J. Pharm. Pharmacol. 2000, 52: 1437–1449
Received March 27, 2000
Accepted July 19, 2000

Transdermal Delivery of the Potent Analgesic Dihydroetorphine: Kinetic Analysis of Skin Permeation and Analgesic Effect in the Hairless Rat

SATOSHI OHMORI, TERUAKI HAYASHI, MASAMI KAWASE, SETSUO SAITO, KENJI SUGIBAYASHI AND YASUNORI MORIMOTO

Faculty of Pharmaceutical Sciences, Josai University, Sakado, Saitama 350-0295, Japan

Abstract

Dihydroetorphine is an extraordinarily strong opioid analgesic. To assess its effectiveness after topical application in hairless rats we have examined the kinetic analysis of skin permeation through excised skin and the in-vitro reservoir effect of skin, and have investigated the predictability of plasma concentration and analgesic effect following in-vivo transdermal application.

Dihydroetorphine was moderately permeable from an aqueous suspension through excised hairless rat skin. Dihydroetorphine flux from drug-dispersed pressure-sensitive adhesive tape was threefold that from the applied aqueous suspension. The fluxes through the abdominal and the dorsal skin during tape application fitted the Fickian diffusion equation well after the tape was removed peeling off the outer layer of the stratum corneum. The relationship between the plasma concentration and the analgesic effect was examined for four different rates of infusion of dihydroetorphine. A non-linear pharmacokinetic disposition was observed. Following abdominal (0·28 cm^2, 20 μg) and dorsal (0·50 cm^2, 35 μg) applications of the dihydroetorphine tape, plasma concentration (0·2–0·8 ng mL^{-1}) and analgesic effect were maintained at a suitable level, for more than 8 h, until removal of the tape. These profiles were predictable using the combined equation for percutaneous absorption, disposition and the analgesic effect, but the analgesic effect was slightly lower than the predicted value.

The results show that it was possible to control the plasma concentration and the analgesic effect of dihydroetorphine by topical application of the analgesic using pressure-sensitive adhesive tape in the hairless rat. It was possible to predict the result using mathematical modelling.

There are many reports on the usefulness of the topical application of opioid analgesics such as morphine (Sugibayashi et al 1989), buprenorphine (Stinchcomb et al 1996) and fentanyl (Southam 1995). Continuous pain relief is necessary for the treatment of illness and for improvement in the quality-of-life for end-stage cancer patients. Transdermal drug delivery is the easiest way for continuous delivery if the drug is adequately permeable through the skin. However, the outer layer of the skin, the stratum corneum, is a significant barrier for the introduction of many drugs (Franz et al 1992). The aforementioned opioid analgesics are reported to be permeable through rat and human skin with the aid of permeation enhancers to produce sufficient pharmacological effects. However, severe enhancers often cause irritation to the skin (Franz et al 1992; Finnin & Morgan 1999). If a more potent opioid analgesic was used for this therapeutic system, it may be possible to achieve continuous relief of severe pain without the aid of enhancers.

The novel opioid analgesic dihydroetorphine (Figure 1) has an analgesic effect more than 1000-fold stronger than morphine (Bentley & Hardy 1967; Tokuyama et al 1993). Dihydro-etorphine began to be used clinically for pain relief in China in the 1980s (Wang et al 1999; Zang 1999), but its abuse increased soon after it was marketed because of its dependent liability under unrestricted control

Correspondence: Y. Morimoto, Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan.

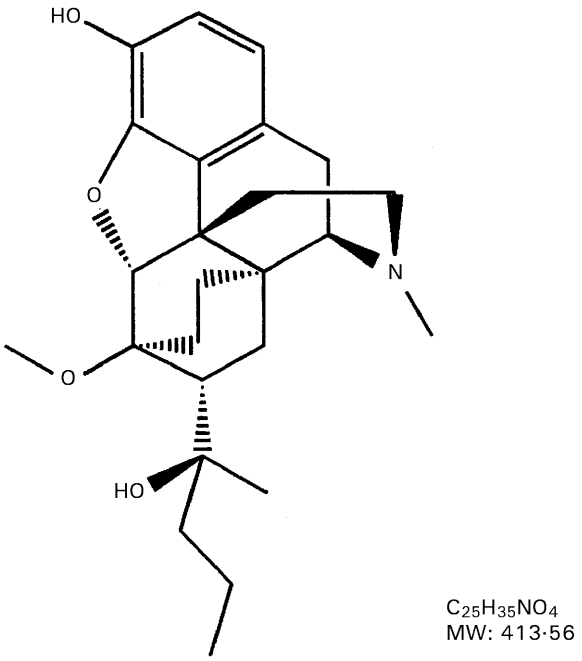

Figure 1. Chemical structure of dihydroetorphine.

(WHO Expert Committee 1999). In 1999, the United Nations registered dihydroetorphine as one of the most strictly controlled narcotic drugs.

Dihydroetorphine can be administered intravenously or sublingually. A sublingual dose produces a quick onset of analgesic effect (within 20 min), but its duration is relatively short (within 1·5–5·3 h) after dosing (Wu & Sun 1991). Therefore, it has to be administered several times per day for continuous pain relief.

In a previous study (Ohmori et al 2000b), it was confirmed that dihydroetorphine was quickly distributed to the central nervous system (< 2 min) and was eliminated smoothly (half-life 37·7 min) after an intravenous injection in hairless rat. Furthermore, after intracutaneous administration dihydroetorphine was highly available (bioavailability $> 0{\cdot}7$) and produced a quick-onset of analgesic effect (< 5 min), unlike the availability after oral administration which was low (bioavailability $< 0{\cdot}004$) due to extensive first-pass metabolism. Its rapid efficacy and elimination potential make it feasible for input-phase controlled drug delivery. In addition, its potency and some physiological properties (melting point and lipophilicity) are suitable for transdermal delivery (Finnin & Morgan 1999).

Guo et al (1997) reported in-vitro snake skin permeation of dihydroetorphine using the permeation enhancer Azone. Chen et al (1996) reported that the transdermal delivery of dihydroetorphine using a drug-reservoir system with Azone showed a stable blood concentration and analgesic effect for 32 h in Wistar rats. However, Azone has a significant permeation enhancing effect and leads to skin irritation (Okamoto et al 1988). Etorphine, structurally similar to dihydroetorphine but with a potency one-quarter of dihydroetorphine, was permeable through mice and cadaver skin without permeation enhancers, but to accomplish the daily dose an application area of 50 cm^2 was required (Jolicoeur et al 1992). If dihydroetorphine has an equivalent permeability to etorphine, its application area would be smaller which would enable practical use.

In this study we have kinetically analysed the skin permeation and reservoir effect of dihydroetorphine in excised abdominal and dorsal skin of hairless rat in-vitro. We have compared fluxes through the skin from aqueous suspension and drug-dispersed pressure-sensitive adhesive tape in-vitro. We have estimated the effective plasma concentration range of dihydroetorphine using various rates of infusion in hairless rat in-vivo. We have evaluated also the plasma concentration and analgesic effect following topical application of dihydroetorphine tape, and their predictability by mathematical calculation in-vivo.

Materials and Methods

Animals

Male hairless rats (WBN/ILA-Ht strain) (250–350 g; 12–16-weeks old) were supplied by Life Science Research Center of Josai University (Saitama, Japan) and Ishikawa Experimental Animal Laboratory (Saitama, Japan). The animals were maintained at $24 \pm 1^{\circ}C$ under a 12-h light–dark cycle and had free access to a standard rodent diet and clean drinking water. The experiments were performed in accordance with the Guide for Laboratory Animal Experiment adopted by Josai University.

