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Research Paper

Buprederm™, a New Transdermal Delivery System of Buprenorphine: Pharmacokinetic, Efficacy and Skin Irritancy Studies

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Purpose. The pharmacokinetics, analgesic efficacy, and irritancy potential of Buprederm™, a new transdermal delivery system of buprenorphine, was evaluated.
Methods. Single and multiple dose pharmacokinetic studies were conducted in mice and rabbits. The analgesic efficacy and skin irritation potential were determined by tail flick and writhing tests in mice and by the Draize dermal scoring system in rabbits.
Results. Fast absorption of buprenorphine into the bloodstream was observed in mice and rabbits after Buprederm™ application. The peak buprenorphine level in plasma was achieved between 1 and 24 h, and the effective therapeutic drug concentration was maintained for 72 h. No significant accumulation of buprenorphine was seen after multiple consecutive applications of patches to rabbits with a 4-day dosing interval. Buprederm™ induced prolongation of tail-flick latency in a dose- and time-dependent manner. Maximum analgesic effect was attained between 3 and 6 h and was maintained for 24 h after patch application. No skin irritation was demonstrated in rabbits after repeated Buprederm™ application.
Conclusions. Buprederm™ was shown to be efficacious by achieving the effective buprenorphine concentration in the blood and brain sufficient to maintain an analgesic effect for 72 h, and was also shown to be safe following multiple applications.

KEY WORDS: analgesic effect; Buprederm™; pharmacokinetics; skin toxicity; transdermal delivery of buprenorphine.

INTRODUCTION

Chronic pain is disabling, has a profound negative impact on the lives of patients and their families, and places a substantial burden on the health care system. Effective management of chronic pain is therefore an essential goal for clinicians and health care professionals (1–4).

Treatment of persistent pain usually requires administration of long-acting opioid analgesics which are considered the most effective option for the treatment of chronic moderate to severe pain (5–8). Among the opioids, morphine was first used in clinical practice followed by fentanyl, methadone, diamorphine, pethidine and buprenorphine (9–13). It is known that existing analgesics (*i.e.*, methadone) require rather long treatment times of up to 3 weeks, have a pain relief rate of only 70%, and are accompanied by severe side effects and withdrawal symptoms (14–16). In addition, fentanyl, a full μ-opioid receptor agonist, is known to be severely addictive (17). On the other hand, buprenorphine is known to have good tolerability and excellent treatment efficacy (18–19).

Buprenorphine is a semi-synthetic, highly lipophilic oripavine derivative that acts as a high affinity partial agonist of μ-opioid receptors (20–22). It has a two times higher affinity to the opiate receptor and about a 30 to 40 times higher analgesic effect than morphine. However, it is almost devoid of morphine's side effects of dependence, tolerance and constipation, and has a wider safety profile (21, 23, 24). Its therapeutic effect is relatively stronger than other analgesics with a much longer duration. Buprenorphine is used as an analgesic for malignancy-related pain, post-operative pain, pain associated with myocardial infarction, and other acute and chronic pain.

For many years, parenteral and sublingual formulations of buprenorphine were commercially available. However, these dosing regimens had many disadvantages such as inconvenient management of chronic pain caused by the need for frequent administrations per day, and the possibility of the manifestation of drug toxicity due to sudden peaks in plasma drug concentrations. Therefore, transdermal delivery systems (TDS) have recently been introduced to overcome such disadvantages by maintaining a constant blood drug

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ABBREVIATIONS: BBB, Blood–brain barrier; BTDS, Buprenorphine transdermal system; HPLC, High performance liquid chromatography; IS, Internal standard; LC/MS/MS, Liquid chromatography-tandem mass spectrometry; LOQ, Limit of quantification; ME, Maximum effect; MeOH, Methanol; , ; MRM, Multiple reaction monitoring; *P-gp*, P-glycoprotein; PK/PD, Pharmacokinetic/pharmacodynamic; S.D., Standard deviation; S.E.M., Standard error of means; TDS, Transdermal delivery system.

concentration at an effective level for analgesia and eliminating the frequent dosing (25–31) required for the management of chronic pain.

The buprenorphine TDS, Transtec®, is a transdermal matrix patch formulation, where the active drug is incorporated into a polymer matrix, which also serves as the adhesive layer. It is available in three strengths (20, 30, 40 mg as 1.78 mg/cm^2) designed for a 72 h application period (32). There is a delay in the onset of the therapeutic effect due to the rate-controlled slow release. Reaching the effective therapeutic concentration more rapidly after application of a single patch would be helpful to improve poor pain relief in patients as needed.

Hence, we have developed a new transdermal hydrogel patch, Buprederm™, designed for faster onset and to release buprenorphine at a controlled rate over 72 h at dosages of 28, 42, 56 mg (2.4 mg/cm^2). It is composed of a pressure sensitive adhesive, an impermeable polymer backing film, and a hydrogel layer containing active drug (buprenorphine HCl) and absorption enhancer. The pressure sensitive adhesive provides adhesion strength that firmly attaches the transdermal preparation to the skin for reliable drug permeation. The impermeable polymer backing film provides occlusive conditions to protect the hydrogel from contamination. Finally, the absorption enhancer maintains desirable blood drug concentrations by facilitating drug transport across the skin into the systemic circulation.

In this study, we evaluated the *in vivo* permeation of buprenorphine in mice and rabbits after single and multiple applications of Buprederm™ (BTDS; buprenorphine transdermal system), respectively. The pharmacokinetics of buprenorphine in the blood and brain, a target organ for drug action, was evaluated to predict pharmacodynamics at expected clinical doses. Analgesic efficacy of Buprederm™ was determined in mice using tail flick tests and writhing tests, and its irritancy potential was evaluated in rabbits using Draize's method.

MATERIALS AND METHODS

Materials and Formulation

The three dosage forms of Buprederm™ (0.24, 0.8, 2.4 mg/cm^2 with a size of 1×1 cm^2 for the mouse study and 2.4 mg/cm^2 with sizes of 1.87×1.87 cm^2 and 2.5×2.5 cm^2 for the rabbit study) were prepared by the transdermal delivery group at Samyang R&D Center using proprietary hydrogel matrix technology. These patches were stored at room temperature until use. Buprenorphine hydrochloride (HCl), norbuprenorphine and naltrindole (internal standard) were purchased from MacFarlan Smith Ltd. (UK), Cerilliant (USA) and Sigma (USA), respectively, and stored refrigerated and protected from light. All other reagents were of analytical grade. Buprenorphine for injection was formulated for administration as solutions in distilled water before injection to animals.

