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Transdermal permeation of apomorphine through hairless mouse skin from microemulsions

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Abstract

The in vitro transdermal absorption of apomorphine from microemulsions was studied using the skin of the hairless mouse as a membrane. Two microemulsions (no. 1 and 2) were prepared and thickened both containing 3.9% of apomorphine hydrochloride. The lipophilicity of the drug was increased by forming apomorphine–octanoic acid ion-pairs. The fluxes of the drug from the microemulsions through hairless mouse skin were 100 μg h^{-1} cm^{-2} from no. 1 and 88 μg h^{-1} cm^{-2} from no. 2. Apomorphine in microemulsions, protected from light with antioxidants, showed no degradation for up to 6 months.

Keywords: Apomorphine; Transdermal permeation; Microemulsions; Hairless mouse skin

1. Introduction

Apomorphine is a non-selective agonist of D1 and D2 dopamine receptors. Several studies have shown it to be valuable in treating 'on–off' fluctuations in patients with Parkinson's disease. However, the short duration of response when apomorphine is administered intravenously or subcutaneously—about 1 h—and the side effects it produces (postural hypotension, sedation, nausea and vomiting) have limited its clinical use. These side effects can be controlled by a peripheral dopamine antagonist, domperidone, which does not cross the blood-brain-barrier; apomorphine, administered subcutaneously with penjects or continuous infusion minipumps, is now used in Parkinson's disease for the treatment of severe 'on–off' fluctuations, a complication that is observed in 50% of patients after 5 years disease evolution.

The pharmacokinetics of apomorphine in man (Gancher et al., 1989) was monitored after subcutaneous injection and intravenous infusion, showing the drug to be rapidly and completely absorbed from subcutaneous tissue; this effect can be correlated with the rapid onset of clinical effects. The brief duration of the drug's clinical action can be explained by its rapid clearance. The absorption of apomorphine after administration by various routes, such as oral and sublingual, has also been studied (Gancher et al., 1991).

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The transdermal transport of apomorphine by iontophoresis may be a promising strategy for treating Parkinson's disease; in vitro (van der Geest et al., 1997a) and in vivo (van der Geest et al., 1997b) studies have addressed this question: the in vivo studies confirmed the relation between drug transport and applied current density, lack of subcutaneous deposits and the achievement of a steady-state after 15 min. Skin irritation may be a side effect.

In previous studies, we used thickened microemulsions to obtain in vivo transdermal transport of azelaic acid (Gasco et al., 1990) (Colonna et al., 1996) and in vitro transdermal transport of nifedipine through hairless mouse skin (Boltri et al., 1994). Drug transport from microemulsions is facilitated, as the drug is completely dissolved in the microemulsion and relatively high concentrations can be carried as a consequence of the supersolvent properties of microemulsions; the dispersed phase can also act as a reservoir, making it possible to maintain an almost constant concentration in the continuous phase. Pseudo-zero-order kinetics can thus be achieved.

The aim of the present research was to incorporate apomorphine in microemulsions and to study 'in vitro' whether thickened microemulsions are feasible for the drug's transdermal transport after their topical application on hairless mouse skin.

2. Experimental

2.1. Materials

R-apomorphine hydrochloride was from Acef (Parma, I), octanoic acid, isopropylmyristate, decanol, hexanoic acid, benzylic alcohol and 1,2 propanediol were from Sigma (Milan, Italy), Epikuron 200 (95% phosphatidylcholine) was a kind gift from L. Meyer (Magonza, D), colloidal silica: Aerosil 200 was from Degussa (Frankfurt, D), glycocholate and taurocholate were kind gifts from PCA (Basaluzzo, I). The oil phase comprised a mixture of isopropylmyristate–decanol 1.5:1 (v/v) containing 1% of ascorbylpalmitate. In all the experiments, water was used with the addition of 2‰ ascorbic acid.