Chemicals

Free base dihydroetorphine was synthesized from codeine by reported procedures (Bentley & Hardy 1967; Barber & Rapoport 1975). Styrene–isoprene-styrene block co-polymer (Cariflex TR-1107) was purchased from Shell Chemical Co. (Tokyo, Japan). Rosin ester (KE-311) was purchased from Arakawa Chemical Co. (Osaka, Japan). Isopropyl myristate and p-hydroxybenzoic acid methyl ester were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). Trypsin was purchased from Wako

Pure Chemical Co. (Kyoto, Japan). Buprenorphine hydrochloride was kindly supplied by Otsuka Pharmaceuticals (Tokyo, Japan). All other reagents were of analytical grade.

Preparation of dihydroetorphine aqueous suspension

Excess amounts of dihydroetorphine and isopropyl myristate were added to distilled water and were dissolved sufficiently by sonication. The mixture was left overnight. The undissolved drug and isopropyl myristate were separated just before use.

Preparation of pressure-sensitive adhesive tape

The matrix-type pressure-sensitive adhesive tape was prepared by the casting method (Morimoto et al 1992a). Dihydroetorphine, styrene–isoprene-styrene block co-polymer, rosin ester and isopropyl myristate were dissolved in chloroform, and then the mixture was gently stirred at ambient temperature. The mixture was cast onto polyethylene terephthalate film as the backing layer using Baker's Film Applicator (Ueshima Seisakusho, Tokyo, Japan). The adhesive tape was kept at 60°C for 15 min and then at ambient temperature for 16 h to evaporate the chloroform. The tape was then covered with siliconized polyethylene terephthalate film as a release liner. The tape was stored at ambient temperature until use. Immediately before application, the tape was pulled out circularly to an appropriate area. The thickness of the adhesive layer of the tape was 70 μm. Dihydroetorphine content of the tape was 66 $\mu g\,cm^{-2}$, and at least 80% of the drug in the tape was dispersed when observed under a light microscope. A placebo tape was prepared by the same method excluding the dihydroetorphine.

Skin preparation

The rat abdominal and dorsal regions were carefully shaved, and skin excised under diethylether anaesthesia. Subcutaneous tissue was carefully removed from the skin (intact skin). Stripped skin was obtained by stripping the stratum corneum from the skin with adhesive cellophane tape (Nichiban Co., Tokyo, Japan) 20 times. The dorsal split skin was prepared by cutting off the dermis side of the dorsal skin using a cryostat microtome. The thickness of each skin preparation was measured with a slide calliper. Other pieces of abdominal and dorsal skin (16 cm^2) were immersed in 1% trypsin at 37°C for 24 h, and then the stratum corneum was carefully isolated. After sufficient rinsing and desiccation, the isolated stratum corneum of both skins was weighed.

In-vitro skin permeation studies

In the aqueous suspension application study, the excised skin preparations were inserted between two halves of side-by-side diffusion cells encompassed by a 37°C water jacket with a volume of 2·5 mL and 0·95 cm^2 effective diffusion area. As a pretreatment of vehicles, isopropyl myristate saturated solution and distilled water were filled into the donor and receiver compartments of the diffusion cell which were in contact with the stratum corneum side and dermis side of the skin, respectively. Applied vehicles in each compartment were stirred with a magnetic bar driven by a constant-speed synchronous motor (Scinics, Tokyo, Japan). After the overnight-pretreatment, donor and receiver solutions were replaced by dihydroetorphine aqueous suspension and fresh distilled water, respectively, and then sampling of the receiver solution was started as described below. In the tape application study, the dermis side of the skin was attached to one-half of the side-by-side diffusion cells. The dihydroetorphine tape was applied to the stratum corneum side of the skin, and then distilled water was filled into the receiver compartment of the diffusion cell. In both application studies, the receiver solution was withdrawn at appropriate times for measurement of the permeated drug, and distilled water was added to maintain a constant volume in the diffusion cell. The suspension and tape were removed from the skin at appropriate times, and the receiver solution was sampled for evaluation of the reservoir capacity in the skin preparation.

Intravenous infusion of dihydroetorphine

Rats were anaesthetized with diethylether. A femoral vein was cannulated with polyethylene tubing for infusion of dihydroetorphine and a femoral artery was cannulated for blood sampling. After surgery each rat was placed in a Bollman cage and allowed to recover from the anaesthetic for 2 h. After the basal analgesic effect was measured, dihydroetorphine in saline was infused at 0·9, 1·8, 3·6 and 9·0 $\mu g\,h^{-1}\,kg^{-1}$ for 4 h through the venous cannula (0·5 $mL\,min^{-1}$). Analgesic effects were measured at appropriate times during and after the infusion, and in succession with each effect measurement, blood was withdrawn from the arterial cannula. To determine the lowest concentration of dihydroetorphine a 1-mL blood sample was required, whereas a blood sample less than

1 mL was enough to determine the higher concentration (Ohmori et al 2000a). Therefore, the blood sampling was set at five to nine times per animal dependent on the predicted concentration in the samples, with the total volume of blood samples not exceeding 1% of the body weight. Blood samples were placed in heparinized tubes and the plasma was separated by centrifugation. The brain was excised after decapitation, and then the cerebellum was removed from the brain. The brain was homogenized with 2 vols methanol. Supernatant of the homogenate was separated by centrifugation (9000 *g*) at 4°C. Plasma and supernatant samples were stored at −20°C until analysis.

In-vivo application of dihydroetorphine tape on skin

Rats were anaesthetized with diethylether and a femoral artery was cannulated with polyethylene tubing for blood sampling. The tip of the cannula was drawn through the skin on the back of the neck so that the awakening rats were able to move freely. After surgery the animals were allowed to recover from the anaesthesia for 2 h. After the basal analgesic effect was measured, dihydroetorphine tape was applied onto the abdominal skin (0·28 cm^2, 20 μg drug) for 8 h or onto the dorsal skin (0·50 cm^2, 35 μg drug) for 24 h. Analgesic effects were measured at appropriate times during and after the application of the tape, and in succession with each effect measurement, blood was withdrawn from the arterial cannula. Blood sampling was set at four to six times per animal, with the total volume of blood samples not exceeding 1% of the body weight. Plasma was separated by the same manner as in the infusion study and was stored at −20°C until analysis.

Measurement of dihydroetorphine concentrations in the receiver solution

A sample of the receiver solution (100 μL) was mixed with an equal volume of p-hydroxybenzoic acid methyl ester as an internal standard (10 μg mL in acetonitrile). After centrifugation (9000 *g*), the supernatant (20 μL) was applied to a high-performance liquid chromatography system (LC-10A, Simadzu Co., Kyoto, Japan). The phosphate buffer (1/30 M, pH 5·2)–acetonitrile–methanol (70 : 21 : 9) mobile phase was delivered at 1·0 mL min^{-1} through Nucleosil $5C_{18}$ (4·6 mm i.d. × 250 mm, Macherey Nagel, Germany) under 40°C. Dihydroetorphine and internal standard were detected at 215 nm.

Measurement of dihydroetorphine concentrations in rat plasma and brain

Dihydroetorphine concentrations in the plasma and brain supernatants were measured by liquid chromatography–tandem mass spectrometry (Ohmori et al 2000a). Briefly, an exact volume less than 0·5 mL of the sample was mixed with 0·1 mL buprenorphine methanol solution as an internal standard (10 ng mL^{-1} in methanol) and 50 mM phosphate buffer (pH 6·0). These mixtures were applied to a Bond Elut Certify cartridge column (3 mL/130 mg, Varian, Harbor City, CA) that had been conditioned with methanol and 50 mM phosphate buffer (pH 6·0). The column was washed sequentially with 100 mM acetic acid and methanol. The eluent with 2% ammonium hydroxide in ethyl acetate was collected and evaporated. The residue was dissolved in acetonitrile–water (80 : 20) and a sample was applied to a LC-MS-MS system (API-300, Perkin Elmer-SCIEX, Foster City, CA). An acetonitrile–50 mM ammonium acetate (95 : 5) mobile phase was delivered at 0·3 mL min^{-1} through Inertsil ODS-2 (5 μm, 2·1 mm i.d. × 150 mm, GL Science, Tokyo, Japan) under 40°C. The analytical conditions of API-300 were the same as reported by Ohmori et al (2000a). The limit of quantitation with acceptance criteria as intra- and inter-assay precision within 20% was 0·05 ng mL^{-1} in plasma and 0·15 ng g^{-1} in brain.

