Pharmacokinetic and Pharmacodynamic Evaluations of a Potent Analgesic, Dihydroetorphine, in Hairless Rat

SATOSHI OHMORI, TERUAKI HAYASHI, MASAMI KAWASE, SETSUO SAITO, and YASUNORI MORIMOTO
Faculty of Pharmaceutical Sciences, Josai University, Saitama, Japan
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ABSTRACT
To evaluate the pharmacokinetic and pharmacodynamic characteristics of a novel opioid analgesic, dihydroetorphine (DHE), concentrations of DHE and its glucuronide (DG) in plasma and central nervous system (by liquid chromatography-tandem mass spectrometry) and the antinociceptive effect (by tail-immersion test) were measured after intravenous (i.v., 2 μg/kg), intracutaneous (i.c., 2 μg/kg), subcutaneous (s.c., 2 μg/kg), intraperitoneal (i.p., 10 μg/kg), and oral (p.o., 200 μg/kg) administrations in hairless rats. An elimination half-life of plasma DHE concentration was 37.2 min after i.v. injection. Brain DHE concentration reached a maximum within 6 min after i.v. injection, and the concentration ratio in brain to plasma was 5.17. Relative bioavailabilities of DHE to i.v. injection (100%) were 70.8, 79.8, 16.7, and 0.37% after i.c., s.c., i.p., and p.o. administrations, respectively. Area under the plasma concentration-time curve ratios of plasma DG to DHE concentrations after i.v., i.c., s.c., i.p., and p.o. were 1.76, 3.26, 4.74, 14.5, and 290, respectively. Antinociceptive effects appeared rapidly after i.v., i.c., and s.c. administrations but were diminished after i.p. and p.o. administrations, and these effects were closely related to the brain DHE concentrations. DHE was excreted mainly as DG in bile (89.5% of the dose) by 240 min after i.v. injection. Serum protein binding of DHE was 83.4%, which was not influenced by DG. Glucuronidation of DHE was detected in the liver, intestine, and kidney in vitro but was minimal in the skin and brain. In conclusion, DHE was rapidly distributed to the brain in relation to producing the antinociceptive effect, and then it was rapidly metabolized to the pharmacologically inactive DG.

Dihydroetorphine, 7,8-dihydro-7α-[1-(R)-hydroxy-1-methylbutyl]-6,14-endo-ethanotetrahydro-orarypinovine (DHE, Fig. 1), is a selective μ-opioid receptor agonist (Wang et al., 1995). DHE produces an extraordinarily strong analgesia, which is 1,000 to 12,000 times more potent than morphine and 2 to 13 times more potent than etorphine (Bentley and Hardy, 1967; Tokuyama et al., 1996; Aceto et al., 1997). In the case of continuous exposure, a physical dependence was found in rodents (Tokuyama et al., 1994; Aceto et al., 2000). Unfortunately, DHE has a potent reinforcing effect and discriminate stimulus effect like some other opioids in drug self-administration studies in rat and monkey (Beardsley and Harris, 1997; Martin et al., 1997). However, these studies have indicated some clinical advantages of DHE over other opioids, because the abuse-liable potency ratios of DHE to morphine (1500 to 3000) were somewhat less than the antinociceptive potency ratio of them (Beardsley and Harris, 1997). In addition to the antinociceptive effect, DHE shows antiallodynic action (Martin et al., 1998), detoxification for opioid addictions (Wang et al., 1992), an antitussive effect (Kamei et al., 1994), and an immunosuppressive effect (Wu et al., 1998).

In China, DHE began to be clinically used for relief of pain in 1981 and was registered as an analgesic for severe pain in 1992. Unexpectedly, the abuse of DHE increased rapidly soon after it was marketed; therefore, the Government of China notified the restriction of the use of DHE in 1993. Epidemiological studies show that the majority of abusers took DHE to avoid withdrawal syndrome of heroin or other opiates (Liu et al., 1995), because of its psychological dependence-producing properties, cheap market prices, and less restricted control (Report from the WHO Expert Committee on Drug Dependence, 1999). In March 1999, the United Nations decided to include DHE in Schedule I of the Single Convention on Narcotic Drugs of 1961, and the Convention as amended by the 1972 Protocol. In these circumstances, DHE has been used clinically for relief of pain under restricted control (Wang et al., 1999).

Tokuyama et al. (1996) investigated the antinociceptive effect profiles of DHE following various routes of administration in mice. Antinociceptive effects appeared quickly and disappeared smoothly after systemic or local injection of DHE. When DHE was directly injected into the central nervous system, the antinociceptive effect was extremely potent. On the other hand, the antinociceptive effect resulting from...
oral administration of DHE was minimal. There are obvious discrepancies regarding the pharmacological profiles and dependence liabilities between DHE and morphine (Tokuyama et al., 1994). It is unclear whether these results may be caused by pharmacokinetic properties or pharmacological features. The disposition of DHE is not fully understood because the quantitative method to measure its disposition is not sensitive enough to measure low doses of DHE. As a first step in our studies for the evaluation of pharmacokinetic properties of DHE, we developed a sensitive method for DHE quantification in rat plasma and brain tissue by liquid chromatography-tandem mass spectrometry (LC-MS-MS) (Ohmori et al., 2000a).