Animals and Treatment Group

Animals in this study were handled in accordance with the provisions of "the Principles of Laboratory Animal Care" (NIH publication #85-23, revised in 1985). Male ICR (Institute of Cancer Research) mice for single dose pharmacokinetics and analgesic efficacy studies and male New Zealand White rabbits for multiple dose pharmacokinetics and skin toxicity studies were supplied by Charles River Laboratories (Orient, Korea). Animals were allowed to adapt to the environment in the laboratory for more than 1 week where constant temperature and humidity were maintained. Then, apparently healthy animals were selected based on their general condition and used for the experiment. Animals were allowed free access to food and water. Subjects were divided into groups of 8–12 animals for the pharmacokinetics and analgesic studies and groups of six animals for the skin toxicity study (33).

Single Dose Pharmacokinetic Study in Mice

The hair on the dorsal area of the mouse was shaved 1 day prior to the beginning of the experiment and one sheet of patch (0.24, 0.8, and 2.4 mg/patch, size: 1×1 cm^2) was applied to the shaved skin. The doses of Buprederm™ to mice (0.24–2.4 mg/patch) was selected based on body surface area, metabolic rate and analgesic effect, which were equivalent to 1/17–1/170 of the clinical dose (2.4 mg/cm^2, 42 mg/patch), respectively. To prevent partial peeling and to ensure proper contact with the skin, the patch was affixed using adhesive and an elastic bandage (Coban™, 3M Health care, USA). Mice were sacrificed at 0.5, 1, 3, 6, 12, 24, 48 and 72 h (n=8) after application of the patch, and blood samples were taken from the abdominal artery using a heparin-treated needle and centrifuged at 1,500 g for 10 min. The brain was removed, washed with normal saline and weighed. Both plasma and brain samples were stored at −70°C until analysis. For pharmacokinetic comparison, a subcutaneous dosing group was included as a control (n=8). The subcutaneous (*s.c.*) dose of buprenorphine (0.25 mg/kg) was selected based on the dose which was known to elicit analgesia in mice (34–35).

Single and Multiple Dose Pharmacokinetic Studies in Rabbits

A single dose pharmacokinetic study was carried out to ensure clinical effectiveness as the drug concentration has to be maintained above a minimum effective level throughout the course of therapy. However, accumulation of drug may occur if drug intake exceeds elimination and hence, a multiple dose pharmacokinetic study was performed in rabbits to assess this behavior (36). Hair on the dorsal area of the rabbit was shaved 1 day prior to the beginning of the experiment and one sheet of Buprederm™ (2.4 mg/cm^2, size: 1.87×1.87 cm^2) was applied to the shaved skin for the single dose studies. In the multiple dose studies, the patches were applied repeatedly every 4 days (3 days attachment and 1 day detachment) for a period of 28 days (n=8). The dose of Buprederm™ to rabbits was selected based on body surface area, metabolic rate and analgesic effect, and was equivalent to 1/5 of the clinical dose. After 72 h, the patch was removed and the application site was rinsed with water. On day 4, a fresh patch was applied to a different site on the skin. To ensure firmer adhesion of the

patch and to prevent removal of Buprederm™ by the animal, the patch was affixed using adhesive tape (Tegaderm™, 3M Health care, USA) and wrapped with an elastic bandage (Coban™, 3M Health care, USA). Blood samples (1 ml) were taken from a marginal ear vein using a heparin-treated needle at 0, 1, 3, 6, 12, 24, 48 and 72 h in the single dose study. In addition, blood was collected at 24, 48, 72, and 96 h after the first through sixth patch applications and at 0, 1, 3, 6, 12, 24, 48 and 72 h after the last (seventh) patch application in the multiple dose study. The blood samples were immediately centrifuged at 1,500 g for 10 min. Plasma (400 μl) was separated and stored at −70°C until analysis. The control group received buprenorphine. HCl subcutaneously (0.1 mg/kg) to elicit analgesia in the rabbits (n=3) (37).

Analytical Method

Plasma and brain concentrations of buprenorphine and norbuprenorphine, an active metabolite, were determined by an established and validated bioanalytical method using LC/MS/MS (38–42). In brief, plasma samples were spiked with naltrindole (internal standard, I.S.) in deproteination solvent (MeOH) and vortexed. After centrifugation, the supernatant was injected onto the column. The brain tissues were homogenized on ice with 6 volumes of ice-cold distilled water and the homogenate was spiked with I.S. in the extraction solvent (1 N NaOH/MeOH/ethyl acetate = 1/8/160) and vortexed. After centrifugation, the supernatant was evaporated and the residue was reconstituted with methanol for LC/MS/MS analysis. For analysis of buprenorphine and norbuprenorphine, a tandem quadrupole mass spectrometer (Quattro Ultima Pt, Micromass, UK) coupled with an HPLC system (1100 series, Agilent, USA) was utilized. The separation was performed on a Capcell pak C_{18} (2.0×150 mm, 5 μm, Shiseido, Japan) column using a mobile phase consisting of 10 mM acetate buffer (pH 4.2) and acetonitrile with a linear gradient (55/45→30/70, *v/v*) for 15 min at a flow rate of 0.2 ml/min. The injection volume was 10 μl. Mass spectra were recorded with positive electrospray ionization (ESI^+) and analysis was performed using multiple reaction monitoring (MRM) with a specific transition at 468.55→55.05 for buprenorphine, 414.5→83.1 for norbuprenorphine, and 414.5→55.3 for naltrindole.

The standard curve was linear over the concentration range of 0.5–100 ng/ml (0.2–50 ng/ml) and 1.4–140 ng/g for buprenorphine in mice (rabbit) plasma and mice brain sample, and 2–100 ng/ml (1–50 ng/ml) and 7–140 ng/g for norbuprenorphine in mice (rabbit) plasma and mice brain sample, respectively, with a typical correlation coefficient of r=0.9946 or higher. The LOQ of buprenorphine and norbuprenorphine was 0.5 (0.2) and 2.0 (1.0) ng/ml in mice (rabbit) plasma and 1.4 and 7.0 ng/g in mice brain, respectively. The mean intra- and inter-day assay coefficients of variation were <9% and <7% for buprenorphine and <8% and <10% for norbuprenorphine in plasma and brain, respectively, over the concentration range studied (n=5 at each concentration). The mean accuracy was 92–109% and 88–106% for buprenorphine and 94–103% and 90–103% for norbuprenorphine in plasma and brain, respectively.

Pharmacokinetic Data Analysis

The area under the curve to the last measurable concentration (AUC_{last}) was calculated by the linear trapezoid rule. The maximum buprenorphine concentration (C_{max}) and the corresponding peak time (t_{max}) were determined by inspection of the individual drug plasma concentration–time profile. The average drug concentration in plasma during the 72 h of patch application (C_{avg}) was calculated by dividing $AUC_{72\,h}$ by the dosing interval (τ=72 h).

