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Evaluation of Fentanyl Transdermal Patches in Rabbits: Blood Concentrations and Physiologic Response

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In the study reported here, we sought to evaluate transdermal fentanyl patches for their ability to achieve detectable plasma concentrations with minimal adverse effects in New Zealand White rabbits. Fentanyl patches were applied to the dorsum after removing hair either by clipping or by application of a depilatory agent. Blood samples were collected every 12 h for a total of 96 h (24 h after patch removal) for determination of plasma fentanyl concentration. At those times, rabbits were assessed for changes in body temperature, heart rate, respiratory rate, and body weight.

In rabbits with clipped hair, where rapid hair re-growth was not a mitigating factor, mean plasma fentanyl concentration reached a mean (± SEM) peak of 1.11 ± 0.32 ng/ml at 24 h, decreased to 0.77 ± 0.21 ng/ml at 72 h, and was negligible at 96 h. In rabbits with depilated hair, peak concentration was obtained at 12 h (6.7 ± 0.57 ng/ml) and decreased gradually to 0.27 ± 0.06 ng/ml at 72 h. In a second group of fentanyl-treated rabbits in which hair started growing back within 24 h, plasma fentanyl concentration was not detectable. Control and fentanyl-treated rabbits with clipped hair had no effect from the experimental manipulations other than slight loss in body weight. In the depilatory group, two rabbits appeared moderately sedated during the initial 12-h period, and had decreased respiratory rate for 24 h.

In conclusion, rabbits tolerate the transdermal fentanyl patch well. Hair regrowth in rabbits may present a complicating factor that impedes dermal absorption of fentanyl. The application of a depilatory agent lead to early and rapid absorption of fentanyl causing undue sedation in some rabbits and lack of sustained plasma concentrations for the desired three-day period.

Provision of quality veterinary care for laboratory animals requires careful consideration of postprocedural pain relief. Assessment of need for postoperative analgesia is often based on previous veterinary experience with the particular species, the level of invasiveness of the procedure, and the general assumption that postoperative analgesia is warranted if that same procedure performed in humans is considered discomforting. Providing appropriate concentrations of analgesia in laboratory animals faces several major challenges. The ability to accurately and objectively determine the level of pain and/or discomfort in an animal is difficult. Typically, an animal is subjectively assessed for general behavior, decrease in mobility or abnormal gait and posture, decreased food and water intake, and abnormal vocalization (1-3) Pain scoring systems have been applied in a number of animal studies, including rodents (4, 5) and other species (6, 7). These systems provide some usefulness, but are usually study specific.

Many of the most potent analgesics have a short duration of action (1 to 4 h), requiring frequent administration and repeatedly perturbing the patient. Individual responses to pain and individual variation in drug absorption hamper the ability to provide consistent levels of pain relief to all patients.

Transdermal fentanyl patch systems are used in human medicine principally to provide pain relief to chronic cancer patients (8-12). Fentanyl is a potent synthetic opioid of the 4-anilinophenylpiperidine class, with predominantly mu receptor agonist activity. Its potency in humans is reported to be 75 times that of morphine on an equi-analgesic basis (13, 14). It is lipid and water soluble, and is rapidly eliminated from the plasma and metabolized by N-dealkylation and hydroxylation in humans, with urinary excretion of metabolites (15, 16).

Transdermal fentanyl patches come in 4 dosage levels: 25, 50, 75, and 100 µg/h. This is achieved by increasing the size of the patch and, thus, the surface area (10, 20, 30, or 40 cm^2, respectively) in contact with skin. The dose per hour rate indicates the average amount of drug that is theoretically delivered to the patient's circulation per hour. The patches contain sufficient fentanyl to continuously release for a period of 72 h.

Use of transdermal continuous-release analgesia in veterinary medicine has the potential to avoid pitfalls associated with more typical routes of administration. The patch can be applied while the patient is anesthetized for surgery, causing no additional stress. Also, continuous release ensures more stable plasma concentrations compared to the peaks and troughs that are associated with other routes of administration, also minimizing side effects. In addition, dependence on personnel for timely administration of repeated doses of analgesic is abrogated. Finally, use of a transdermal delivery system does not pose physical restrictions on the animal, which might occur with continuous intravenous administration, and avoids the risk of infection associated with repeated intravenous dosing.