There are few reports concerned with the pharmacokinetics of DHE in rodents using the radiolabeled drug (Huang et al., 1988). In their study, the radioactivity in the body might have included both the unchanged form and major metabolites. It is possible that DHE is extensively transformed into its glucuronide (DG) in rodents, because DHE has a phenolic hydroxyl group in the 3-position of its structure like morphine (Milne et al., 1996) or buprenorphine (Ohtani et al., 1994). A measurement of the precise concentrations of the unchanged form and the metabolites is necessary to understand the profiles of the pharmacological effect and the adverse effects. The purposes of this study are 1) to make clear the elimination of the unchanged form, production of metabolites, distribution into the central nervous system (CNS), and excretion into bile or urine of these compounds after systemic administration of DHE in hairless rats; 2) to compare with bioavailabilities of DHE following various extravascular administrations; and 3) to estimate the contribution of metabolites to the antinociceptive effect of DHE. These purposes will contribute to the determination of the optimal therapeutic use of DHE and to the design of appropriate pharmaceutical formulations.

Materials and Methods

Animals. In all experiments male hairless rats (WBN/ILA-Ht strain) weighing 200 to 300 g (10–14 weeks old), supplied by Life Science Research Center of Josai University (Saitama, Japan) and Ishikawa Experimental Animal Laboratory (Saitama, Japan), were used. Animals were kept in a room that was maintained at 24 ± 1°C under 12-h light/dark cycle and had free access to a standard rodent diet and clean drinking water. These experiments were performed in accordance with the Guide for Laboratory Animal Experiment adopted by Josai University.

Chemicals. Free base DHE was synthesized from codeine using the reported procedures (Bentley and Hardy, 1967; Barber and Rapoport, 1975). Buprenorphine hydrochloride was kindly supplied from Otsuka Pharmaceuticals (Tokyo, Japan) and was used as a quantitative internal standard. β-Glucuronidase was purchased from Boehringer Mannheim (GmbH, Germany). UDP-glucuronic acid trisodium salt was purchased from Wako Pure Chemical (Osaka, Japan). Acetonitrile, methanol, ethyl acetate, and water were of high-performance liquid chromatography (HPLC) grade. All other reagents were of analytical grade.

Plasma and CNS Organ Sampling after DHE Administrations. Rats were cannulated with polyethylene tubing into a femoral vein for injection of DHE (only for the i.v. injection group) and into a femoral artery for blood sampling (for the all administration group) under diethyl ether anesthesia. The tip of the cannula was drawn through the skin on the back of the neck so that awakening rats were able to move freely. The animals were left for 2 h after surgery to recover from the anesthesia. DHE was dissolved in saline, and it was given by five routes of administration. For the i.v. injection group (n = 67), 2 μg/kg DHE was injected through the venous cannula. For the intracutaneous (i.c.) injection group (n = 16), 2 μg/kg DHE was injected into a shallow layer of the dorsal skin. For the s.c. injection group (n = 36), 2 μg/kg DHE was injected under the dorsal skin. For the p.i. injection group (n = 35), 10 μg/kg DHE was injected. For the p.o. administration group (n = 24), 200 μg/kg DHE was administered by a stainless feeding needle. Blood samples were withdrawn from the arterial cannula at appropriate times after the various administrations. Blood sampling was set at one to four times per body, but a total volume of blood samples did not exceed 1% of the body weight. Blood samples were placed in a heparinized tube, and plasma was separated by centrifugation. Brain and spinal cord as CNS organs were excised after decapitation, and then the brain was dissected into two sections: the cerebellum, which does not contain a μ-opioid receptor, and the remaining regions, including the frontal cortex, thalamus, caudate, midbrain, and medulla, which are μ-opioid receptor-rich tissues like the spinal cord (Kawai et al., 1991; Abbruscato et al., 1997). Each sample was homogenized with 2 volumes of methanol. The supernatant of the homogenate was taken by 9000g centrifugation at 4°C. Plasma and supernatant samples were stored at −20°C until analysis.

Bile and Urine Sampling after DHE i.v. Injection. Rats were cannulated with polyethylene tubing into a femoral vein for injection of DHE and into the bile duct and bladder for the bile and urine collections under diethyl ether anesthesia. The animals were placed in a Bollman cage, and saline infusion (1 ml/min) through the venous cannula was maintained to ensure the bile and urine was collected. After the bile and urine flow rate became constant, 2 μg/kg DHE was administered by a stainless feeding needle. Blood samples were collected at appropriate intervals and were stored at −20°C until analysis.