Tail Flick Test

To assess the analgesic potency of Buprederm™, a tail flick test was performed (43). Prescreened mice were divided into five groups (0, 0.24, 0.8, 2.4 mg/patch and 0.25 mg/kg, *s.c.*, n=12) and were treated as described above (single dose pharmacokinetic study in mice). The pain threshold was measured before (baseline) and after drug treatment at 1, 3, 6, and 24 h after Buprederm™ attachment and at 0.5, 1, 3, and 6 h after subcutaneous injection. Mean baseline latency was calculated from three repeated measurements (20 min interval) before treatment. The tail flick analgesiometer (LE7106, Panlab, S.L., Spain) emits radiant heat to the tail at a distance 1.5 cm from the tip in mice. The time from the onset of heat to the withdrawal of the tail (tail-flick latency) was measured. The intensity of the radiant heat was adjusted so that the baseline latencies were between 2.5 and 3.5 s. To avoid causing tissue damage, the heat stimulus automatically switched off at 10 s (cut-off latency). Analgesic potency was calculated by the following equation.

$$\%\mathrm{ME}(\text{maximum effect}) = (\mathrm{TL} - \mathrm{BL})/(\mathrm{CL} - \mathrm{BL}) \times 100$$

TL: test latency, BL: baseline latency, CL: cut-off latency (10 s)

Writhing Test

The writhing test is described in detail elsewhere (44). Prescreened mice were divided into four groups (0.24, 0.8, 2.4 mg/patch and 0.075 mg/kg, *s.c.*) and were treated as described above (single dose pharmacokinetic study in mice). The writhing response was induced by intraperitoneal injection of 10 ml/kg of 0.8% *v/v* acetic acid in distilled water. The intensity of nociception was quantified by counting the total number of writhings that occurred between 10 and 20 min after acetic acid injection. The writhing response consists of a contraction of the abdominal muscles together with a stretching of the hind limbs.

Skin Irritancy Test

The skin irritancy potential of Buprederm™ upon *in vivo* application, with and without drug was carried out after single application and repeated application. The backs of rabbits were clipped free of fur with an electric clipper 24 h prior to application of a patch. Just prior to Buprederm™ application, each rabbit received two diagonal epidermal abrasions, in an area of skin approximately 2.5×2.5 cm square, with a sterile needle at one test site and one control site while the skin at another site remained

intact. Buprederm™ (2.4 mg/cm^2, 2.5×2.5 cm^2) and placebo patch (without drug, 2.5×2.5 cm^2) were then applied to each site, two sites per rabbit. The patches were backed with impervious plastic wrap and covered with a non-reactive tape. The entire test site was wrapped with an elastic bandage and the animals were returned to their cages. After 24 h of exposure, each patch was removed and the test site was rinsed with tap water. At 24 h and 72 h after application, the test sites were examined and scored for signs of erythema and edema according to the Draize dermal scoring criteria (45). For repeated application, Buprederm™ and placebo patches were applied to the shaved skin repeatedly every 4 days (3 days attachment and 1 day detachment), for a period of 28 days. After the last application (seventh), the skin reaction was observed and scored at 24 h following removal of the patches. The Primary and Cumulative Irritation Index described below were calculated and the irritancy potential of Buprederm™ after single and repeated application was evaluated.

The Primary and Cumulative Irritation Index

0.0–0.4 Negligible
0.5–1.9 Slight
2.0–4.9 Moderate
5.0–8.0 Severe

Data Analysis

The data from the pharmacokinetic studies and efficacy studies were expressed as a mean value ± standard deviation (S.D.) and a mean value ± standard error of means (S.E.M.), respectively. An unpaired, two-tailed Student's *t* test was used to determine the significance of differences between two group means. One-way analysis of variance (ANOVA) was used to assess the statistical significance of difference among means of more than two groups. A p value <0.05 was considered significant.

RESULTS

Single Dose Pharmacokinetic Study in Mice

The plasma (Fig. 1A) and brain (Fig. 1B) concentration–time curves upon single patch application to mice at three different doses are shown in comparison with subcutaneous (*s.c.*) administration of buprenorphine (Fig. 1C). Following the single dose of Buprederm™, plasma buprenorphine concentrations increased rapidly within 0.5 h after patch application for all three doses (0.24, 0.8, 2.4 mg/patch) and remained elevated for 72 h. The highest drug concentration in the plasma (C_{max}=3.3±0.6, 9.3±1.4, 32.7±7.8 ng/ml) was achieved between 1 and 24 h after patch application. The C_{max} and $AUC_{72\,h}$ increased dose-proportionally indicating dose-independent linear pharmacokinetics. Similarly, brain buprenorphine concentrations increased rapidly reaching