The transdermal fentanyl patch has been used in several veterinary species, including dogs (7, 17-20), cats (21, 22), goats (23), and swine (24). To the authors' knowledge, its use in rabbits has not been reported. In the study reported here, we sought to evaluate the ability of the transdermal fentanyl patch to obtain plasma fentanyl concentrations in laboratory rabbits comparable to those in

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other species, and to assess the drug's effect on basic physiologic and behavioral parameters.

Materials and Methods

Animals. New Zealand White, 3.5-to 4.0-kg female rabbits were purchased from Blue and Gray Rabbitry (Unionville, Va.). These rabbits are tested twice yearly for *Pasteurella multocida*, *Bordetella bronchiseptica*, *Encephalitozoon cuniculi*, *Treponema cuniculi*, and cilia-associated respiratory bacillus. Results of testing performed in July 2000 were negative for all of the aforementioned agents. The rabbits were singly housed under standard conditions (18-21°C , 30 to 70% humidity), fed Harlan High Fiber Rabbit Chow ad libitum, and had access to automatic water providers. All animal experiments were performed with strict attention to humane care and use, and met full approval by the University of Virginia's Institutional Animal Care and Use Committee.

Materials. The 25 µg/h transdermal fentanyl patch (Duragesic, Janssen Pharmaceutica, Titusville, N.J.) was used. This size patch was chosen on the basis of pharmacokinetic data obtained from studies performed in cats (21, 22). Rabbits that had skin biopsy specimens taken were anesthetized with ketamine (45 to 50 mg/kg of body weight) and xylazine (5 mg/kg) given intramuscularly. Neet for Sensitive Skin (distributed by Premier Inc., Greenwich, Conn.) was applied to the hair of rabbits in the depilatory experimental group. Fentanyl citrate (A. H. Robins Comp., Cherry Hill, N.J.) was purchased from the University of Virginia Pharmacy to prepare calibration samples used in the radioimmunoassay and to run intraassay and interassay controls.

Experimental design: Experiment 1. Rabbits were randomly assigned to two groups, a control group (group 1, n = 4) and a fentanyl group (group 2, n = 5). Prior to blood sample collection, the rabbits were weighed, and heart rate, respiratory rate, and rectal body temperature were measured. A 3-ml blood sample was collected from the central ear artery and placed in a heparinized tube for subsequent centrifugation and plasma collection. Plasma samples were stored at -20°C until assayed. In the fentanyl treatment group (group 2), hair was clipped on the dorsum in the interscapular region and the patch was applied according to manufacturer's instructions. Rabbits were returned to their cages and re-examined over the next three hours for any immediate adverse effects. At 12-h intervals, rabbits were observed in their cages for level of alertness and willingness to move around the cage when gently touched. Appetite and fecal and urine output were subjectively assessed for normal quantity and quality. Heart rate, respiratory rate, and rectal temperature were measured, and blood samples were collected. At 72 h, the patch was removed. The final measurements were taken at 96 h. At the end of the study, two rabbits in each group were anesthetized, an area on the dorsum where the patch had been applied was aseptically prepared, and a skin biopsy specimen was taken, using a 4-mm biopsy punch. The wound was closed with a single interrupted suture.

Experiment 2. An additional fentanyl group with clipped hair (group 3, n = 4) was treated in a manner similar to that of group 2. A fourth group of rabbits (group 4, n = 6) had hair removed 24 h prior to patch application, using Neet for Sensitive Skin, as an alternative method of hair removal. The Neet was applied for four minutes, with subsequent washing and drying of the skin. All other parameters of the study remained similar.