Measurement of DHE Concentrations. DHE concentrations in plasma and CNS supernatants were measured by LC-MS-MS as reported previously (Ohmori et al., 2000a). The same procedure was applied to bile and urine samples. Briefly, a volume less than 0.5 ml of the sample was mixed with 3 ml of 50 mM phosphate buffer (pH 6.0) and 0.1 ml of buprenorphine methanol solution as an internal standard. The sample was mixed with 3 ml of 50 mM phosphate buffer (pH 6.0) and 0.1 ml of buprenorphine methanol solution as an internal standard.
standard (10 ng/ml). This mixture was applied to a Bond Elut Certify cartridge (3 ml/130 mg; Varian, Harbor City, CA) that had been conditioned with 3 ml of methanol and 3 ml of 50 mM phosphate buffer (pH 6.0). Then the column was washed with 3 ml of 100 mM acetic acid and 2 ml of methanol. After air was fully passed through the column, 4 ml of 2% ammonium hydroxide in ethyl acetate was poured into the column. The eluant was collected and dried under a stream of nitrogen. The residue was successively dissolved in 0.1 ml of acetonitrile/water (80:20), and its aliquot (20 μl) was applied to LC-MS-MS analysis.

The LC-MS-MS instrument used was an API-300 equipped with an ion-spray interface (PerkinElmer-SCIEX, Foster City, CA) and connected with an LC-10A system (Shimadzu, Tokyo, Japan). For LC separation, acetonitrile/50 mM ammonium acetate (95:5) as a mobile phase was delivered at 0.3 ml/min through an Inertsil ODS-2 column (5 μm, 2.1 mm i.d. × 150 mm; GL Science, Tokyo, Japan) under 40°C. The analytical conditions of the API-300 were the same as those of our previous report (Ohmori et al., 2000a). The precursor and product ions on the multiple reaction monitoring were 414/414 m/z for DHE and 468/468 m/z for buprenorphine. Calibration was constructed in a range from 0.05 to 10 ng/ml. The limit of quantification, which was decided by intra-assay precision within 20% (Shah et al., 1992), was 0.05 ng/ml in plasma, bile, and urine and 0.15 ng/g in CNS organs.

Measurement of DG Concentrations. For the measurement of DG concentrations, 50 μl of β-glucuronidase (200 U/ml using 4-nitrophenyl-β-D-glucuronide as a substrate) and 1 ml of 0.3 M acetate buffer (pH 6.0) were added to 0.1 ml of sample. Supernatant of brain was dried beforehand and then redissolved in the same buffer. The mixture was incubated at 37°C for over 16 h. Then 2 ml of 50 mM phosphate buffer (pH 6.0) and 0.1 ml of buprenorphine methanol solution (10 ng/ml) were mixed with the hydrolyzed mixture. Subsequent preparations were the same as those in the procedure for the measurement of DHE. DG concentrations were calculated by subtracting the DHE concentration of the untreated sample from that of the β-glucuronidase-treated sample.

Measurement of the Antinociceptive Effect. The antinociceptive effect was determined using the tail-immersion test (Ouellet and Pollack, 1997) in a room that was maintained at 24 ± 1°C. Rats were loosely wrapped in a cloth for calming, and the tail emerged from the cloth. The distal two-thirds of the tail was immersed in hot water, and the latency time for a flick of the tail or to struggle was measured. The temperature of water was adjusted in the range of 50–55°C so that the mean basal latency was approximately 2 s. If the basal latency of an individual animal was in excess of 3 s, the individual was excluded from the experiment. The latency time was determined before drug administration (predrug latency) and appropriate time intervals after the i.v., i.c., s.c., i.p., and p.o. administrations of DHE (postdrug latency). A cut-off time was adopted as 10 s to prevent damage to the tail. The antinociceptive effect was expressed as the percentage of the maximum possible effect (%MPE): %MPE = [postdrug latency – predrug latency]/cut-off latency × 100.

Determination of Protein Binding. Plasma protein binding of DHE was determined by the ultrafiltration method. DHE was spiked in blank plasma at 0.5 to 100 ng/ml. In addition, we prepared plasma samples withdrawn from rats administered with DHE i.v. or p.o. One milliliter of each sample was poured into a micropartion device (MPS kit) with an ultrafiltration membrane (YMT membrane; Amicon, Beverly, MA). Free DHE was filtered by centrifugation at 1000g with a 23” fixed-angle rotor for 20 min. Buprenorphine methanol solution as an internal standard was added to an aliquot of filtrate. After centrifugation, the supernatant was injected into the LC-MS-MS system.

Determination of in Vitro Glucuronidation Activity. Liver, small intestine, kidney, skin, and brain were excised after decapitation. The tissues were rinsed and perfused by ice-cold 1.15% potassium chloride. Then the homogenate was centrifuged at 9000g under 4°C for 15 min. The supernatant (postmitochondrial fraction) was stored at −50°C until the in vitro glucuronidation assay. A half-milliliter of the postmitochondrial fraction, 0.5 ml of 0.15 M Tris-HCl buffer (pH 7.4) containing 10 μg/ml DHE, and 1 ml of the same buffer containing 10 mM UDP-glucuronic acid, 0.05% Triton X-100, 15 mM magnesium chloride, 2 mM L-ascorbic acid were mixed in a test tube. The solutions for liver, intestine, and kidney were incubated at 37°C for 30 min, and those for skin and brain were incubated for 180 min. The incubations were terminated by adding 4 ml of ice-cold acetonitrile. The supernatant was taken by 9000g centrifugation and divided into two portions. Both portions were dried, and then one portion was deconjugated by β-glucuronidase. DHE contents in both portions were measured. The formation of DG was calculated as glucuronidation activity in vitro by subtracting the DHE concentration in the glucuronidase-treated portion from that in the untreated portion.