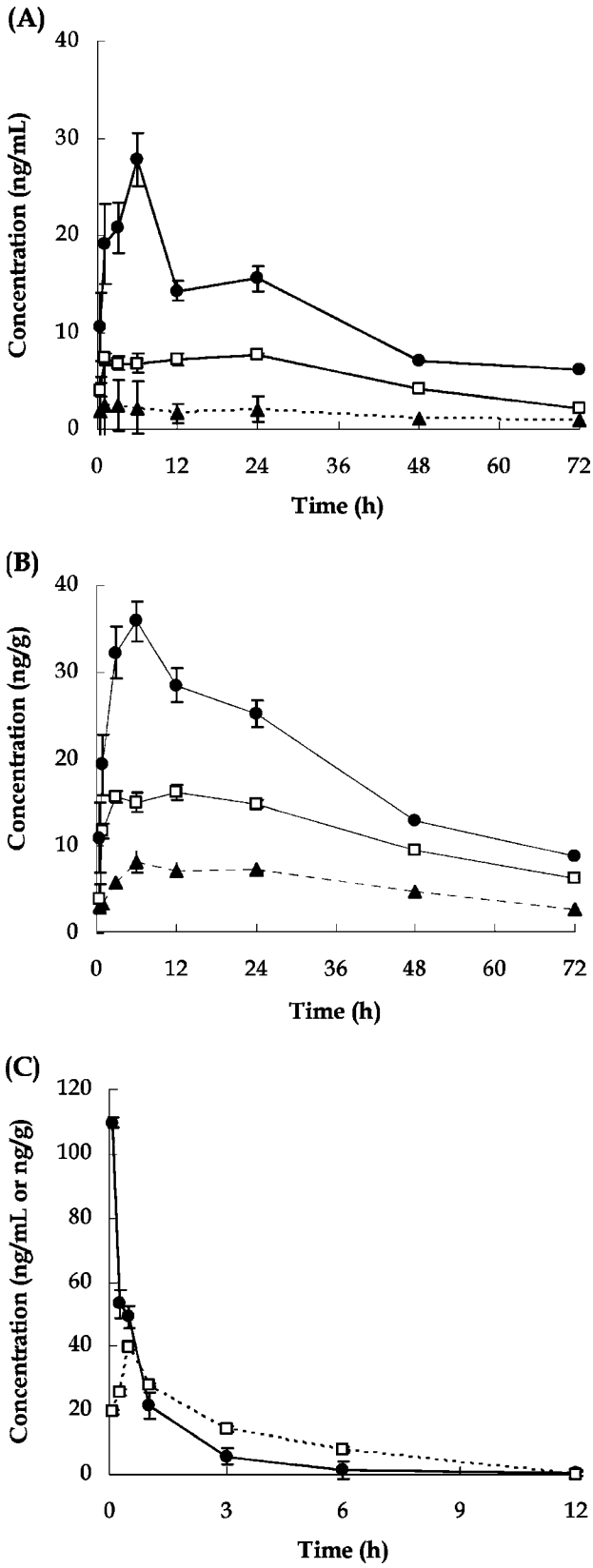

Fig. 1. Mean plasma (**A**) and brain (**B**) concentration–time curves of buprenorphine in mice (n=8) after dermal application of Buprederm™ (0.24 mg/patch: *closed triangle*, 0.8 mg/patch: *open square*, 2.4 mg/patch: *closed circle*) or subcutaneous injection (**C**) of buprenorphine HCl at 0.25 mg/kg (plasma: *closed circle*, brain: *open square*). Plasma and brain concentration of buprenorphine was measured up to 72 h. The data represent the means and standard deviation (S.D.).

Table 1. Pharmacokinetic parameters of buprenorphine after Buprederm™ application or subcutaneous administration of buprenorphine·HCl to mice

Treatment	Plasma				Brain		
	AUC_{last} (ng·h/ml)	C_{max} (ng/ml)	t_{max} (h)	C_{avg} (ng/ml)	AUC_{last} (ng·h/g)	C_{max} (ng/g)	t_{max} (h)
0.24 mg/patch	104±16	3.3±0.6	1–24	1.4±0.2	384±58	10.0±2.4	3–24
0.8 mg/patch	373±63	9.3±1.4	1–24	5.2±0.9	828±64	18.6±2.8	3–24
2.4 mg/patch	815±127	32.7±7.8	1–24	12.3±1.8	1352±139	42.2±10.0	3–24
s.c. injection 0.25 mg/kg	79±11	109.7±31.8	0.1	N.A.	101±14	42.3±6.8	0.25

C_{max} the highest observed plasma concentration, t_{max} the time at which C_{max} occurred, $AUCl_{last}$ the area under the curve to the last measurable concentration (72 and 12 h for patch application and *s.c.* injection, respectively), C_{avg} average drug concentration in plasma during 72 h of patch application ($C_{avg} = AUC_\tau/\tau$, τ=72 h), *N.A.* Not applied.
Pharmacokinetic parameters were expressed as the mean ± S.D. (n=8), except t_{max} (shown as a range).

C_{max} (10.0±2.4, 18.6±2.8, 42.2±10.0 ng/g for 0.24, 0.8, 2.4 mg/patch, respectively) between 3 and 24 h after patch application. The $AUC_{72\,h}$ and C_{max} values increased less than dose-proportionally over the dose range of 0.24–2.4 mg/patch. On the other hand, buprenorphine concentrations in the plasma and brain declined rapidly following *s.c.* administration and were below the LOQ at 6 h after administration. The pharmacokinetic parameters determined in the plasma and brain tissue are provided in Table 1.

Norbuprenorphine, an active metabolite, was not detected in plasma or the brain for all groups suggesting its concentration was below the level of detection. In fact, norbuprenorphine does not seem to contribute to the analgesic efficacy of Buprederm™ since norbuprenorphine has only 1/4 the intrinsic analgesic activity of buprenorphine and a low permeability into the brain (~10%), and therefore may not be clinically significant (45).

Single and Multiple Dose Pharmacokinetic Studies in Rabbits

To simulate the clinical situation, where more than one patch will be applied consecutively for a prolonged period of time, a multiple dose pharmacokinetic study was conducted in rabbits. Patches were replaced every 4 days (3 days attachment and 1 day detachment) over a 28 day period according to the clinical dosing schedule.

After application of a single patch, the plasma buprenorphine concentration increased rapidly, reached near its maximum concentration at 3 h, maintained this concentration for 24 h (C_{max}=0.97±0.49 ng/ml), and slightly declined thereafter. The effective therapeutic concentration (0.5–1.0 ng/ml) was maintained until its removal, i.e., 72 h ($AUC_{72\,h}$= 51.9±22.6 ng·h/ml, C_{avg}=0.72±0.31 ng/ml). After removal of the patch, the buprenorphine concentration in plasma declined to 0.27±0.61 ng/ml at 96 h. On the other hand, following single *s.c.* administration (0.1 mg/kg) of buprenorphine·HCl to rabbits (n=3), plasma buprenorphine concentrations reached maximum levels at 15 min, declined rapidly, and were below the LOQ by 12 h (Fig. 2A and B).

After multiple applications of the patch, the values of $AUC_{72\,h}$ (53.1±12.9 ng·h/ml) and C_{max} (1.01±0.27 ng/ml) obtained from each dose were not statistically different, indicating no accumulation of drug with subsequent applications (Fig. 2C). This was further evidenced by the similar pharmacokinetic parameters obtained after the last patch (seventh) application (C_{max}=0.84±0.37 ng/ml, AUC_{72h}= 44.1±15.7 ng·h/ml) compared with those from the single patch application (Fig. 2A, Table 2).

Tail Flick Test

Figure 3 shows the results of the analgesic effect of transdermal and *s.c.* administration of buprenorphine·HCl to mice determined by tail flick latency. Analgesic efficacy of three patch dosages (0.24, 0.8, 2.4 mg/patch) were compared over time (1, 3, 6, and 24 h after application) with repeated measurements at each time point.

Buprederm™ at all dosages produced a prolongation of tail-flick latency. For the low dose group (0.24 mg), the analgesic effect appeared after 3 h, maximum latency was attained at 6 h (%ME of 53.4), and was maintained for 24 h after patch application. For the medium (0.8 mg) and high (2.4 mg) dose groups, the analgesic effect appeared after 1 h, and maximum latency was attained at 6 h (%ME of 92.2) and 3 h (%ME of 94.9), respectively, and decreased slightly to 68.1% and 82.2% at 24 h after patch application. No significant differences between groups were observed at baseline. Increasing the dose above 0.8 mg/patch had little effect on the maximum degree of analgesia that was produced, but a higher dose did provide a more rapid onset of action. The buprenorphine administered by *s.c.* injection also induced significant increases in pain threshold with the maximum analgesic effect attained at 0.5 h (%ME of 81.7) and declined at 6 h after injection.