Plasma fentanyl concentration determination. Fentanyl radioimmunoassay kits were purchased from Diagnostic Products Corp. (Los Angeles, Calif.). The assay is based on competitive binding of fentanyl for antibody sites immobilized on polypropylene tubes. ^{125}I-Labeled fentanyl provided in the kit is used as the competitive substance against fentanyl in the samples. Calibration samples used in these experiments contained 0, 0.25, 0.5, 1.0, 2.5, and 7.5 of fentanyl ng/ml of normal pooled rabbit plasma. Assay sensitivity was 0.08 ng/ml, intra-assay coefficient of variance (CV) was 3.3 to 4.0%, and interassay CV was 4.9 to 6.8%. Plasma samples were stored at -20°C until assayed. All samples were assayed in duplicate, and the counts per minute (CPM) were averaged. Total count was obtained by measuring radioactivity (CPM) of ^{125}I-labeled fentanyl solution alone in an uncoated tube (no antibody present). Nonspecific binding (NSB) of ^{125}I-labeled fentanyl was determined by measuring the radioactivity of the zero calibrator plus ^{125}I-labeled fentanyl solution in an uncoated tube. To determine the binding of each pair of tubes as a percentage of maximal binding (MB), average NSB CPM was first subtracted from each of the samples' average CPM. Percentage bound was calculated, using the following formula:

Percentage bound = (Net counts/Net MB counts) × 100 where MB binding equals the net count of the 0 ng/ml calibrator. A calibration curve was plotted on a logit-log graph, as recommended by the manufacturer to provide the most linear relationship and allow regression analysis. The slope and y-intercept were determined from a regression line and were used to calculate fentanyl concentrations in the experimental samples. Performance of the assay was assessed, comparing rabbit plasma control samples and rabbit plasma samples to which fentanyl was added (spiked) with the human control samples supplied with the assay kit. Rabbit and human samples behaved equivalently.

Statistical analysis. Area under the curve (AUC) was calculated for each animal's fentanyl concentration profile, using the trapezoid rule. A non-parametric test was chosen due to unequal variance in AUC measurements among treatment groups. Differences in AUC fentanyl concentration between groups 2 and 4 were tested for significance, using the Wilcoxon rank-sum test. Repeated measures analysis of variance (ANOVA) models were used to test for differential treatment effects over time for heart rate, respiratory rate, rectal temperature, and body weight. Tests of interest from these models were the global F-tests for treatment-by-time interaction. A repeated measures regression model was used to determine the rate of fentanyl decrease after patch removal while adjusting for the correlation between measurements taken from the same animal. An F-test was used to test for differences in slope for each of the treatment groups.

Results

Effect of fentanyl patches on rabbits. Subjective assessments of mental alertness, activity, fecal and urine output, and appetite revealed similar results for all rabbits in experiment 1, which included control rabbits and those with fentanyl patches applied after clipping hair from the dorsum. There was no apparent adverse effect of the fentanyl patch on the rabbits in these groups. In experiment 2, two rabbits from group 4 (fentanyl; hair removed with depilatory agent) appeared moderately to heavily sedated for the first four to eight hours. This was manifested by sternal or lateral recumbency and reluctance to move when stimulated. Patches were applied at approximately 9 a.m.. Activity increased by 4 p.m., and rabbits were clinically normal by the next morning.

There was no significant difference in heart rate, respiratory rate, or rectal temperature between fentanyl and control groups in experiment 1, as measured by ANOVA and the global F-test for differences in treatment effect over time. However, both groups lost body weight during the four-day period (2% in controls and 3.5% in fentanyl). In Experiment 2 respiratory rates of rabbits in group 4 were decreased shortly after the time of application until the 24-h time point. Mean (± SEM) respiratory rate at t = 12 h was 81 ± 18 breaths/min for group-4 rabbits versus 139 ± 13 for controls (group 3). At t = 24 h, respiratory rate for group-4 rabbits was 84 ± 36 versus 103 ± 7 for controls. By t = 36 h, respiratory rate had returned to normal.

Plasma fentanyl concentrations. There was no detectable fentanyl in any of the control rabbit plasma samples from experiment 1, as depicted in Fig. 1. Fentanyl concentration measured in plasma samples from group-2 rabbits indicated a gradual increase in concentration during the first 24 h, a plateau over the next 48 h, and a rapid decrease after patch removal at 72 h (Fig. 1). Peak fentanyl concentration of 1.12 ± 0.32 ng/ml (mean ± SEM) was apparent at 24 h. At 72 h, mean (± SEM) fentanyl concentration was 0.77 ± 0.21 ng/ml. Median area under the curve was 26.7 (range, 6.9 to 99.7), an indirect measure of the total amount of fentanyl absorbed. The rate of decrease after patch removal from t = 72 to t = 96 h was 0.028 ± 0.006 ng/ml/h.