Calculation of Pharmacokinetic Parameters. Pharmacokinetic parameters in each administration group were calculated by the extended least square method, MULTI-ELS (Yamaoka and Tanaka, 1987). In the i.v. injection group, plasma DHE concentrations (Cp) were fitted to eq. 1:

\[ C_p = \left( A + \eta_a \right) \exp\left(-\alpha + \eta_\alpha t\right) + \left( B + \eta_\beta \right) \exp\left(-\beta + \eta_\beta t\right) \times \exp\left(\epsilon\right) \] (1)

where A and α and B and β are the means of the ordinate axis intercept and the first order rate constant on initial phase (A, α) and terminal phase (B, β) of the logarithmic concentration-time profile. The terms ηa, ηB, ηα, and ηβ are the corresponding interindividual variations. ε was the individual variation within the group. The half-life of both phases, t1/2α, and t1/2β, were calculated by 0.693/α and 0.693/β, respectively. The area under the plasma concentration-time curve (AUC) and area under the first moment curve (AUMC) were calculated by AUC = A0/α and AUMC = A0/α2, respectively. Mean residence time (MRT) was calculated by AUC/AUMC. Total body clearance (Clint) was calculated by dose/AUC. Steady-state volume of distribution (Vdss) was calculated by Clint × MRT.

DG concentrations in plasma (CpDG) and DHE concentrations in CNS organs (CpCNS) were fitted to a physiological hybrid model (Rowland and Tozer, 1995), eqs. 3 and 4, respectively, with substitution of A, B, α, and β calculated from eq. 1:

\[ C_{DG} or C_{CNS} = R \times \left[ \frac{A}{S - \alpha} \exp\left(-\alpha t\right) - \exp\left(-St\right) + \frac{B}{S - \beta} \exp\left(-\beta t\right) - \exp\left(-St\right) \right] \times \exp(\epsilon) \] (2)

where, for the calculation of CDG,

\[ R = k_{DG} V_j / V_{DG} + \eta_{DGVjVyDG} \text{ and } S = k_{DG} + \eta_{DGk} \] (3)

and, for the calculation of CPCNS,

\[ R = Q/V_{CNS} + \eta_{VQVJVCNS} \text{ and } S = R(\text{KpCNS} + \eta_{KpCNS}) \] (4)

In eq. 3, kDG and kDGk were the formation and elimination rate constants of DG, respectively, and Vj and Vj DG were the distribution volume of DHE in the central compartment and of DG in the body compartment, respectively. In eq. 4, Q/V CNS was the plasma flow rate per tissue weight in each CNS organ, and KpCNS was the DHE distribution ratio in CNS to plasma. In eqs. 3 and 4, η and ϵ were the same as described above.

In the i.c. and s.c. injection groups, plasma DHE concentrations were fitted to eq. 5:

\[ C_p = \left( A + \eta_a \right) \exp\left(-\alpha + \eta_\alpha t\right) + \left( B + \eta_\beta \right) \exp\left(-\beta + \eta_\beta t\right) - \left( A + \eta_a \right) + \left( B + \eta_\beta \right) \exp\left(-k_e + \eta_{k_e} t\right) + \epsilon \] (5)
where \( k_a \) was the first order rate constant of absorption phase, and \( \eta_a \) and \( \epsilon \) were the same as described above. The half-life of absorption phase was calculated by 0.693/\( k_a \). AUC was calculated by \( A/A + B/B - (A + B)/k_a \).

In the i.p. and p.o. administration groups, plasma DHE concentrations were calculated by:

\[
C_p = (A + \eta_a)[\exp(-k_a + \eta_a)t] - \exp(-k_a + \eta_a)t + \epsilon, \tag{6}
\]

where \( k_a \) was the first order constant rate in the elimination phase, and \( \eta_a \) and \( \epsilon \) were the same as described above. The half-life of the elimination phase was calculated by 0.693/\( k_e \). AUC was calculated by \( A/(k_e - k_a) \).

Bioavailability (\( F \)) after extravascular administration was calculated by using the mean of \( \text{Cl}_{\text{tot}} \) in the i.v. injection group (i.v.) and the mean of AUC and dose in the extravascular administration groups (e.v.), \( \text{Cl}_{\text{tot}}(\text{i.v.}) \times \text{AUC(\text{e.v.})/Dose(\text{e.v.})} \). The AUC of the plasma DG concentration-time curve (\( \text{AUC}_{\text{DG}} \)) and of DHE concentration in the CNS organs-time curve (\( \text{AUC}_{\text{CNS}} \)) were calculated by trapezoidal rule.