A potential problem with the repeated measurements used in this experiment is that repeated exposure to heat may have, by itself, altered the accuracy of pain threshold over repeated trials as evidenced by an increase in pain threshold at 24 h in the control group receiving the placebo patch.

Writhing Test

Injection of 10 ml/kg of 0.8% (*v/v*) acetic acid into the intra-peritoneal cavity of mice produced writhing characterized by length way stretching of the abdomen and extension of the hind limbs. The effect of buprenorphine on acetic acid-induced writhing behavior in mice was determined at 0.5 and 24 h following subcutaneous injection and patch application, respectively, and is shown in Fig. 4. Control animals receiving the placebo patch produced an average of 33.4±4.2 writhes in 10 min. Buprederm™ at all doses reduced the number of

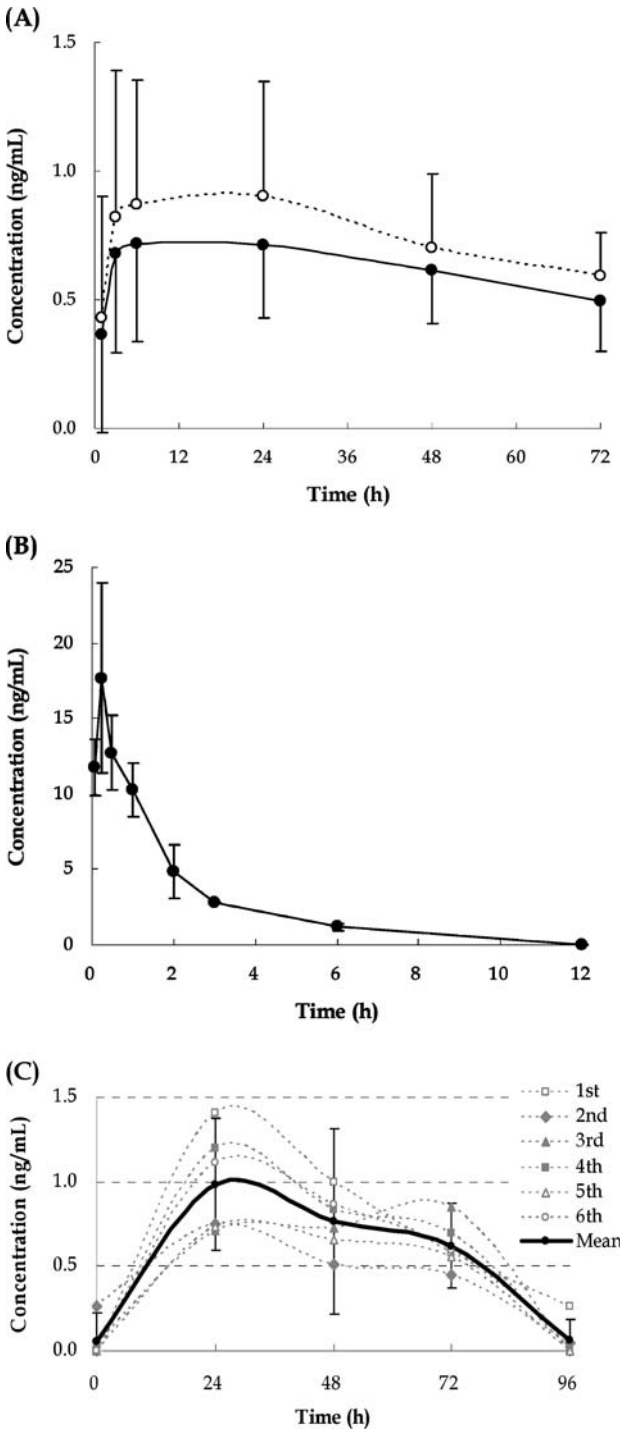

Fig. 2. Mean plasma concentration–time curves of buprenorphine in rabbits after **A** single (*open circle*) and multiple dermal application (seventh dose, *closed circle*) of Buprederm™ (8.4 mg/patch, size: 1.87×1.87 cm^2) for 72 h (n=8) or **B** subcutaneous injection of buprenorphine·HCl at 0.1 mg/kg (n=3). The data represent the means and standard deviation (S.D.). **C** Plasma concentration *vs.* time curves of buprenorphine in rabbits after multiple (×6) applications of Buprederm™ (n=8). Mean plasma concentration of buprenorphine from first to sixth dose is shown as a *solid line*. Effective therapeutic range of buprenorphine in plasma after dermal application of buprenorphine is 0.5–1.0 ng/ml.

writhes; 23.3±4.7, 15.4±3.4 (p<0.01 *vs.* control), and 17.6±3.4 (p<0.01 *vs.* control) for the 0.24, 0.8 and 2.4 mg/patch, respectively. The antinociceptive effects of the 0.8 and 2.4 mg groups at 24 h following patch application were similar indicating that increasing the dose above 0.8 mg had little effect on the degree of analgesia which was produced. On the other hand, subcutaneous buprenorphine·HCl at 0.075 mg/kg reduced the number of writhes to 6.9±3.3 (p<0.01) at 0.5 h after injection.

Skin Irritancy Test

In the skin irritancy test, the scores of erythema and edema in intact and abraded skin were tallied for all rabbits at 24 and 72 h. No erythema, eschar, or edema appeared at the treatment sites during the observation period (mean score: 0 for all points). The primary and cumulative irritation index of the Buprederm™ was calculated to be "0"; therefore, the irritancy potential of Buprederm™ after single and repeated application was negligible according to the Draize dermal scoring criteria.

DISCUSSION

The transdermal delivery system of buprenorphine has contributed to advances in the effective pharmacological management of chronic pain in recent years. The recently introduced buprenorphine transdermal delivery system (Transtec®) is a matrix type patch formulation. The active drug is homogeneously incorporated into an adhesive polymer matrix that also controls its release by a matrix diffusion mechanism. However, this typical matrix system has a significant lag time (12–24 h) to reach clinically effective concentrations (0.1–0.5 ng/ml) (32).

Therefore, a new buprenorphine hydrogel matrix system, Buprederm™ was developed for a faster onset of therapeutic effects using a combination of hydrophilic polyhydric alcohol and fatty acid ester as absorption enhancers incorporated in aqueous polyvinyl alcohol as a hydrogel base. Based on the pharmacokinetics of buprenorphine, a target flux 2.2–3.1 μg/cm^2·h was required across human skin from 17.5 cm^2 patch to reach therapeutically effective target plasma concentrations in humans (0.5–0.7 ng/ml) (46). And the steady state flux of 2.7 μg/cm^2·h was achieved from Buprederm™ (2.4 mg/cm^2, 17.5 cm^2, 42 mg/patch), which was demonstrated in ex vivo permeation study using human skin (data not shown).