Measurement of analgesic effect

The analgesic effect was determined using the tail-immersion test (Ouellet & Pollack 1997). In the dihydroetorphine infusion study the tail of the rat was extended from the Bollman cage. In the transdermal application study using the dihydroetorphine tape, rats were loosely wrapped in a cloth from which the tail was extended. Two-thirds of the tail was immersed in hot water (50–55°C) and the latent time for flick of the tail or struggle was measured. Basal latency was approximately 2 s and did not exceed 3 s. A cut-off time of 10 s was adopted to prevent tail damage. Analgesic effect was expressed as % of the maximum possible effect (% MPE): % MPE = (post-drug latency − pre-drug latency)/(cut-off latency − pre-drug latency) × 100.

Calculation of kinetic parameters

Figure 2 shows the scheme of the mathematical model for skin permeation, disposition and the analgesic effect of dihydroetorphine.

Parameters for skin permeation. It was postulated that dihydroetorphine permeation through the

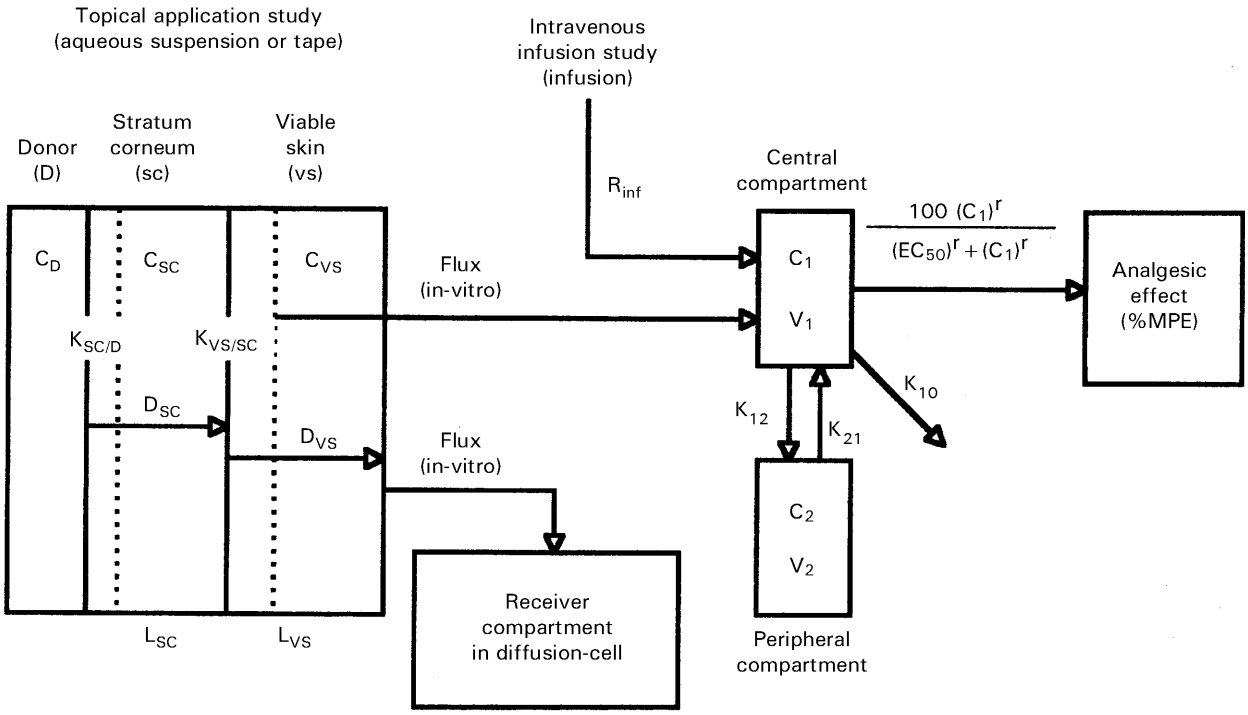

Figure 2. Scheme of the mathematical model for skin permeation, disposition and production of the analgesic effect. C_D, C_{SC}, C_{VS}, C_1, C_2, dihydroetorphine concentrations in each layer or compartment; $K_{SC/D}$, $K_{VS/SC}$, partition coefficient of dihydroetorphine between layers; D_{SC}, D_{VS}, diffusivity of dihydroetorphine in each layer; L_{SC}, L_{VS}, thickness of each layer; R_{inf}, infusion rate of dihydroetorphine; Vd_1, Vd_2, volume of distribution in each compartment; k_{12}, k_{21}, k_{10}, first-order rate constant for transfer between compartments and elimination of dihydroetorphine; EC50, dihydroetorphine concentration producing 50% of the maximum effect; r, sigmoidicity factor. In the topical application study, the drug in the donor enters the receiver compartment of the diffusion-cell in-vitro and enters the central compartment of the body in-vivo through the skin. The dotted line in the stratum corneum indicates the outer layer that peels off with the removal of the donor. The dotted line in viable skin indicates the drug that enters into the blood vessel (central compartment) in-vivo. In the intravenous infusion study, dihydroetorphine directly entered the central compartment in the body in-vivo.

stratum corneum (sc) and viable skin (vs) occurred in accordance with the mono- or bi-layer diffusion model based on Fick's Second Law without any specific binding and/or enzymatic degradation of the drug in each skin layer (Tojo 1987). Dihydroetorphine concentrations in stratum corneum (C_{SC}) and viable skin (C_{VS}) at a position x and at time t, are represented as follows:

$$aC_{SC}/at = D_{SC}a^2C_{SC}/ax^2 \quad (1)$$

$$aC_{VS}/at = D_{VS}a^2C_{VS}/ax^2 \quad (2)$$

where D_{SC} and D_{VS} are diffusivity in each layer of skin. Initial and boundary conditions in the skin were set as follows:

$$\text{at } t = 0 \text{ and } 0 \le x \le L_{SC} + L_{VS}, C_{SC} = C_{VS} = 0 \quad (3)$$

$$\text{at } t > 0 \text{ and } x = 0, C_{SC} = K_{SC/D}C_D \quad (4)$$

$$\text{at } t > 0 \text{ and } x = L_{SC}, C_{VS} = K_{VS/SC}C_{SC} \text{ and } D_{SC}dC_{SC}/dx = D_{VS}\,dC_{VS}/dx \quad (5)$$

$$\text{at } t > 0 \text{ and } x = L_{SC} + L_{VS}, C_{VS} = 0 \quad (6)$$

where L_{SC} and L_{VS} are the thickness of each layer of skin, C_D is the effective drug concentration in the donor compartment (D) as a solubility of the drug in the suspension and in the tape, and $K_{SC/D}$ and $K_{VS/SC}$ are the partition coefficients of dihydroetorphine between layers. The flux of dihydroetorphine through the skin into the receiver compartment in the diffusion cell, j, is represented as follows:

$$j = -D_{VS}(dC_{VS}/dx)_{x=Lsc+Lvs} \quad (7)$$

Partial differential equations 1 and 2, and ordinary differential equations 5 and 7, are approximated by the explicit finite-difference equation (Crank 1975). The number of spatial mesh in each layer was 10, which satisfied the degree of accuracy in this study.