Simulation of plasma DG and brain DHE profiles after extravascular administrations were calculated by using glucuronidation and the distribution parameters obtained by eqs. 3 and 4, and absorption and disposition parameters were obtained by eqs. 5 and 6. In particular, plasma DG concentrations after i.p. and p.o. administrations were calculated by eq. 7 involving bioavailability (\( F \)) as a consideration of first-pass glucuronidation.

\[
C_t = \frac{k_{DG}V_i}{FV_{DG}} \times \left[ \frac{A}{k_{DGel} - k_a} \left[ \exp(-k_{DGel}t) - \exp(-k_{DGel}a) \right] \right. \\
\left. + \frac{A}{k_{DGel} - k_a} \left[ \exp(-k_{DGel}t) - \exp(-k_{DGel}a) \right] \right] \tag{7}
\]

### Results

#### DHE Concentrations in Plasma and CNS Organs after i.v. Injection

DHE and DG concentrations in plasma and CNS organs after i.v. administration (2 \( \mu \)g/kg) are shown in Fig. 2. Initial DHE concentration in plasma was 3.87 ng/ml at 2 min after the injection, and then it decreased rapidly (Fig. 2A). Some individuals exhibited a concentration below 0.05 ng/ml at 120 or 180 min after the injection. An elimination profile was properly fitted to the biexponential equation (eq. 1). The \( t_{1/2a} \) and \( t_{1/2b} \) of DHE in plasma were 3.77 and 37.2 min, respectively. AUC, \( \text{Cl}_{\text{tot}} \), and \( \text{Vd}_{\text{a}} \) were 66.3 ng · min/ml, 30.2 ml/min/kg, and 1081 ml/kg (Table 1).

The glucuronide concentration in plasma increased over the DHE concentration, and it reached a calculated maximum concentration at 16 min after i.v. injection of DHE (Fig. 2A). Then it decreased exponentially in relation to the elimination of DHE. DG concentration was 3 to 5 times higher than DHE concentration in the elimination phase. The AUC of DG concentration was 118 ng · min/ml, and the AUC ratio of DG to DHE was 1.76 (Table 1).

The DHE concentrations in the CNS organs increased quickly and reached a maximum within 6 min after the i.v. injection (Fig. 2B). The calculated maximum concentration was 10.4 ng/g of tissue in the brain, excluding the cerebellum and 4.86 ng/g of tissue in cerebellum with \( K_p \) values of 5.17 and 1.77, respectively. The DHE concentration in the spinal cord was similar to that of brain, excluding the cerebellum, and the DHE concentration in the CNS organs decreased biphasically, depending upon the plasma concentration. The DG concentration in the brain was detected in some individuals at 30 min after DHE injection (data not shown), but it was less than 10% of the DHE concentration in brain.

#### DHE Concentrations in Plasma and CNS Organs after Extravascular Administrations

Figure 3 shows the DHE and DG concentrations in the plasma and brain after extravascular administrations. Plasma DHE profiles were properly fitted to eqs. 5 or 6. DHE concentrations in the plasma reached a maximum from 9 to 15 min in all dosing groups. The calculated maximum concentration and AUC were 1.11 ng/ml and 47.0 ng · min/ml in 2 \( \mu \)g/kg i.c. injection (Fig. 3A), 0.97 ng/ml and 52.9 ng · min/ml in 2 \( \mu \)g/kg s.c.

### Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Calculated Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A ) (ng/ml)</td>
<td>4.51 ± 1.24</td>
</tr>
<tr>
<td>( \alpha ) (min(^{-1}))</td>
<td>0.184 ± 0.011</td>
</tr>
<tr>
<td>( B ) (ng/ml)</td>
<td>0.778 ± 0.063</td>
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<tr>
<td>( \beta ) (min(^{-1}))</td>
<td>0.0186 ± 0.0031</td>
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<tr>
<td>( k_{DG} \cdot V_i/V_{DG} ) (min(^{-1}))</td>
<td>0.0682 ± 0.0200</td>
</tr>
<tr>
<td>( k_{DG} ) (min(^{-1}))</td>
<td>0.0326 ± 0.0122</td>
</tr>
<tr>
<td>( QV_{\text{brain}} ) (ml/min/g)</td>
<td>0.832 ± 0.161</td>
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<tr>
<td>( K_{P,2min} )</td>
<td>5.17 ± 1.46</td>
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<tr>
<td>( QV_{\text{cerebellum}} ) (ml/min/g)</td>
<td>0.608 ± 0.259</td>
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<tr>
<td>( K_p,\text{cerebellum} )</td>
<td>1.77 ± 1.80</td>
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<tr>
<td>( t_{1/2a} ) (min(^{#}))</td>
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</tr>
<tr>
<td>( t_{1/2b} ) (min(^{#}))</td>
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</tr>
<tr>
<td>AUC (ng · min/ml(^{#}))</td>
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<tr>
<td>MRT (min(^{#}))</td>
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<td>( \text{Cl}_{\text{tot}} ) (ml/min/kg(^{#}))</td>
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</tr>
<tr>
<td>( \text{Vd}_{\text{a}} ) (ml/kg(^{#}))</td>
<td>1081</td>
</tr>
<tr>
<td>( \text{AUC}_{\text{DG}} ) (ng · min/ml(^{#}))</td>
<td>117</td>
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<tr>
<td>( \text{AUC}_{\text{DG}}/\text{AUC} )</td>
<td>1.76</td>
</tr>
<tr>
<td>( \text{AUC}_{\text{cerebellum}} ) (ng · min/ml(^{#}))</td>
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</tr>
<tr>
<td>( \text{AUC}_{\text{cerebellum}}/\text{AUC} )</td>
<td>4.86</td>
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<tr>
<td>( \text{AUC}_{\text{cerebellum}} ) (ng · min/ml(^{#}))</td>
<td>106</td>
</tr>
<tr>
<td>( \text{AUC}_{\text{cerebellum}}/ \text{AUC} )</td>
<td>1.60</td>
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</table>

\(^{\#}\) The mean value.