In the second experimental group, fentanyl concentrations in group-4 rabbits (depilatory hair removal) rapidly increased during the first 12 h to peak at 7.24 ± 0.73 ng/ml. Concentration decreased at a constant rate after that point, as shown in Fig. 2 (0.37 ± 0.18 ng/ml at t = 60 h), even prior to patch removal. Median area under the curve (AUC) was 155.4 (range 67.2 to 235.8). The rate of decrease from t = 72 to t = 96 h was 0.009 ± 0.007 ng/ml/h. The rate of decrease in fentanyl concentration between the 2 treatment groups after patch removal indicated a trend toward statistical significance ($P = 0.067$). Rabbits whose hair was clipped (group 3) had extremely rapid hair regrowth. In this group, plasma samples did not contain detectable concentrations of fentanyl. A patch from one of the rabbits in this group is shown in Fig. 3A revealing a substantial amount of hair attached to the patch and regrowth at the site of removal (Fig. 3B). These photographs were taken at t = 72 h. The calculated AUC for the two treatment groups (groups 2 and 4) were compared and are presented in Fig. 4. The difference in these values was significant ($P < 0.01$).

Pathologic examination. Microscopic evaluation of tissues was performed by collecting skin biopsy punch specimens at t = 96 h from four rabbits, two each from groups 1 and 2. Samples were sent to a pathologist blinded as to the nature of the study. The histopathologic report indicated mild superficial dermatitis in control rabbits and moderate superficial and perivascular dermatitis in fentanyl-treated rabbits, indicating that presence of the patch induced mild inflammatory changes. After patch removal, the skin did not appear grossly inflamed. In group-4 rabbits, the depilatory cream elicited erythematous reactions in the area of application. This erythema was still present, but decreased 24 h later when the patch was applied.

Discussion

Rabbits treated with transdermal fentanyl patches tolerated the patches well. Plasma fentanyl concentrations within the range considered analgesic in humans (0.5 to 2.0 ng/ml) had no impact on heart rate, respiratory rate, or rectal temperature. The slight weight loss in rabbits of the control and fentanyl groups was attributed to the frequent handling and observation of the rabbits, and we suspect that body weight returned to normal several days after completion of the study, although it was not measured. Dermatitis was evident in control and fentanyl-treated rabbits, but was characterized as more evident in the fentanyl-treated animals. Irritation from the hair clippers may have resulted in the mild inflammatory changes. The cause of the additional inflammation associated with patch application is not known. The adhesive agent, the patch material, the fentanyl, or the vehicle solution may be causally related.

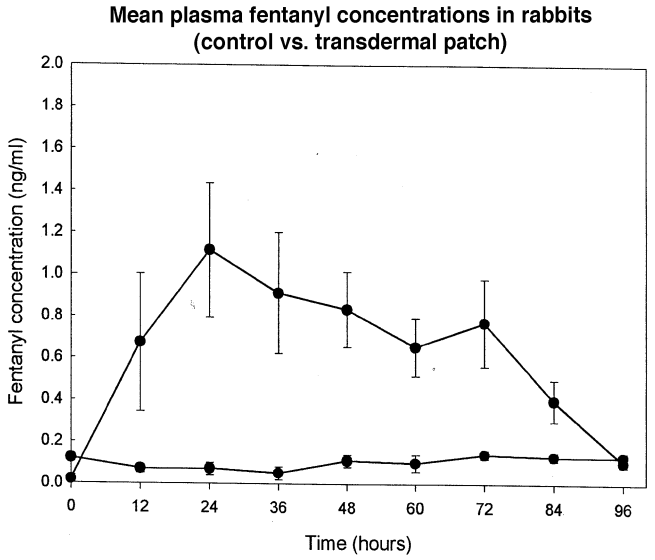

Figure 1. Mean (± SEM) fentanyl concentrations in plasma samples from control (no fentanyl patch) and hair-clipped fentanyl-treated (25 µg/h transdermal fentanyl patch) rabbits of from experiment 1. Plasma concentrations were determined by radioimmunoasssay (RIA) every 12 h from t = 0 to 96 h. Fentanyl-treated rabbits had concentration within the range considered to be analgesic in humans (mean value of 0.83 ng/ml between t = 12 and t = 72 h).