The fluxes before removing the donor were fitted to equation 7 by the weighted least-square method

using a quasi-Newton algorithm on solver-function of Microsoft Excel 97. Initially, the flux through the stripped skin was fitted to equation 7 combined with the mono-layer diffusion model, a more simple case than the equations described above, by using fixed L_{VS}, and thereby $K_{VS/D}C_D$ and D_{VS} were estimated. Secondly, the flux through the intact skin was fitted to equation 7 combined with the bi-layer diffusion model by using a fixed value for L_{VS}, $K_{VS/D}C_D$, D_{VS} and L_{SC}, and substituting $K_{VS/SC}$ by $K_{VS/D}C_D/K_{SC}/DC_D$, and thereby $K_{SC/D}C_D$ and D_{SC} were estimated. The flux at steady state and the lag time for the quasi-steady state were calculated from theoretical values by Fick's First Law (Franz et al 1992).

Pharmacokinetic parameters. Pharmacokinetic parameters of dihydroetorphine in the infusion study were calculated by fitting a linear two-compartment model with a zero-order-rate input function, equation 8, to the mean plasma concentration, Cp.

$$Cp = \frac{R_{inf}}{Vd_1(\alpha-\beta)} \times \left[\frac{k_{21}-\alpha}{\alpha}\{\exp(-\alpha t_1)-1\}\exp(-\alpha t_2) + \frac{\beta-k_{21}}{\beta}\{\exp(-\beta t_1)-1\}\exp(-\beta t_2)\right] \quad (8)$$

where R_{inf} and Vd_1 are the infusion rate of the drug and the distribution volume of the central compartment, respectively. α and β are the slopes of distribution and elimination phase on the logarithmic plasma concentration–time curve, respectively, and k_{21} is the first-order rate constant for transfer from the peripheral- to the central-compartment. t_1 and t_2 are the time during infusion and after infusion, respectively.

Pharmacodynamic parameters. Pharmacodynamic parameters were calculated by fitting a sigmoidal effect–concentration relationship, equation 9, to the mean plasma concentration and the mean % MPE in each infusion group.

$$\%\,MPE = 100 \times (Cp)^r/((EC50)^r + (Cp)^r) \quad (9)$$

where the maximum effect (MPE) is 100, EC50 is the dihydroetorphine concentration producing 50% of MPE and r is the sigmoidicity factor describing the shape of the relation curve.

Simulation of percutaneous absorption, plasma concentration and analgesic effect

The release profiles after the donor was removed in-vitro and the time course of plasma concentration and analgesic effect during and after the topical application of the tape in-vivo were simulated by numerical solution combined with skin permeation, disposition and the analgesic effects. After the donor was removed from the intact or stripped skin, the boundary condition as in equation 4 was expressed as follows (Tojo 1988):

$$\text{at } t > 0 \text{ and } x = 0,\ dC_{SC}/dx = 0 \text{ or } dC_{VS}/dx = 0, \text{ respectively} \quad (10)$$

On the assumption that the outer layer of the stratum corneum is peeled off by removing the donor, several mesh on finite-difference approximation were deleted from the outer layer of the stratum corneum, so that equation 10 was held at the appropriate position x, as an integral number, not zero. Half-life of the flux after removing the donor was calculated from the theoretical flux by the log-linear regression.

Results

In-vitro skin permeation of dihydroetorphine from an aqueous suspension

Figure 3 shows the flux and the cumulative amount of dihydroetorphine that permeated through the excised abdominal skin from the aqueous suspension. The flux gradually increased after the application of the suspension and reached a steady-state level within a few hours. The steady-state flux through the stripped skin was 3·6-fold higher than that of the intact skin. These fluxes and cumulative amounts during the suspension application fitted well the mono- or bi-layer diffusion model for stripped and intact skin, respectively (solid lines in Figure 3). The obtained kinetic parameters are listed in Table 1. Initial concentrations at the boundary between the donor solution and stripped skin or intact skin, $K_{SC/D}C_D$ or $K_{VS/D}C_D$, were approximately the same. However, the diffusivity, D_{VS}, was 110-fold higher than D_{SC}. After the suspension was removed at 8 h (vertical dotted lines in Figure 3), the flux decreased exponentially which was well predicted by the diffusion model with equation 10 at position $x = 0$ (solid lines in Figure 3). Half-lives of the flux from the intact and stripped skin after removing the suspension were approximately the same (Table 1).

In-vitro skin permeation of dihydroetorphine from the tape

Figure 4 shows the flux and cumulative amount of dihydroetorphine that permeated through the

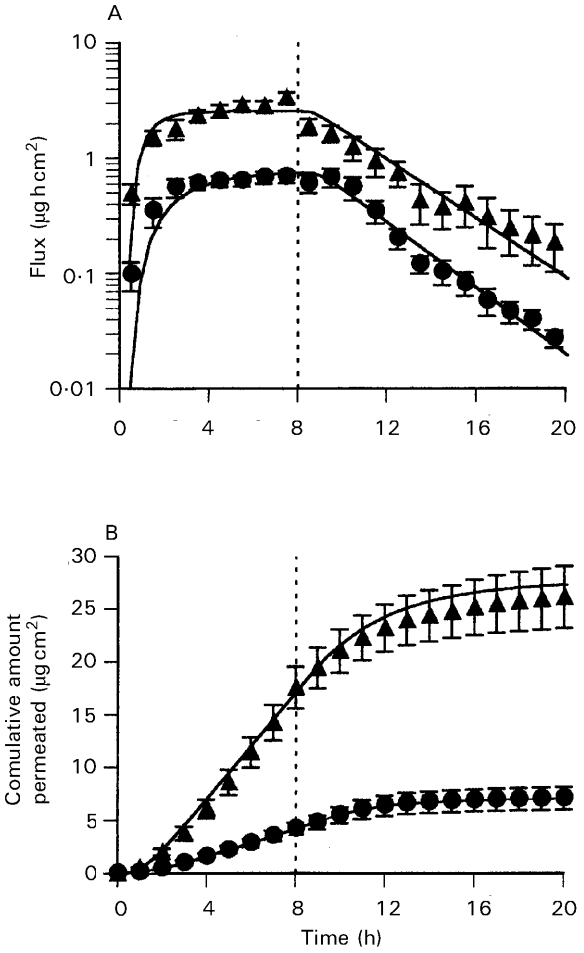

Figure 3. In-vitro permeation of dihydroetorphine from suspension through the excised abdominal skin of hairless rats. Each point represents the mean and standard error (n = 4). ●, Intact skin; ▲, stripped skin. Vertical dotted lines indicate the time of removal of the donor solution. Solid lines indicate the calculated mean values obtained by fitting to the mono- or bilayer diffusion model.

excised abdominal and dorsal skin preparations from the tape formulation. The steady-state flux through the intact dorsal skin was one-half of that through the intact abdominal skin. The flux was 3·1-fold higher than that after the application of the suspension. The kinetic parameters calculated from the diffusion models are listed in Table 1. Dry weight of the stratum corneum of the dorsal skin was 2·8-fold higher than that of the abdominal skin, so that the thickness of these skins was estimated as 15·9 μm for abdominal skin and 45·8 μm for dorsal skin. The $K_{SC/D}C_D$ for abdominal and dorsal skin was 11–47-fold higher than that of the suspension applied, although $K_{VS/D}C_D$ was the same order. Although the difference between D_{VS} values for abdominal and dorsal skin was less than 2, D_{SC} for dorsal skin was sixfold that of abdominal skin. In the stripped skin, the release profiles and cumulative amount permeated following the removal of the tape were well predicted using the diffusion model with equation 10 at position $x = 0$ (solid lines in Figure 4). In intact skin the release profiles were preferably described by the model with equation 10 at position $x = 0{\cdot}6\ L_{SC}$ for abdominal skin and $x = 0{\cdot}7\ L_{SC}$ for dorsal skin (dashed lines in Figure 4). This postulated whether the outer six or seven layers of the stratum corneum were peeled off when removing the tape. It was visible to peel off of several outer layers of the skin when removing the tape. Half-lives of the theoretical flux calculated with peeling off the stratum corneum significantly decreased from that calculated without peeling off the stratum corneum in the abdominal and dorsal skin. To confirm the suitability of these models, the simulation of the flux and cumulative amount through the dorsal split skin by using kinetic parameters (Table 1) was determined. It was possible to predict them satisfactorily from the diffusion model supposing that the peeling off of the stratum corneum by removing the tape occurred at position $x = 0{\cdot}4\ L_{SC}$ on the dorsal split skin (dashed line in panel C and D of Figure 4). The difference between the peeled position on the dorsal intact skin and split skin might be caused by the application period of the tape in-vitro.