\(^{\#}\) Calculated by trapezoidal rule.
in plasma; DG in plasma. In particular, plasma DG in C and D were predicted by eq. 6. Dashed lines indicate the predicted mean values of DHE in brain and calculated mean value of DHE in plasma obtained by fitting to eqs. 5 or the mean and the standard deviation (s.c. (2 m treatments in hairless rats. When DHE was given i.v., the antinociceptive effect-time profiles and its relationship with plasma and brain DHE concentrations after i.v. (2 µg/kg), i.c. (2 µg/kg), s.c. (2 µg/kg), i.p. (10 µg/kg), and p.o. (200 µg/kg) administrations in hairless rats. When DHE was given i.v., the antinociceptive effect was produced quickly and was continued 100% MPE until 30 min after injection. Some individuals recovered to predose latency at 90 min. Antinociceptive effects in the i.c. and s.c. injection groups reached a maximum in the range from 15 to 30 min after injection, and then it was decreased to the predose level after 120 min. The maximum value of antinociceptive effect in the i.p. injection group was 56.7%. Antinociceptive effect was not produced in the p.o. administration group. The relation curves between plasma DHE concentration and the effect were shifted to the right side in i.p. and p.o. administrations (Fig. 4B). On the other hand, brain DHE concentration was closely related to the antinociceptive effect in all administration groups (Fig. 4C).

Fig. 3. DHE and DG concentrations in plasma and brain, excluding cerebellum after i.c. (2 µg/kg, A), s.c. (2 µg/kg, B), i.p. (10 µg/kg, C), and p.o. (200 µg/kg, D) administrations in hairless rats. Each point represents the mean and the standard deviation (n = 4–5). ●, DHE in plasma; ○, DG in plasma; ▲, DHE in brain; ◆, DG in brain. Each solid line indicates a calculated mean value of DHE in plasma obtained by fitting to eqs. 5 or 6. Dashed lines indicate the predicted mean values of DHE in brain and DG in plasma. In particular, plasma DG in C and D were predicted by eq. 7 with F = 0.167 and 0.0037, respectively.

Table 2

Pharmacokinetic parameters of dihydroetorphine after extravascular administrations

The parameters were calculated by the extended least square method and are represented as the mean and the standard deviation of interindividual variation (s.c.).

<table>
<thead>
<tr>
<th>Route and Dose</th>
<th>Intracutaneous, 2 µg/kg</th>
<th>Subcutaneous, 2 µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (ng/ml)</td>
<td>1.04 ± 0.41</td>
<td>0.350 ± 0.065</td>
</tr>
<tr>
<td>k (min⁻¹)</td>
<td>0.220 ± 0.048</td>
<td>0.205 ± 0.038</td>
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<tr>
<td>α (ng/ml)</td>
<td>1.89 ± 0.13</td>
<td>1.06 ± 0.11</td>
</tr>
<tr>
<td>β (ng/ml)</td>
<td>0.0658 ± 0.0113</td>
<td>0.0524 ± 0.0239</td>
</tr>
<tr>
<td>t₀/2α (min)</td>
<td>11.9 ± 5.2</td>
<td>13.2 ± 3.0</td>
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<tr>
<td>t₀/2α (min)</td>
<td>50.6 ± 18.9</td>
<td>42.8 ± 14.4</td>
</tr>
<tr>
<td>AUC (ng ∙ min/ml)</td>
<td>47.0 ± 22.9</td>
<td>52.9 ± 28.0</td>
</tr>
<tr>
<td>Fₚ</td>
<td>0.708 ± 0.308</td>
<td>0.798 ± 0.398</td>
</tr>
<tr>
<td>AUCbrain/AUC</td>
<td>153 ± 73</td>
<td>251 ± 123</td>
</tr>
<tr>
<td>AUCbrain/Min/min/ml</td>
<td>178 ± 91</td>
<td>233 ± 124</td>
</tr>
<tr>
<td>AUCbrain/AUC</td>
<td>3.78 ± 2.11</td>
<td>4.40 ± 2.64</td>
</tr>
</tbody>
</table>

**a** The mean value.

**b** Calculated by trapezoidal rule.

Antinociceptive Effect after i.v. or Extravascular administrations of DHE. Figure 4 shows the antinociceptive effect-time profiles and its relationship with plasma and brain DHE concentrations after i.v. (2 µg/kg), i.c. (2 µg/kg), s.c. (2 µg/kg), i.p. (10 µg/kg), and p.o. (200 µg/kg) administrations in hairless rats. When DHE was given i.v., the antinociceptive effect was produced quickly and was continued 100% MPE until 30 min after injection. Some individuals recovered to predose latency at 90 min. Antinociceptive effects in the i.c. and s.c. injection groups reached a maximum in the range from 15 to 30 min after injection, and then it was decreased to the predose level after 120 min. The maximum value of antinociceptive effect in the i.p. injection group was 56.7%. Antinociceptive effect was not produced in the p.o. administration group. The relation curves between plasma DHE concentration and the effect were shifted to the right side in i.p. and p.o. administrations (Fig. 4B). On the other hand, brain DHE concentration was closely related to the antinociceptive effect in all administration groups (Fig. 4C).

