentanyl patches have been used to provide analgesia in a variety of animal species including dogs, cats, horses, pigs, goats, rabbits, and sheep.[2-14] Although the exact serum fentanyl concentrations that are associated with adequate analgesia in these species are unknown, it has been suggested[11] that the values range from 0.5 to 3.0 ng/mL; these values of serum fentanyl concentration are similar to those which have been determined to provide analgesia in humans.[11,20] The serum fentanyl concentration needed to provide analgesia in camelids is also unknown, but if the value is similar to requirements of other animal species and humans, then fentanyl would have to be supplied transdermally at a dosage in excess of 300 µg/h to reach adequate serum concentrations. On the basis of the rapid rate of increase in serum fentanyl concentration and attainment of steady-state concentration after transdermal administration of fentanyl, we predict that transdermal fentanyl patches could be used to provide serum fentanyl concentrations in llamas that are within the range that has been shown to provide analgesia in other species. Without doubt, application of 4 or more patches in an adult llama could be fairly expensive at current prices; however, the dosage of butorphanol (an opioid agonist-antagonist) required to provide analgesia in llamas is lower than that required in other species,[21] and it is possible that fentanyl could also provide adequate analgesia in llamas at a dosage that is lower than that required in other species.

a. Abbocath-T, Abbott Animal Health, Abbott Park, Ill.
b. Duragesic, Janssen Pharmaceutical, Titusville, NJ.
c. Fentanyl kit, product No. 104010, Neogen Corp, Life Sciences Division, Lexington, Ky.
d. Elastikon, Johnson & Johnson, New Brunswick, NJ.

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