Plasma concentration and analgesic effect by infusion of dihydroetorphine

Figure 5 shows the plasma and brain concentrations and analgesic effect during and after 4-h-continuous infusion of dihydroetorphine at rates of 0·9, 1·8, 3·6 and 9·0 $\mu g\,h^{-1}\,kg^{-1}$. Pharmacokinetic and pharmacodynamic parameters are listed in Table 2. Steady-state plasma concentrations increased with the infusion rate from 0·9 to 3·6 $\mu g\,h^{-1}\,kg^{-1}$. At the 9·0 $\mu g\,h^{-1}\,kg^{-1}$ infusion, however, the plasma concentration promptly rose. As a result of the pharmacokinetic analysis, the volume of distribution of the central compartment apparently decreased at the 9·0 $\mu g\,h^{-1}\,kg^{-1}$ infusion. The rats were severely anaesthetized and their respiration was extremely weak, suggesting that an abnormal disposition of dihydroetorphine occurred. Dihydroetorphine indicated the linear disposition below 1·2 $ng\,mL^{-1}$ of plasma concentration. However, the dihydroetorphine concentration in the brain was approximately threefold that in the plasma even at 9·0 $\mu g\,h^{-1}\,kg^{-1}$ (Figure 5, open symbols, concentration data). The analgesic effects increased with the infusion rate, and reached 100% maximum effect at 3·6 $\mu g\,h^{-1}\,kg^{-1}$ infusion. The mean EC50

Table 1. Kinetic parameters for the skin permeation of dihydroetorphine through the excised skin of hairless rats.

Skin preparation	Thickness (μm)			Initial concn (mg cm^{-3})		Diffusivity (cm^2 h^{-1})	
	L_{SC}	L_{VS}[a] (in-vitro)	L_{VS} (in-vivo)	$K_{SC/D}C_D$[b]	$K_{VS/D}C_D$[c]	D_{SC}[b]	D_{VS}[c]
Abdominal skin (suspension)							
Stripped	—	550	—	—	0·350	—	$3{\cdot}97 \times 10^{-4}$
Intact	15·4[e]	550	—	0·386	(0·350)	$4{\cdot}42 \times 10^{-6}$	($3{\cdot}97 \times 10^{-4}$)
Abdominal skin (tape)							
Stripped	—	443	—	—	0·532	—	$8{\cdot}23 \times 10^{-4}$
Intact	15·4[e]	578	154[g]	18·3	(0·532)	$2{\cdot}81 \times 10^{-7}$	($8{\cdot}23 \times 10^{-4}$)
Dorsal skin (tape)							
Stripped	—	1205	—	—	0·348	—	$1{\cdot}47 \times 10^{-3}$
Intact	43·4[f]	1437	434[g]	4·24	(0·348)	$1{\cdot}69 \times 10^{-6}$	($1{\cdot}47 \times 10^{-3}$)
Split[d]	43·4[f]	499	—	(4·24)	(0·348)	($1{\cdot}69 \times 10^{-6}$)	($1{\cdot}47 \times 10^{-3}$)

Skin preparation	Steady state flux[h] (μg h^{-1}cm^{-2})	Lag time for quasi-steady state[h] (h)	Half-life of flux after removal of the donor[h] (h)	
			Without peeling of stratum corneum	With peeling of stratum corneum
Abdominal skin (suspension)				
Stripped	2·52	1·23	2·36	—
Intact	0·70	2·40	2·10	—
Abdominal skin (tape)				
Stripped10^{-4}	9·81	0·38	0·74	—
Intact	2·14	3·06	5·47	2·14
Dorsal skin (tape)				
Stripped	4·16	1·52	3·05	—
Intact	1·07	6·97	8·90	4·52
Split[d]	1·42	2·96	—	2·68

[a]Measured by slide calliper. [b]Parameters for the stratum corneum were obtained by fitting the bi-layer diffusion model to the mean values of the dihydroetorphine flux through the intact skin using fixed parameters for viable skin given in parenthesis. [c]Parameters for viable skin were obtained by fitting mono-layer diffusion model to the mean dihydroetorphine flux through the stripped skin. [d]Parameters obtained from the intact skin data were used for simulation. [e]Sato et al (1991). [f]Calculated from the thickness of abdominal stratum corneum corrected by the dry-weight ratio of isolated dorsal stratum corneum (2·79 mg cm^{-2})/abdominal stratum corneum (0·99 mg cm^{-2}). [g]Calculated from 10 times the thickness of stratum corneum. [h]Parameters were calculated from theoretical values illustrated in Figures 3 and 4.

and r, calculated using equation 9, were 0·708 ng mL^{-1} and 3·03, respectively.

Prediction of the plasma concentration and analgesic effect following topical application of dihydroetorphine tape

After abdominal (0·28 cm^2, 20 μg) and dorsal (0·50 cm^2, 35 μg) tape application, the plasma concentration and analgesic effect increased to the effective concentration until tape removal (Figure 6). The plasma concentration during the application of the tape was similar to the predicted value calculated using the combined equation. However, the analgesic effects were slightly lower than the predicted values.

Discussion

Dihydroetorphine shows several suitable properties for transdermal delivery, as summarized by Finnin & Morgan (1999): "the daily systemic dose should be $\leq$20 mg", the sublingual dose of dihydroetorphine is $\leq$160 μg (Li et al 1996); "the molecular weight should be $<$500 Da", dihy-