DHE Excretion into Bile and Urine after i.v. Injection. Cumulative excretion ratios of DHE in bile and urine after i.v. injection (2 µg/kg) in the bile duct- and bladder-cannulated rats are shown in Fig. 5. DHE and DG were excreted in bile and urine, and the cumulative excretion ratio of the total amount reached 95.5% of the dose by 240 min after i.v. injection. DHE was found to be excreted as 89.5% of the dose as DHE in urine, and 0.17% of the dose as DG in urine, and 0.17% of the dose as DHE in bile. The bile flow decreased suddenly to 10% of the basal flow after injection of DHE, and then it recovered by 120 min.

Protein Binding of DHE in Rat Serum. Figure 6 shows the protein binding of DHE in spiked serum and serum withdrawn from rats administered with DHE. Binding in...
DHE-spiked serum was unity, the mean of 83.4% in the range of 0.5 to 100 ng/ml (Fig. 6, closed symbols). Bindings in serum withdrawn from rats injected 2 mg/kg, i.v. was 84.3% (mean of four data, Fig. 6, open circles) and rats administered 200 mg/kg, p.o. was 80.4% (mean of four data, Fig. 6, open squares).

In Vitro Glucuronidation Activity in Rat Tissue. Glucuronidation activities of DHE in the postmitochondrial fraction of the liver, small intestine, kidney, skin, and brain are shown in Fig. 7. DHE was markedly metabolized to DG in the liver, intestine, and kidney, but metabolism was minimal in the skin and was not detected in the brain.

Discussion

Although DHE is one of the most strictly controlled narcotic drugs, its pharmacokinetic properties and their relation to the pharmacological effects are largely unknown. In this paper, we evaluated the pharmacokinetic and pharmacodynamic characteristics of DHE in hairless rats. We sufficiently accomplished our three objectives. First, we clearly demonstrated that DHE was promptly distributed into the CNS, which is the pharmacological target site for opioid analgesics (Reisine and Pasternak, 1996), after systemic administration. Then, DHE was rapidly eliminated by metabolism to DG and was completely excreted to bile. Second, the bioavailability of DHE was quite different in accordance with the route of administration. DHE was not at all available after
The antinociceptive effects of DHE rapidly appeared and then disappeared by 120 min after administration via various routes (Fig. 4A). Many authors have ascribed the antinociceptive effects of DHE to its quick onset and short duration (Wang et al., 1995; Kamei et al., 1996; Tokuyama et al., 1996; Aceto et al., 1997). It was demonstrated that these pharmacological profiles were due to its rapid distribution into the CNS organs (within 6 min after i.v. injection) and elimination. The $t_{1/2\beta}$ (37.7 min) of plasma DHE concentration after systemic administration was shorter than other opioids, such as 57 min of morphine (Ouellet and Pollack, 1997), 2.4 h of buprenorphine (Ohtani et al., 1995), 73 min of fentanyl, and 79 min of sufentanyl, and was somewhat longer than 25 min of alfentanil (Cox et al., 1998), in rats. It was reported that DHE has a relatively low physical dependence at intermittent doses, but in the case of continuous exposure of DHE by infusion (Aceto et al., 2000) or repeated injection with 1- to 2-h intervals (Tokuyama et al., 1994) significant withdrawal symptoms were observed. This strongly suggests that the rare physical dependence by intermittent doses of DHE is caused by the rapid elimination from the body. In these reports, DHE was injected i.p. in which DHE was extensively metabolized to DG, thus DG might affect the physical dependence of DHE.

In this study, DHE was abundantly distributed to the $\mu$-opioid receptor-rich tissue (brain except cerebellum with 5.17 of Kp) in comparison with receptor-absent tissue (cerebellum with 1.77 of Kp). These values were clearly higher than <1 of morphine for brain (Milne et al., 1996) and similar to 3.8 to 5.5 of etorphine for the brainstem (Tavani et al., 1979). Ohtani et al. (1995) reported that the buprenorphine concentrations in rat brain and cerebellum were 3- to 7-fold and 1- to 2-fold higher than that in plasma, respectively, and the concentration difference between these organs, as a specific binding concentration, was closely related to their antinociceptive effects. Antinociceptive effects were closely related to the DHE concentration in the brain rather than that in the plasma (Fig. 4C). However, it was unclear that the antinociceptive effect of DHE was correlated with the specific binding concentration as described above, because DHE in the brain reached a distribution equilibrium more quickly than buprenorphine (Ohtani et al., 1995).