In the present study, pharmacokinetics, analgesic potency and irritancy potential of Buprederm™ was evaluated in mice and rabbits. For pharmacokinetic and efficacy studies, hair was removed from dorsal area of mice and rabbit 1 day before dosing. Extra care was taken to avoid damaging the skin during the shaving procedure, however, shaving may have removed some of the stratum corneum. So we kept animals under observation for 24 h for any untoward effects of shaving before the patch application. Increases in permeation through damaged skin are a function of the barrier property of skin for the test compound and so that the greatest increases in penetration were obtained with the drugs that are most poorly absorbed (47). Hence, buprenor-

Table 2. Pharmacokinetic parameters of buprenorphine in plasma after dermal application of Buprederm™ or subcutaneous administration of buprenorphine·HCl to rabbits

Treatment	AUC_{last} (ng·h/ml)	C_{max} (ng/ml)	C_{avg} (ng/ml)	t_{max} (h)
Single patch application (8.4 mg/patch, n=8)	51.9±22.6	0.97±0.49	0.72±0.31	3–24
Multiple patch application (seventh dose) (8.4 mg/patch, n=8)	44.1±15.7	0.84±0.37	0.61±0.22	3–24
s.c. injection 0.1 mg/kg (n=3)	28.8±4.0	17.7±6.3	N.A.	0.25

C_{max} the highest observed plasma concentration, t_{max} the time at which C_{max} occurred, $AUCl_{last}$ the area under the curve to the last measurable concentration (72 and 12 h for patch application and *s.c.* injection, respectively), C_{avg} average drug concentration in plasma during 72 h of patch application ($C_{avg} = AUC_\tau/\tau$, τ=72 h), *N.A.* Not applied.
Pharmacokinetic parameters were expressed as the mean ± S.D. (n=8), except t_{max} (shown as a range).

phine which is highly lipophilic to cross the skin barrier may be considered to be affected to the least extent.

Occlusive condition was provided using adhesive and elastic bandage to ensure good contact with the skin and to prevent patch removal by the animal without affecting integrity of the skin barrier and blood flow. However, the occlusive condition may affect drug absorption from the patch into the systemic circulation (48–51). That is to say, the occlusion of the skin under the patch and the adhesive or elastic bandage may entrap sweat which in turn serves to hydrate the skin (specifically, the stratum corneum), thus facilitating drug penetration across the skin. Skin occlusion may also cause skin irritation due to decreased skin "breathing," however, no skin irritation was observed after repeated Buprederm™ application. In this study, to minimize such effects we use non-woven fabric backing film and fabric bandage which have a good airflow.

The doses of Buprederm™ to mice and rabbits were selected based on body surface area and metabolic rate, which were equivalent to 1/170 (0.24 mg/patch) and 1/5 (8.4 mg/patch) of the human dose (42 mg/patch), respectively. In addition, 1/52 (0.8 mg/patch) and 1/17 (2.4 mg/patch) of the human dose were chosen for maximum analgesic effect in mice.

In a single dose pharmacokinetic study in mice, buprenorphine was detected at 0.5 h after Buprederm™ application, the first time point, suggesting no lag time to reach detectable drug levels. The peak drug concentration was attained between 1 and 24 h reaching plateau at constant steady-state plasma concentration with zero-order absorption of buprenorphine from Buprederm™, which would imply that only 1 h was required for buprenorphine in the BTDS to equilibrate with the skin and subcutaneous tissues. Afterwards, buprenorphine concentration was declined gradually until the patch was removed at 72 h indicating that the buprenorphine absorption rate was not sustained for the duration of patch application. The steady-state pharmacokinetic profile that is typical of transdermal delivery system depends on constant drug input. The rates of drug input from TDS into systemic circulation are controlled by penetration barriers (skin) and may be described in Fick's law term: the drug delivery rate varies in direct proportion to the drug concentration in the matrix and drug diffusion coefficient. In reality, the drug permeation through skin may not be constant and varies during patch application period possibly due to changes in skin properties, decrease of drug concentration in the matrix and depletion of enhancers in the process of drug delivery. The deviation from the steady-state plasma levels of buprenorphine after 24 h may partly be attributed to depletion of the volatile penetration enhancer that is successfully applied due to the unique features of hydrogel matrix system and to changes in the drug concentration in the patch to a certain extent since 5–10% of the loading

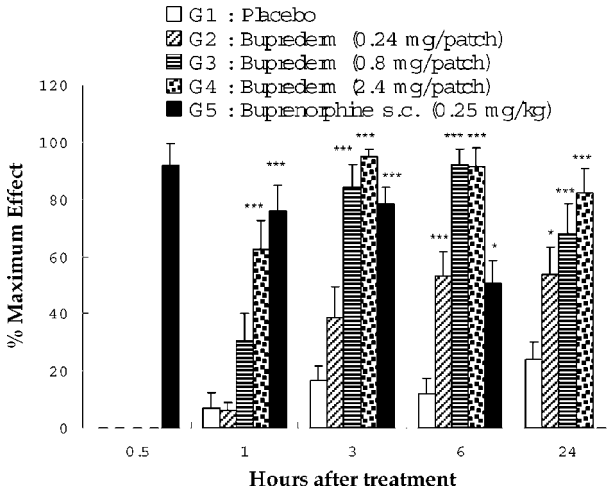

Fig. 3. The analgesic effect on thermal pain in mice (n=12) after dermal application of Buprederm™ (0.24, 0.8, 2.4 mg/patch) and subcutaneous injection of buprenorphine·HCl (0.25 mg/kg) was determined by a standard tail-flick test. The data represent the mean ± S.E.M. (n=12). Significantly different from G1 (placebo patch) *P<0.05, ***P<0.001.