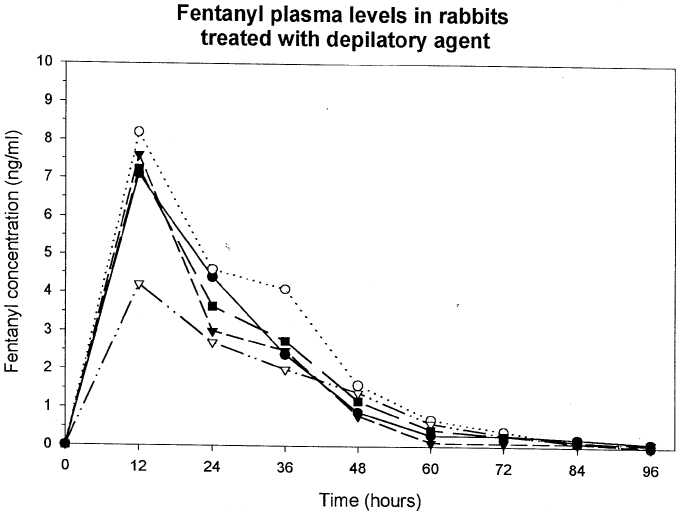

Figure 2. Plasma fentanyl concentrations over time for individual rabbits pretreated with a depilatory agent 24 h prior to patch application. Fentanyl concentration increased rapidly during the first 12 h (6.7 ± .57 ng/ml), plateaued between 24 and 36 h (3.4 to 3.0 ng/ml) and gradually decreased to baseline by t = 72 h.

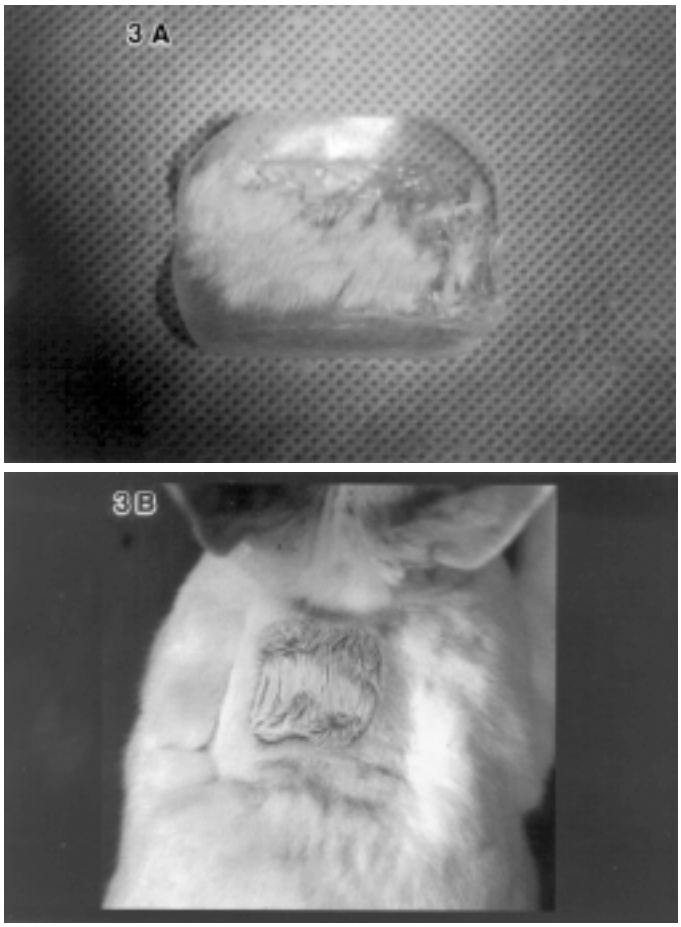

Figure 3. In rabbits of experiment 2, hair regrowth after clipping occurred rapidly, substantially inhibiting fentanyl absorption through the skin (n = 4). There was no detectable plasma fentanyl concentration in these rabbits on the basis of results of RIA determination. (A) One of the patches with new hair growth adhering is shown in this photograph, taken at the time of patch removal (t = 72 h). (B) Site of patch removal from the same rabbit at the same time.