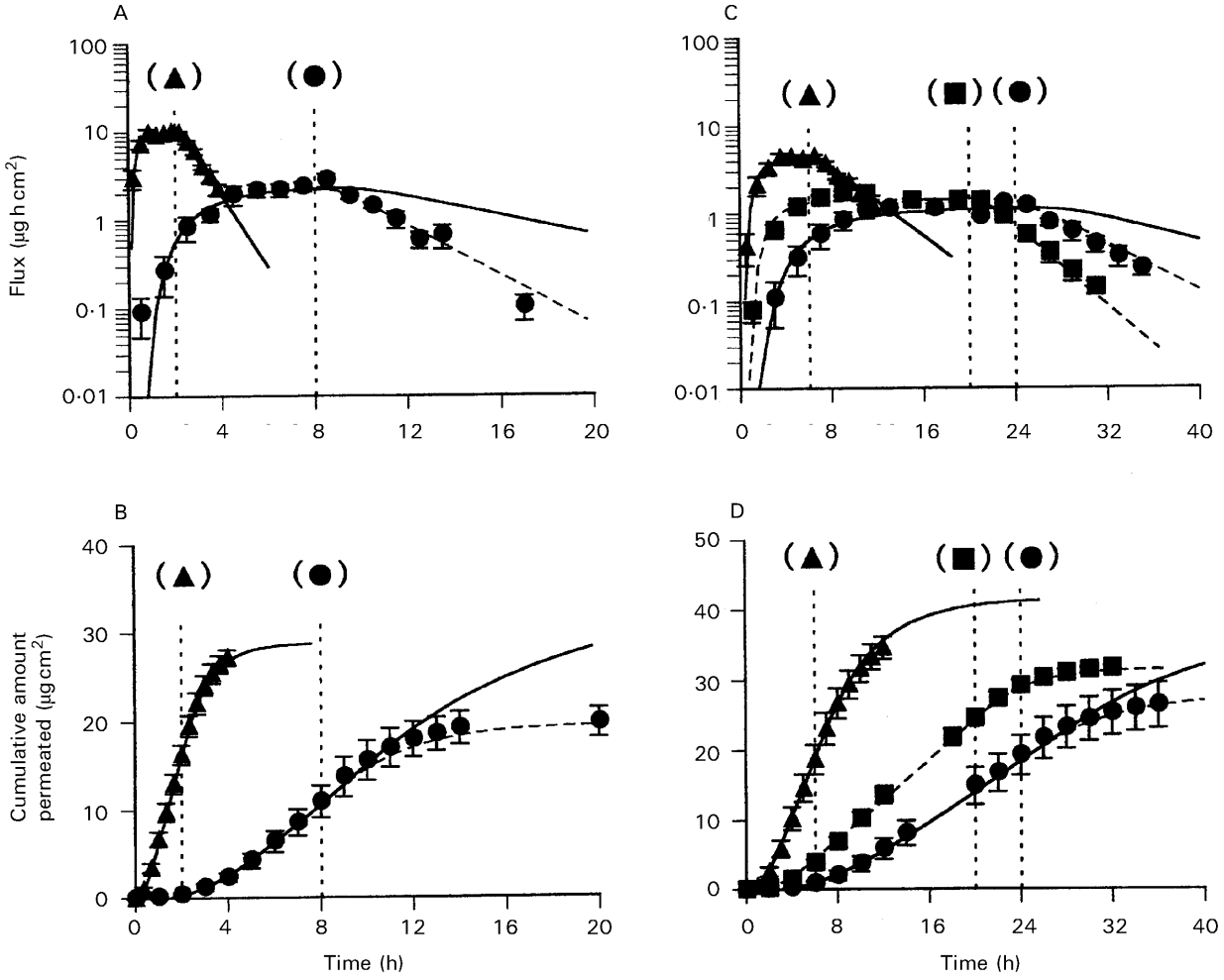

Figure 4. In-vitro permeation of dihydroetorphine from the tape formulation through the excised abdominal (A, B) and dorsal (C, D) skin of hairless rats. Each point represents the mean and standard error (n = 4). ●, Intact skin; ▲, stripped skin; ■, split skin. Vertical dotted lines indicate the time of removal of the tape. Solid lines indicate the calculated mean values obtained by fitting to the mono- or bi-layer diffusion model. Dashed lines indicate the calculated mean values predicted by the diffusion model after peeling off the outer layer of the stratum corneum when removing the tape.

droetorphine is 413 Da; "the log P (octanol/water) should be in the range 1–3", log P is 2·13 for dihydroetorphine (unpublished data); "the melting point should be $< 200°C$", the melting point is 205°C for dihydroetorphine (Bentley & Hardy 1967); "direct skin irritation should not be presented", only a slight irritation is observed for dihydroetorphine with the permeation enhancer Azone (Chen et al 1996). Finnin & Morgan mentioned another two factors that are required: "hydrogen-bonding groups should be ≤ 2", dihydroetorphine has five groups, and "immunogenicity should not be presented", this has not yet been proved.

In this study, it was confirmed that dihydroetorphine was permeable enough through hairless rat skin to produce an analgesic effect. Using drug-dispersed pressure-sensitive adhesive tape, the flux of dihydroetorphine through the intact abdominal skin was enhanced threefold with a 47-fold increase of the initial concentration in stratum corneum and a 16-fold decrease of D_{SC} in comparison with application of suspension with saturated isopropyl myristate (Table 1). These results are different from our previous report (Morimoto et al 1992a) that the flux of isosorbide dinitrate was the same between isopropyl myristate saturated aqueous suspension and pressure-sensitive adhesive. It was suggested that hydration of the stratum corneum caused a decrease of solubility and an increase of diffusion resistance of dihydroetorphine owing to its hydrophobicity (log P 2·13) dissimilar to the more hydrophilic isosorbide dinitrate (log P 1·34) (Morimoto et al 1992b). Isopropyl myristate has been used widely as a vehicle in cosmetic and pharmaceutical formulations because of its emollient properties and safety (Suh & Jun 1996). In this study, it was used as an emollient additive rather than a permeation enhancer because its enhancing

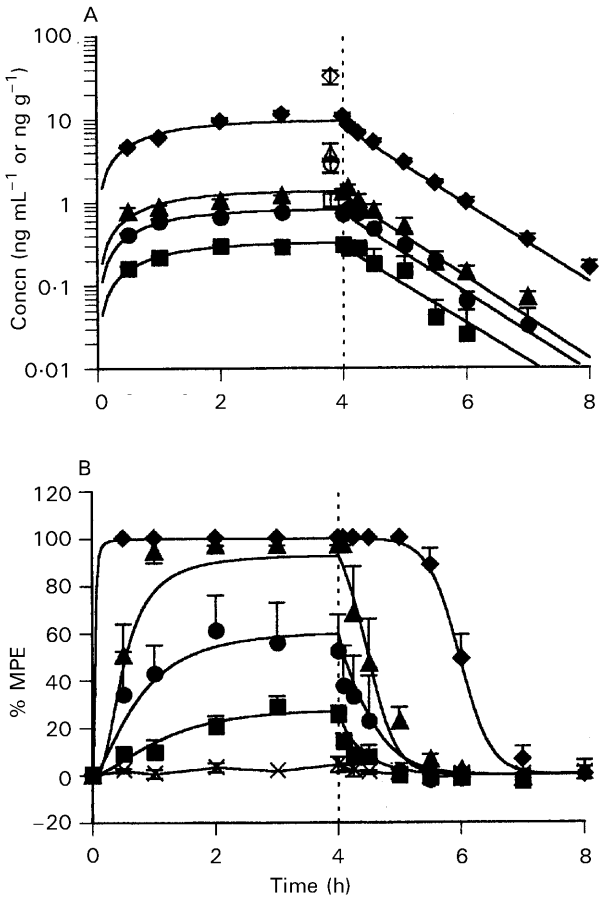

Figure 5. Dihydroetorphine concentrations in plasma and brain, and analgesic effect (% maximum effect; % MPE) during and after 4-h continuous infusion in hairless rats. Each point represents the mean and standard error (n = 4-5). ■□, 0·9 μg h^{-1} kg^{-1}; ●○, 1·8 μg h^{-1} kg^{-1}; ▲△, 3·6 μg h^{-1} kg^{-1}; ◆◇, 9·0 μg h^{-1} kg^{-1}; ×, saline infusion (1 mL h^{-1}). Closed and open symbols of the concentration data indicate dihydroetorphine concentrations in the plasma and brain, respectively. Vertical dotted lines indicate the termination time of infusion. Solid lines indicate the calculated mean values obtained by fitting to the linear two-compartment model with zero-order input and the sigmoidal effect-concentration equation, respectively.

ratio of dihydroetorphine permeation was less than 2 (Ohmori et al 2000c).

From microscopic observations of the various contents of the dihydroetorphine tape, at least 80% of the drug was dispersed in the tape. The flux of dihydroetorphine was stable for 8 and 24 h until the tape was removed from the abdominal and the dorsal intact skin, in which cumulative amounts transferred into the skin were nearly 30 μg cm^{-2}, more than 40% of the contents in the tape (Figure 4B and D). Therefore, it is postulated that the boundary concentrations on the skin surface were regular during these periods. If the tape was applied for a longer time, the fluxes might continue until the dispersed drug was lost.