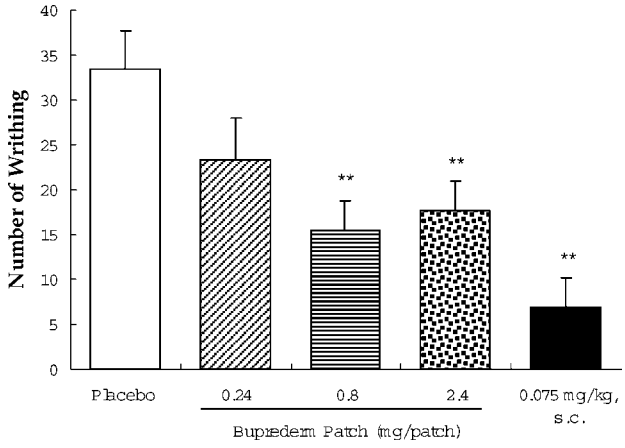

Fig. 4. Analgesic effect on chemical pain, acetic acid-induced writhing syndrome, in mice (n=12) was determined at 24 h after dermal application of Buprederm™ (0.24 mg/patch, 0.8 mg/patch, 2.4 mg/patch) and at 0.5 h after subcutaneous injection of buprenorphine·HCl (0.075 mg/kg). The data represent the mean ± S.E.M. Significantly different from G1 (placebo patch) **P<0.01.

dose seemed to be absorbed based on the transdermal delivery rate derived from experimental pharmacokinetics in mice. In fact, non-steady-state pharmacokinetic profiles have been demonstrated in estradiol patch (52) and nicotine patch (53) due to depletion of enhancers and decrease in nicotine depot in the patch (~10% of the total dose), respectively.

The overall effective concentration was maintained for up to 72 h both in the blood and brain for all three doses (0.24, 0.8, 2.4 mg/patch). The therapeutic range for buprenorphine was not well defined, which primarily reflect the complexity of the overall response and the lack of sensitive, discriminatory and objective measures of efficacy. Numerous studies conducted in patients with tumor-related or postoperative pain have shown that the required buprenorphine plasma concentration for the relief of moderate to severe pain lies between 0.1 and 1 ng/ml with 0.1 ng/ml as a minimum effective concentration (46). The relationship between plasma concentration and its analgesic effects has not fully elucidated in animals. Understanding of PK/PD relations is complicated by the fact that systemic drug concentrations can fluctuate significantly over a dosage interval, and the therapeutic effect may also exhibit fluctuations that are either in phase with systemic drug concentration, or are phase-shifted as a result of the time required to achieve target site distribution. The PK/PD analysis in rats showed a counterclockwise hysteresis between the concentration of buprenorphine in plasma and the analgesic effect and both the target site distribution and the receptor binding kinetics contribute to the observed hysteresis (54–55) which has also shown in the present study. From these studies, the effective buprenorphine concentrations in mice are estimated to be 1–10 ng/ml in plasma and 3–12 ng/g in brain, which appear to be tenfold higher than the effective concentration in human. Also, it has been reported that the opioid μ-receptor was localized in various brain regions at a density of ~30 pmol/g of rat brain and suggested that low receptor occupancy (less than 30%, lower than 8 ng/g) will be satisfactory for the onset of an analgesic effect (51, 56). Therefore, Buprederm™ at all three strengths (0.24, 0.8, 2.4 mg/patch) was able to keep the drug level in the brain high enough to maintain analgesic activity for 72 h. Overall, there was a good relationship between drug plasma/brain pharmacokinetics and analgesic effect.

When $AUC_{72\,h}$ and C_{max} of buprenorphine in plasma were normalized with applied dose (dose normalization), dose-independent, linear pharmacokinetics were observed. On the other hand, $AUC_{72\,h}$ and C_{max} in the brain increased less than dose-proportionally suggesting dose-dependant, non-linear kinetics in the brain. These results suggest that buprenorphine was transported from Buprederm™ across skin into the systemic circulation with linear kinetics, and distributed into target tissue compartment, the brain tissue with non-linear kinetics. To reach the brain, buprenorphine in the systemic circulation have to cross the blood–brain barrier (BBB) via the trans-cellular route (57), and therefore, only lipophilic compounds can readily cross the endothelial cells via passive diffusion. Due to high lipophilicity, it is believed that buprenorphine readily penetrate the BBB. The observed less than dose proportional increase of $AUC_{72\,h}$ and C_{max} in brain over the dose range of 0.24–2.4 mg/patch may suggest involvement of transporters. *In vivo* and *in vitro* studies have demonstrated that various opioids, including morphine, fentanyl and methadone, interact with P-glycoprotein (P-gp), ATP-dependent efflux pump, which is highly expressed on the brain capillaries and play a role to decrease the xenobiotic permeation into the brain (58–60). Recently, it has shown that P-gp-mediated efflux transport system is involved in buprenorphine transport at the BBB in rats (61), which may contribute to non-linear pharmacokinetics in brain to some extent.

On the other hand, subcutaneous administration of buprenorphine·HCl led to rapid loss of the drug in the blood or brain within 6 h, thus requiring frequent dosing for long-lasting pain management. The concentrations of norbuprenorphine, a major metabolite, in the plasma and brain were below detection limits at all time points. The contribution of norbuprenorphine to the analgesic efficacy of Buprederm™ appears to be negligible due to its low permeability into the brain and lower intrinsic pharmacological activity (1/4 of buprenorphine) (54).

Fast-absorption of buprenorphine into the blood was also shown in a single dose pharmacokinetic study in rabbits after Buprederm™ application. The steady-state plasma concentration was achieved after a short time lag (3 h) and the effective concentration (0.5–1 ng/ml) was maintained for the period of patch application (72 h). During 1 day detachment period, plasma buprenorphine concentrations declined to a level of minimum effective concentration and therefore, analgesic effect can be maintained throughout 4-day dosing schedule. No significant accumulation of buprenorphine after consecutive multiple applications of patch with a 4 day dosing interval was evidenced by the fact that the pharmacokinetic parameters (C_{max}, $AUC_{72\,h}$,) from each patch application were similar to each other and also similar to those of a single patch application. In fact, the accumulation index derived from ratio of C_{max} and $AUC_{72\,h}$ (first to last patch application) was 0.87 and 0.85 (less than 1), respectively, indicating no accumulation of buprenorphine in the body. Furthermore, plasma buprenorphine concentrations declined rapidly to below the lower limit of quantification (except the first patch) at 24 h after removal of patch, suggesting no significant drug accumulation in the skin. Likewise, in the event of patch removal inadvertently or purposefully during 72 h application period, plasma buprenorphine concentration is expected to fall below the lower limit of quantification within 24 h, since buprenorphine concentrations of 0.5–1 ng/ml were maintained for the period of patch application.

The differences in permeability of drugs across skin have been observed between human and animal, which depend on differences in their physiological and biochemical skin structures (62). Many factors such as skin thickness, appendageal openings and lipid content of stratum corneum have been postulated as mechanisms responsible for species differences in TDS. Therefore, feasibility studies for the development of TDS in animals that are similar to human skin are needed for most predictive of transdermal absorption in man. The pig and monkey have been suggested to be the most predictive of percutaneous absorption in man (63), which however varies depending on the physicochemical characteristics of drugs suggesting that there is no general rule to apply (64).