2.2. Preparation of the microemulsions

Apomorphine hydrochloride and octanoic acid, 1,2 propanediol, sodium hexanoate (or octanoate), sodium glycocholate (or taurocholate) were dissolved in water (+ 2‰ ascorbic acid): the mixture was brought to pH 6.0. Epikuron 200 as surfactant and the mixture isopropylmyristate–decanol as oil phase were then added to the aqueous phase. Transparent systems were obtained.

The compositions of the two microemulsions studied—no. 1 and 2—were as follows (wt./wt.): Microemulsion no. 1: aqueous solution (18.4%), oil phase (42.5%); apomorphine hydrochloride (3.9%), Epikuron 200 (7.4%), benzyl alcohol (10.4%), octanoic acid (4.7%), sodium hexanoate (3.6%), sodium glycocholate (4.7%), 1,2-propanediol (4.4%). Microemulsion no. 2: aqueous solution (18.2%), oil phase (42.1%), apomorphine hydrochloride (3.9%), Epikuron 200 (7.3%), benzyl alcohol (7.1%), octanoic acid (4.6%), sodium octanoate (3.5%), sodium taurocholate (5.7%), 1,2-propanediol (7.6%).

2.3. Thickening the microemulsions

The microemulsions were thickened by adding 5.9% (wt./wt.) of Aerosil 200.

2.4. Octanol apparent partition coefficients of apomorphine at pH 6.0 in the presence of increasing amounts of octanoic acid

Octanol was previously saturated with water brought at pH 6.0 and water pH 6.0 with octanol. A known volume of apomorphine hydrochloride (10^{-3} M) at pH 6.0, alone and in the presence of increasing amounts of octanoic acid (at apomorphine:octanoic acid molar ratios: 1:1.2, 1:1.5, 1:2.0, 1:2.2, 1:2.5) was added to a known volume of octanol.

All aqueous samples were studied at pH 6.0. The system was shaken at 25 °C until drug equilibrium was reached. After separation of the two phases, the drug concentration was determined in the aqueous phase by spectrophotometry ($\lambda = 273$ nm) and the apparent octanol/water partition coefficient at pH 6.0 was calculated.

2.5. Characterisation of microemulsion droplets

The average diameter and polydispersity index of the microemulsion droplets were determined by photocorrelation spectroscopy using a 90 PLUS instrument (Brookhaven Instrument, USA) at a fixed angle of 90° and at a temperature of 25 °C. Refractive indices were measured with a thermostated Abbe refractometer. Each system was determined in triplicate.

2.6. HPLC analysis of apomorphine

Apomorphine was analysed by reversed phase HPLC using a Shimadzu AL 9 instrument (Kyoto, Japan). The separations were on a 5 μm ODS column (250×4.6 mm^2; Spherisorb-Waters). The mobile phase was acetonitrile/water/85% phosphoric acid at a ratio 33:67:0.1 (v/v/v). The flow-rate was fixed at 1.2 ml min^{-1}. and the UV detector (Shimadzu, SPD 10-AV) was set at $\lambda =$ 273 nm.

2.7. In vitro release kinetics of apomorphine

2.7.1. From thickened w/o microemulsions no. 1 and 2, using double membrane

Vertical Franz cells were used: a lipophilic/hydrophilic double membrane was employed. Three hundred milligram of the thickened microemulsion were placed on the donor hydrophilic side of the membrane. The receptor cell was filled with 6 ml of water at pH 6.0 added of ascorbic acid 2‰. The contents of the receptor compartment were stirred continuously at 600 rpm by a road shaped rotating magnet and thermostated at 25 °C. At fixed times, the entire contents of the receptor cell were tipped out and the cell was refilled with fresh aqueous solution at pH 6.0.

2.7.2. From thickened microemulsions no. 1 and 2 using hairless mouse skin

The full-thickness dorsal skin of male hairless mice (athymic-nude-Charles River) aged 4–5 weeks was used. The skin was excised, washed, examined for integrity and used for the experiments.