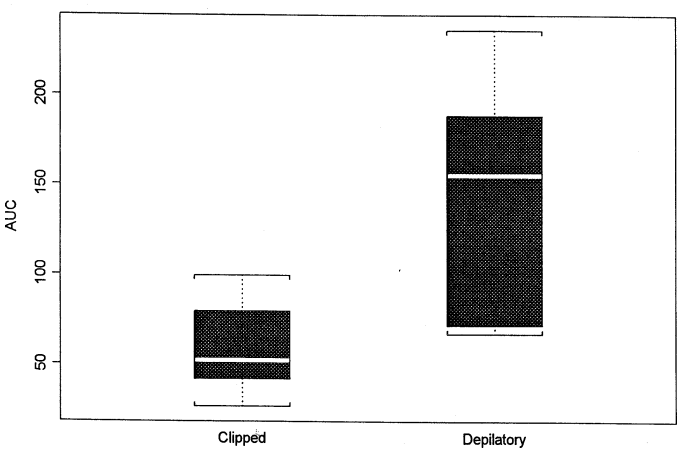

Figure 4. Area under the curve (AUC) was calculated and compared for group-2 (clipped hair) and group-4 (depilatory cream) rabbits that all received fentanyl patches for 72 h. As depicted in this box-and-whisker plot, there was significant ($P < 0.01$) difference between the two groups.

The only side effect seen in the rabbits of this study was moderate sedation and respiratory depression in two rabbits of the depilatory hair removal group, which correlated with high fentanyl concentration measured in these individuals. These rabbits did not receive additional treatment. The patch was left in place, and rabbits were monitored every two hours for the first 12 h. They were fully recovered within 12 to 24 h. The most frequent adverse effects in humans are those typically associated with opioid agents, and include nausea, vomiting, and constipation (12). The most serious adverse impact associated with opiate analgesics is respiratory depression reported to develop in 2% of cancer patients and 4% of postoperative patients (12). The sedation and respiratory depression seen in rabbits with high plasma fentanyl concentration was similar to side effects seen in humans. Mild sedation has been reported in a few dogs treated with either the 75 or 100 µg/h patch (17). A separate study in dogs reported variable degrees of sedation, as well as heart and respiratory rate depression, and hypothermia (20).

A complication encountered in application of the patch in rabbits involved the pattern of cyclic hair growth. In rabbits with slow hair re-growth, fentanyl concentration followed similar pharmacokinetic patterns seen in other species. If hair follicles are in anagen phase at the time of patch application, rapid hair regrowth poses a problem with drug absorption. Hair re-growth completely impaired fentanyl absorption in the group of rabbits acquired and used at a later date (group 3). This points to a noteworthy species difference when applying the transdermal mode of drug delivery to rabbits. Use of the depilatory cream results in a smoother and hairless skin surface on which to apply the patch. However, the cream causes mild inflammatory response in the skin with increased vascularity, as indicated by the erythema still present 24 h after application. This may have led to more rapid absorption of fentanyl resulting in the increased plasma fentanyl concentrations measured. This problem might be avoided by waiting an additional 24 h after depilatory application prior to application of the patch, or by varying the location of placement of the patch. For the purposes of the study reported here, all fentanyl patches were placed in the same general anatomic location.

Plasma fentanyl concentration had wide interindividual variability in this study. This has been reported in humans (11, 25) and animal species (17-19, 22). Reasons for interindividual variability may include variations in cutaneous absorption, uptake by cutaneous vasculature, volume of distribution, and plasma clearance rates. Indeed, the actual delivery rate is often substantially less than the predicted theoretical rate of delivery in animals and humans (19, 26, 27). Species-specific skin characteristics may play a substantial role in percutaneous drug absorption. Factors, such as temperature, vascular perfusion of the skin, hydration status, blood flow, and skin damage, influence transdermal absorption. In humans, as well as swine, there is an extensive direct musculocutaneous arterial supply (24, 28). In many animals, blood is supplied by direct cutaneous arteries and arteries that initially supply muscle, then terminate in the skin. This is particularly well documented in the dog (29). There is, therefore, good reason to believe that differences in vascular anatomy of the skin would impact the rate of fentanyl absorption and the depot effect, which causes gradual release of fentanyl from the dermis into the general circulation. These anatomic differences are likely to contribute to the interspecies variation in pharmacokinetics associated with use of the transdermal patch.