Steady-state flux of dihydroetorphine through the dorsal skin was one-half of that through the abdominal skin (Table 1). We suggested that this discrepancy was caused by the difference in thickness of the stratum corneum. As the result of the kinetic analysis, the diffusivity of dihydroetorphine in the stratum corneum of dorsal skin was sixfold that of abdominal skin. Bronaugh et al (1983) reported that the permeability of water, urea and cortisone in a water vehicle through the abdominal skin of male Osborne-Mendel rats was 3-10-fold that of dorsal skin, dependent on a 2·5-fold difference in the skin thickness as 13·8 μm of abdominal and 34·7 μm of dorsal skin, but there was no significant difference in the lag time. Although several authors have investigated the permeation properties and pharmacological effects following drug application to different sites of the skin, the relation of site-dependency to the permeation rate of the drug should be considered. In our previous report (Morimoto et al 1992b), the permeability coefficient of drugs with moderate lipophilicity, such as dihydroetorphine (log P nearly 2), through human skin was slightly lower than that of rat abdominal skin. This suggests that the dihydroetorphine permeation rate through

Table 2. Pharmacokinetics and pharmacodynamic parameters of dihydroetorphine in hairless rats.

Infusion rate (μg h^{-1} kg^{-1})	Pharmacokinetic parameters[a]				Pharmacodynamic parameters[b]	
	α (h^{-1})	β (h^{-1})	k_{21} (h^{-1})	V_1 (L kg^{-1})	EC50 (ng mL^{-1})	r
0·9	10·4	1·05	5·32	1·35	0·496	2·30
1·8	10·0	1·09	5·38	1·09	0·679	2·28
3·6	10·4	1·13	5·68	1·30	0·691	3·83
9·0	11·5	1·06	4·90	0·38	0·966	3·69
Mean	10·3[c]	1·09[c]	5·46[c]	1·24[c]	0·708	3·03

[a]Parameters were obtained by fitting the linear two-compartment model with zero-order input to the mean dihydroetorphine concentration during and after continuous infusion. [b]Parameters were obtained by fitting the sigmoidal equation to the plasma dihydroetorphine concentration and analgesic effect during and after infusion. [c]Calculated from parameters in 0·9, 1·8 and 3·6 μg h^{-1} kg^{-1}.

human skin will approximate that through dorsal skin rather than abdominal skin of the hairless rat.

Kinetic analysis of skin permeation described well the flux during topical application and after the donor was removed. Mathematical modelling for the release profile from the skin after removal of the transdermal delivery devices has been reported by several authors (Tojo 1988; Kubota & Maibach 1994; Roberts et al 1999). Kubota & Maibach (1994) reported that the half-life of the flux after removing the donor was dominated by the thickness of the skin layer, the diffusivity and the partition coefficient in the skin layer. When the tape was used the half-life of dihydroetorphine was larger than when the suspension was used, due to the smaller diffusivity and the higher partition coefficient in the stratum corneum by the tape. There are few reports regarding the mathematical analysis in consideration with peeling off the stratum corneum by using an adhesive device. In this study, the dihydroetorphine tape caused the peeling off of six or seven of the ten hypothetical layers of the stratum corneum. This indicated that 50% of the dihydroetorphine remaining in the stratum corneum was lost with the tape (Figure 4B, D). These events might occur in the in-vitro condition only, because the stratum corneum layer of excised rat skin was easily removed after four days hydration of the skin in our previous report (Sugibayashi et al 1995). It was unclear if the dihydroetorphine tape would cause the peeling off of the stratum corneum layer during in-vivo application.

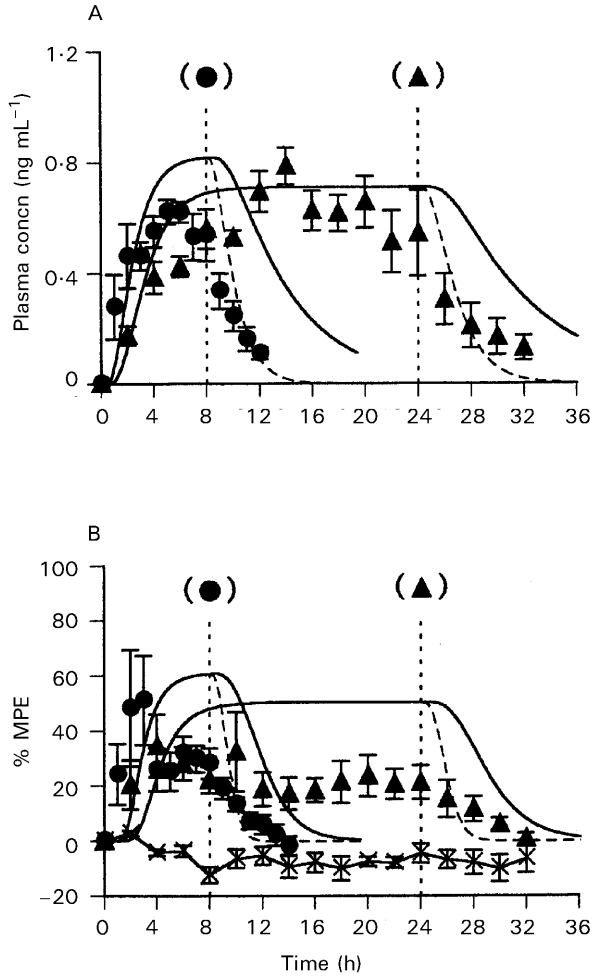

Figure 6. Plasma dihydroetorphine concentration and analgesic effect following topical application of the tape to abdominal and dorsal skin in hairless rats. Each point represents the mean and standard error (n = 4–5). ●, Abdominal skin; ▲, dorsal; × , placebo tape to dorsal skin. Vertical dotted lines indicate the time of removal of the tape. Solid lines indicate the calculated mean values obtained from the combined equation of the diffusion model, compartment model and/or sigmoidal effect–concentration equation. Dashed lines indicate the calculated mean values predicted by models after peeling off the outer layer of the stratum corneum with removal of the tape.

The plasma concentration of dihydroetorphine was maintained in the effective concentration range, but it was somewhat variable during the abdominal and dorsal applications of the tape (Figure 6). The variation of plasma concentration is suggested to have been caused by variation of the drug input rate through the skin, which was influenced by the cutaneous perfusion rate and expansion or contraction of the skin in contact with the tape by movement of the conscious rats. However, the in-vivo thickness of viable skin, which was set at 10-fold of the stratum corneum thickness, was suggested to be acceptable to estimate the in-vivo flux of dihydroetorphine, because the time lag for the onset of plasma concentration after application of the tape was close to theoretical values.

The analgesic effect during application of the tape was slightly lower than the theoretical value calculated from 4-h infusion studies (Figure 6). The plasma concentration was similar to the theoretical value, so that an analgesic tolerance might occur during the application of dihydroetorphine tape for 8 or 24 h. The relationship between the plasma concentration and the analgesic effect during application of the tape had a tendency towards a shift to the right from the theoretical line, but it was not clear (data not shown). Tokuyama et al (1993) reported the development of tolerance to dihydroetorphine analgesia by repeated subcutaneous injection. However, although a continuous infusion of morphine showed apparent tolerance to its analgesic effect in normal rats (Ouellet & Pollack 1997), the tolerance of morphine did not occur in pain-presented rats (Vaccarino et al 1993). We have confirmed that continuous exposure of dihydroetorphine develops analgesic tolerance and rewarding effect in hairless rats, but not in formalin-pain presented hairless rats (unpublished data).