DHE was extensively metabolized to DG in the liver, intestine, and kidney (Fig. 7), and the DG concentration in plasma was detected at 3 to 5 times higher than the DHE concentration (Fig. 2A). It was apparent that the radioactive concentrations in the previous pharmacokinetic study using radiolabeled DHE (Huang et al., 1988) had included the major glucuronide. The bioavailability of DHE after p.o. administration was extremely low, 0.38% of dose. It was caused by the first-pass metabolism in the intestine and/or liver, because DG concentrations after i.p. and p.o. administrations were remarkably high, and it was predictable by simulation with the hypothesis that DHE excluded by the first-pass elimination was converted to DG (Fig. 3, C and D). These results indicate that DHE was extensively metabolized in rats more than other opioids, such as morphine, which was 21% available by oral administration (Milne et al., 1996) and buprenorphine, which was 9.7% available by intraduodenal administration (Brewster et al., 1981). Interestingly, DHE was almost totally metabolized to DG (Fig. 5) but not to a $N$-dealkyl compound like morphine (Milne et al., 1996) or buprenorphine (Ohtani et al., 1994).

Brain DG concentration was clearly detected only after p.o. administration, but it was much less than the plasma DG concentration (Fig. 3D). This result suggests that DG is weakly permeable through the blood-brain barrier. Moreover, it indicates that DG is pharmacologically inactive, because of the antinociceptive effects after p.o. administration was minimal. These properties of DG are similar to those of morphine-3-glucuronide, which has a weak permeability.
through the blood-brain barrier and no antinoceptive activity, and furthermore, it reduced the antinoceptive effect of morphine (Ekblom et al., 1993; Bickel et al., 1996). When DHE was given i.p. and p.o., brain DHE concentrations were lower than the predicted concentrations, and in particular, values were lower than the plasma DHE concentration at the early phase after p.o. administration (Fig. 3D). It is possible that DHE transport into the brain and/or binding to brain tissue is inhibited by DG. It is reported that morphine shows a saturable uptake into isolated rabbit choroid plexus in vitro, but uptake of morphine-3-glucuronide was relatively low (Milne et al., 1996). We confirmed that protein binding of DHE in rat serum was not influenced by DG (Fig. 6), but the change of DHE binding in brain tissue was not determined. These findings indicate that DHE should be administered by a route that allows minimal glucuronidation.

DHE was slightly metabolized in the skin preparation in vitro (Fig. 7). However, glucuronidation activity of DHE could be detected in a particular condition in which Triton X-100 was added to the in vitro assay system, and DG was not detected in the permeation study using freshly excised hairless rat skin (data not shown). DG concentration after i.c. or s.c. injection was rather predictable by simulation in consideration of the first-pass metabolism in skin (Fig. 3, A and B). However, it was unclear that DHE was metabolized through the skin in vivo, because the predicted DG concentrations after i.c. and s.c. administrations were in the range of the mixed variance. It was reported that several compounds were metabolized by glucuronide conjugation in skin microsome with appropriate detergents, which approximate 5% of the liver activity, but glucuronidation in the skin was hardly detected in vivo such as for morphine (Matsuzawa et al., 1994; Hotchkiss, 1998).

Opioid narcotic drugs are available for the relief of severe pain in cancer patients (Reisine and Pasternak, 1996). In this study, DHE exhibited a high availability from cutaneous tissue and rapid elimination from the body. DHE is more suitable for continuous delivery through the skin by a patient-controlled analgesia technique. It is reported that the transdermal application of morphine (Sugibayashi et al., 1989) and fentanyl (Southam, 1995) was feasible for continuous pain relief. Dihydroetorphine indicates several suitable properties for transdermal delivery as summarized by Finnin and Morgan (1999): 1) the daily systemic dose is ≤160 μg (Li et al., 1996); 2) the molecular weight is 413; 3) the log P (octanol/water) is 2.13; 4) the melting point is 205°C (Bentley and Hardy, 1967); 5) the log P is 3.56; 6) the boiling point is 205°C (Bentley and Hardy, 1967); 7) the melting point is 205°C (Bentley and Hardy, 1967); 8) the molecular weight is 413; 9) the log P (octanol/water) is 2.13; 10) the melting point is 205°C (Bentley and Hardy, 1967). DHE transport into the brain and/or binding to brain tissue was slightly lower than the theoretical value calculated by the permeability of morphine (Sugibayashi et al., 1989) and fentanyl (Southam, 1995) was feasible for continuous pain relief. Dihydroetorphine indicates several suitable properties for transdermal delivery as summarized by Finnin and Morgan (1999): 1) the daily systemic dose is ≤160 μg (Li et al., 1996); 2) the molecular weight is 413; 3) the log P (octanol/water) is 2.13; 4) the melting point is 205°C (Bentley and Hardy, 1967); 5) the skin irritation is only slight during topical application of dihydroetorphine with the permeation enhancer, Azone (Chen et al., 1994; Hotchkiss, 1998).

References


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Send reprint requests to: Yasunori Morimoto, Ph.D., Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Josai University, 1-1, Keyakidai, Sakado, Saitama 350-0295, Japan. E-mail: morimoto@josai.ac.jp