In humans, effective analgesia is achieved with plasma concentrations in the range of 0.5 to 2.0 ng/ml, using patches de-

livering 50 to 100 µg/h (13). Using an "on-demand" administration system of fentanyl, Gourlay and co-workers (26) determined that the minimum effective concentration for postoperative pain control in humans was 0.63 ng/ml, with an inter-patient range of 0.23 to 1.18 ng/ml. The plasma fentanyl concentrations achieved in this study were well within this analgesic range throughout the 12- to 72-h period after patch application. Furthermore, smaller species (cats, smaller dogs, and rabbits) appear to require a higher dose of drug on a microgram per kilogram basis than do humans.

Studies evaluating analgesic efficacy of transdermal fentanyl in animal species are scant but attention to and research of this mode of analgesic delivery is expanding. Kyles and co-workers compared transdermal fentanyl with oxymorphone after ovariohysterectomy in dogs and concluded that analgesic effects of the two agents were comparable, with less adverse effects in the fentanyl-treated group (18). Robinson and co-workers concluded that pain relief provided by transdermal fentanyl was similar to or better than epidural morphine after orthopedic surgery (7). Recently a study performed in swine indicated that use of the 50 µg/h patch in 26-kg pigs provided sufficient postoperative analgesia and that the drug was similar to buprenorphine in its ability to control pain (24). That study also involved evaluation of the cost-effectiveness of these two methods of analgesic administration and results indicated that when labor costs were factored in, the fentanyl patch cost approximately a fourth that of buprenorphine.

Buprenorphine, another opioid agent, has gained widespread popularity in laboratory animal medicine, and is often used as the first-choice analgesic. Buprenorphine is a partial mu and kappa agonist that induces profound analgesia along with CNS side effects, and is reported to be effective in rodents for 6 to 12 h (1). The duration of action of buprenorphine in rabbits has been reported to be 8 to 10 h (1, 30). However, because it is parenterally administered, there remains a reliance on personnel to perform timely administration even with its long action of duration. In many research applications, the experimental group size of animals may be large and parenteral administration of analgesics to all individuals two or three times daily could constitute a substantial investment of time.

Various studies of oral administration in rodents have been reported. Buprenorphine has been administered in the drinking water of rats (3, 31) and in flavored gelatin (32). However, most opioids undergo significant first-pass hepatic extraction when administered orally (33). Finally, the maximal analgesic effect of buprenorphine is less than that of morphine and other mu agonists (34-36), and therefore, is less useful for controlling acute and severe pain. For these reasons, the transdermal fentanyl patch is a viable alternative to buprenorphine for postoperative analgesia. However, if the patch were not applied until the time of surgery, buprenorphine or another analgesic would need to be provided to cover the first 12 h until plasma fentanyl concentration is sufficient.

An objective comparison of the analgesic efficacy of the transdermal fentanyl patch and parenteral opioid and/or nonsteroidal anti-inflammatory administration is indicated prior to advocating use of the patch in rabbits. However, to our knowledge, there is no widely accepted model for assessment of pain in rabbits. Analgesic agents developed for clinical use in humans have typically been tested first in rodents for which a variety of tests have been described. These methods involve application of a noxious stimulus and measuring response to that stimulus. Commonly used assays in rodents include the tail flick test, hot plate test, and paw pressure test. Wootton and co-workers (30) developed an analgesiometry system for use in rabbits and other large animals, using infrared heat applied cutaneously. The quantitative factor equaled the amount of time heat was applied before the rabbit's skin twitched. A similar method, using radiant heat, was also used to evaluate morphine analgesia in rats and rabbits (37). This method involved considerable restraint and measured head withdrawal following heating of the nose and mouth. A more invasive method has been electrical stimulation of the tooth pulp (38). Use of a method similar to that developed by Wootton and co-workers may prove to be valuable in assessing analgesic efficacy of transdermal fentanyl for comparison with other available analgesic agents in rabbits.

This study indicates that transdermal fentanyl patches can be safely used in rabbits, and plasma fentanyl concentrations can be achieved that are within the range reported to be analgesic in humans and other species. Further research is warranted to establish therapeutic plasma fentanyl concentration in the rabbit by use of analgesiometry, and the transdermal fentanyl patch should be evaluated in various postoperative situations, such as abdominal, thoracic, and orthopedic procedures.

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