In the present study, when the doses of Buprederm™ to mice (0.24 mg/patch) and rabbits (8.4 mg/patch) selected

based on body surface area and metabolic rate were applied, average plasma levels of buprenorphine (C_{avg}) were 1.4 ng/ml in mice and 0.7 ng/ml in rabbits. On the other hand, steady state plasma concentration of ~0.6 ng/ml is expected in humans based on the ex vivo skin permeation study when clinical dose of Buprederm™ (42 mg/patch) is applied to humans, suggesting that pharmacokinetics of Buprederm™ in humans may well be predicted from rabbit study. Also, the lag time seems to be a little different between mice (1 h) and rabbits (3 h).

The current 4-day dosing regimen (3 days attachment and 1 day detachment) allows administration of multiple doses of Buprederm™ required for the management of chronic pain without drug accumulation, since the steady-state plasma concentration after the first and seventh doses were not statistically different. Therefore, 4-day dosing regimen eliminates the need for frequent dosing, which leads to improved patience compliance compared to the 1-day dosing schedule or other dosing regimen (*i.m.*, *i.v.*, and sublingual). Continuous steady-state administration of BTDS may lead to tolerance. Although the underlying mechanisms for opioid tolerance remain unclear early adaptive processes such as acute receptor desensitization and receptor down-regulation could be crucial (65). Recently, it has shown that buprenorphine regulate μ opioid receptors through down-regulation of μ binding sites (66). Acute administration of buprenorphine induced a large and generalized decrease (62%) in μ opioid binding sites in the rat brain while a less marked down-regulation of μ receptors after chronic administration, thus reducing the likelihood of tolerance development after multiple administration of Buprederm™ with 4-day dosing schedule.

Next, we compared the analgesic effect of Buprederm™ with the standard therapeutic *s.c.* dose of buprenorphine, 0.25 mg/kg and 0.075 mg/kg, using a tail flick test and writhing test in mice, respectively. Over the years, a large body of data on the analgesic effect of buprenorphine in animals has been published, largely in animal models of acute pain (67). Recently, analgesic efficacy of buprenorphine was investigated in a broad panel of rodent models of acute and chronic pain, and showed full analgesic efficacy against persistent/chronic inflammatory and neuropathic pain as well as against acute thermal pain (68). The preclinical testing of drugs in validated pain models should be important for their pharmacological characterization. In contrast to the polymorphic nature of pain described in humans, pain in animals can best be estimated only by examining their reactions to various chemical, thermal, and mechanical stimuli, with the latency or nature of response altered in the "pain" state. The test models for acute pain are easier to perform and to standardize and may also be useful for study of chronic pain. Therefore, antinociceptive activity of Buprederm™ was evaluated using chemical and thermal models of acute pain, acetic acid-induced writhing test and the tail flick test. Such thermal tail-flick tests are most widely and reliably used for revealing the potency of opioid analgesics, useful for predicting analgesic effects in humans (69–70).

Although the magnitude and onset of action varied with the strength of Buprederm™ applied, a prolongation of tail-flick latency was induced in a dose- and time-dependent manner. An increase in dose from 0.24 mg/patch to 0.8 mg/patch increased the maximum level of analgesia. However, increasing the dose above 0.8 mg/patch had little effect on the maximum level of analgesia but instead induced a more rapid onset of pain relief (~3 h). Maximum latency was attained at 6 h for the 0.24 and 0.8 mg/patch and at 3 h for the 2.4 mg/patch, with the analgesic effect maintained for up to 24 h after patch application. The absorption and penetration of buprenorphine through the patch and skin system is mainly by passive diffusion, and the rate of passive membrane diffusion is proportional to the drug's concentration in the patches. Therefore, plasma buprenorphine concentration increased in a linear fashion according to dose and increase in dose decreases the time to reach the maximal effective drug concentration (~10 ng/ml in mice). With high (2.4 mg/patch), medium (0.8 mg/patch) and low (0.24 mg/patch) strength patches, maximal effective plasma concentration as well as maximum latency was attained in about 3, 6 and 6 h, respectively. Increasing the concentration above 10 ng/ml had little effect on the maximum degree of analgesia. In fact, dose response studies of buprenorphine in animals have shown data characterized by flattened or bell-shaped curves. They reveal dose-related increase in efficacy in lower dose, higher doses on the other hand have no greater or even less effects (20–21, 71). Although a bell-shaped response has not always been evident in studies in humans, it may occur at doses above those which are clinically relevant for analgesia. The observed ceiling effect for antinociception may depend on the intensity of the stimulus used to induce pain (72) or may be due to an inherent limitation of the applied tail-flick model such as tail-latencies above the cut-off value. The analgesic effect of Buprederm™ beyond 24 h was not measured due to an increase in the baseline pain threshold caused by the repeated exposure to heat.

The *s.c.* administered buprenorphine·HCl (0.25 mg/kg) induced significant increases in pain threshold at 0.5 to 3 h. However, at 6 h after injection the analgesic activity had rapidly declined to a level similar to that of a low dose of Buprederm™ (0.24 mg/patch) after 24 h. The duration of analgesia induced by *s.c.* administration of buprenorphine was consistent with that reported under comparable testing conditions, where the analgesic activity was significantly reduced at 4 h after injection (20). Our data suggest that Buprederm™ can induce analgesia that is comparable to that induced by repeated subcutaneous injection.

The acetic acid-induced writhing test, a well-established nociceptive test using a chemical stimulus, is known to be more sensitive to opioids than other tests using thermal, mechanical or electrical stimuli (73). Buprederm™ (0.24, 0.8 and 2.4 mg/patch) reduced the acetic acid-induced writhing response significantly compared to the control ($p<0.01$) at 24 h following patch application. The maximal analgesic activity was attained at a dose of 0.8 mg/patch, the same as in the tail flick test. The number of writhing movements was significantly reduced to ~20% of the control at 0.5 h after *s.c.* injection of buprenorphine·HCl as previously reported (74–75).

Finally, the skin irritancy potential of the Buprederm™ was evaluated after single and repeated application according to the Draize's method, and shown to be non-irritant.

CONCLUSION

Buprederm™ developed by Samyang Co. using proprietary hydrogel matrix technology, was shown to achieve the effective buprenorphine concentration in the blood and brain sufficient to maintain an analgesic effect for 72 h with a rapid onset of action. Overall, there was good relationship between the buprenorphine plasma/brain pharmacokinetics and the analgesic effects determined by tail-flick and writhing tests in mice. Moreover, no significant accumulation of buprenorphine or skin irritation was observed after multiple application of Buprederm™ using a 4 day dosing interval.

In conclusion, Buprederm™ is shown to be efficacious, non-irritant to skin and safe upon multiple applications, and therefore, is worthy of further clinical evaluation.

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