In conclusion, it was confirmed that dihydroetorphine was permeable enough through the

abdominal and dorsal skin of hairless rat to produce an analgesic effect. Using drug-dispersed pressure-sensitive adhesive tape, dihydroetorphine was continuously delivered for 8 or 24 h. After the tape was removed, the release profiles of dihydroetorphine from the skin were described by the Fickian diffusion equation with peeling off of the outer layer of stratum corneum. Following the abdominal and dorsal applications of dihydroetorphine tape, the plasma concentration and analgesic effect were maintained at suitable levels until the tape was removed. These results were predictable by mathematical modelling.

Acknowledgements

The authors would like to thank Mr Yohei Sugiyama, Mr Liang Fang and Mr Hidehiko Mizuguchi for their technical assistance.

References

Barber, R. B., Rapoport, H. (1975) Synthesis of thebaine and oripavine from codeine and morphine. J. Med. Chem. 18: 1074–1077

Bentley, K. W., Hardy, D. G. (1967) Novel analgesics and molecular rearrangements in the morphine-thebaine group. III. Alcohols of the 6,14-endo-ethenotetrahydrooripavine series and derived analogs of *N*-allylnormorphine and -norcodeine. J. Am. Chem. Soc. 89: 3281–3292

Bronaugh, R. L., Stewart, R. F., Congdon, E. R. (1983) Differences in permeability of rat skin related to sex and body site. J. Soc. Cosmet. Chem. 34: 127–135

Chen, X. P., Guo, Q. D., Shi, T. S. (1996) Studies on transdermal delivery system of dihydroetorphine hydrochloride. Acta. Pharm. Sin. 31: 770–774

Crank, J. (1975) The Mathematics of Diffusion, Second Edition. Oxford University Press, London

Finnin, B. C., Morgan, T. M. (1999) Transdermal penetration enhancers: applications, limitations, and potential. J. Pharm. Sci. 88: 955–958

Franz, T. J., Tojo, K., Shah, K. R., Kydonieus, A. (1992) Transdermal delivery. In: Kydnieus, A. (ed.) Treatise on Controlled Drug Delivery: Fundamental, Optimization, Applications. Marcel Dekker, New York, pp 341–421

Guo, Q. D., Chen, X. P., Shi, T. S. (1997) Effect of vehicle pH, drug concentration and Azone on the snake skin permeation of dihydroetorphine hydrochloride. Chin. Pharm. J. 32: 349–352

Jolicoeur, L. M., Nassiri, M. R., Shipman, C., Choi, H. K., Flynn, G. L. (1992) Etorphine is an opiate analgesic physicochemically suited to transdermal delivery. Pharm. Res. 9: 963–965

Kubota, K., Maibach, H. I. (1994) Significance of viable skin layers in percutaneous absorption and its implication in mathematical models: theoretical consideration based on parameters for betamethasone 17-valerate. J. Pharm. Sci. 83: 1300–1306

Li, Y. S., Li, J. L., Qu, F. L. (1996) Using dihydroetorphine to control cancer pain. Chin. J. New Drug 5: 122–123

Morimoto, Y., Hatanaka, T., Oguchi, M., Sugibayashi, K., Kobayashi, M., Kimura, M. (1992a) A screening method for percutaneous absorption enhancers appropriate for adhesive matrix devices. S. T. P. Pharma Sciences 2: 253–258

Morimoto, Y., Hatanaka, T., Sugibayashi, K., Ohmiya, H. (1992b) Prediction of skin permeability of drugs: comparison of human and hairless rat skin. J. Pharm. Pharmacol. 44: 634–639

Ohmori, S., Hayashi, T., Kawase, M., Saito, S., Morimoto, Y. (2000a) Quantitative determination of dihydroetorphine in rat plasma and brain by liquid chromatography-tandem mass spectrometry. J. Chromatogr. B 740: 253–263

Ohmori, S., Hayashi, T., Kawase, M., Saito, S., Morimoto, Y. (2000b) Pharmacokinetic and Pharmacodynamic evaluations of a potent analgesic dihydroetorphine in hairless rat. J. Pharmacol. Exp. Ther. (in press)

Ohmori, S., Suguyama, Y., Morimoto Y. (2000c) A protective effect against undesirable increase of dihydroetorphine permeation through damaged skin by using pressure-sensitive adhesive tape with an ethylene-vinyl acetate co-polymer membrane. Biol. Pharm. Bull. (in press)

Okamoto, H., Hashida, M., Sezaki, H. (1988) Structure-activity relationship of 1-alkyl- or 1-alkenylazacycloalkanone derivatives as percutaneous penetration enhancers. J. Pharm. Sci. 77: 418–424

Ouellet, D. M. C., Pollack, G. M. (1997) Pharmacodynamics and tolerance development during multiple intravenous bolus morphine administration in rats. J. Pharmacol. Exp. Ther. 281: 713–720

Roberts, M. S., Anissimov, Y. G., Gonsalvez, R. A. (1999) Mathematical models in percutaneous absorption. In: Bronaugh, R. L., Maibach, H. I. (eds) Percutaneous Absorption. Marcel Dekker, New York, pp 3–55

Sato, K., Sugibayashi, K., Morimoto, Y. (1991) Species differences in percutaneous absorption of nicorandil. J. Pharm. Sci. 80: 104–107

Southam, M. A. (1995) Transdermal fentanyl therapy: system design, pharmacokinetics and efficacy. Anticancer Drugs 6 (Suppl. 3): 29–34

Stinchcomb, A. L., Paliwal, A., Dua, R., Imoto, H., Woodard, R. W., Flynn, G. L. (1996) Permeation of buprenorphine and its 3-alkyl-ester prodrugs through human skin. Pharm. Res. 13: 1519–1523

Sugibayashi, K., Sakanoue, C., Morimoto, Y. (1989) Utility of topical formulations of morphine hydrochloride containing Azone and N-methyl-2-pyrrolidone. Sel. Cancer Ther. 5: 119–128

Sugibayashi, K., Kobayashi, D., Nakagaki, E., Hatanaka, T., Inoue, N., Kusumi, S., Kobayashi, M., Kimura, M., Morimoto, Y. (1995) Differences in enhancing effect of l-methanol, ethanol and their combination between hairless rat and human skin. Int. J. Pharm. 113: 189–197

Suh, H. R., Jun, H. W. (1996) Effectiveness and mode of action of isopropyl myristate as a permeation enhancer for naproxen through shed snake skin. J. Pharm. Pharmacol. 48: 812–816

Tojo, K. (1987) Mathematical modeling of transdermal drug delivery. J. Chem. Eng. Jap. 20: 300–308

Tojo, K. (1988) Concentration profile in plasma after transdermal drug delivery. Int. J. Pharm. 43: 201–205

Tokuyama, S., Takahashi, M., Kaneto, H. (1993) Antinociceptive effect of dihydroetorphine and its tolerance/dependence liability in mice. Biol. Pharm. Bull. 16: 774–777

Vaccarino, A. L., Marek, P., Kest, B., Ben-Eliyahu, S., Couret, L. C., Kao, B., Liebeskind, J. C. (1993) Morphine fails to produce tolerance when administered in the presence of formalin pain in rats. Brain Res. 627: 287–290

Wang, C. Y., Li, H., Cheng, N. S., Zhong, J. H., Wang, B. (1999) An investigation on the situation of utilization of narcotic medications for cancer pain patients. Chin. J. Drug Depend. 8: 210–213

WHO Expert Committee (1999) WHO Expert Committee on Drug Dependence, Thirty-first Report. World Health Organization Technical Report Series 887, World Health Organization, Geneva

Wu, G. Q., Sun, Y. (1991) Dihydroetorphine hydrochloride for moderate and severe cancer pain. Chin. J. Oncol. 13: 64–67

Zang, D. L. (1999) A survey on the utilization of narcotic analgesic drugs in general hospitals of Qingdao. Chin. J. Drug Depend. 8: 204–206