Vertical Franz cell was used; the skin, previously rinsed with normal saline, was sandwiched between the two ground glass panels. The formulation (0.30 g) was gently applied to the donor site of the skin surface, which had an available diffusion area of 1.7 cm^2. The contents of the receptor cell were continuously stirred and thermostated at 25 °C. At appropriate intervals, the entire contents of the receptor chamber were removed for HPLC determination and the cell refilled with fresh aqueous solution at pH 6.0. The experiments were performed in triplicate.

3. Results

3.1. Percentages of apomorphine

The percentages of apomorphine hydrochloride inglobed in microemulsions after thickening were 3.7% (p/p) in both the two microemulsions.

3.2. Characterisation of microemulsions

The average droplet diameter of microemulsion no. 1 was 44.9 nm with a polydispersity of 0.28. The average droplet diameter of microemulsion no. 2 was 74.9 nm with a polydispersity of 0.20. Both microemulsions were added of antioxidants in both the aqueous phase and the lipophilic mixture, since the drug might be oxidized. Stored in the dark, the thickened microemulsions did not undergo any oxidation after 6 months.

Table 1
Octanol log P_{app} at pH 6.0 of apomorphine alone and in the presence of octanoic acid at different molar ratio

Molar ratio (apomorphine:octanoic acid)	Log P_{app}
–	0.30
1:1.2	0.90
1:1.5	2.20
1:2.0	2.31
1:2.2	2.62
1:2.5	2.77

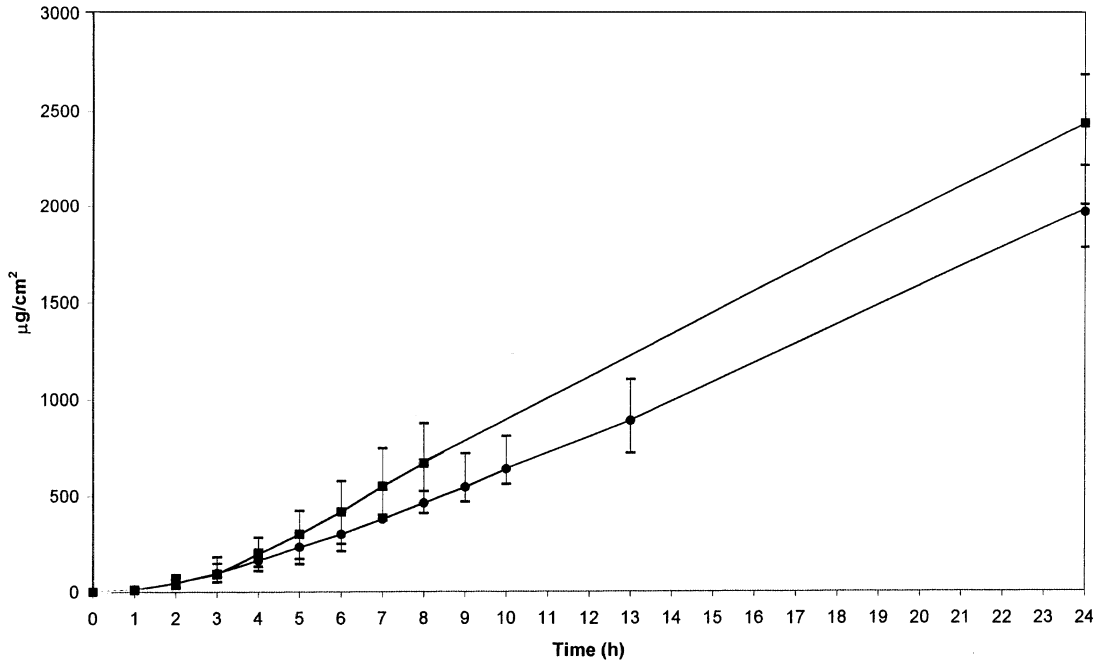

Fig. 1. Permeation profiles of apomorphine from thickened microemulsions: no. 1 (■) and no. 2 (●). Vertical bars indicate S.D.

3.3. Apparent partition coefficients at pH 6.0

Table 1 reports the octanol apparent partition coefficients of apomorphine in the absence and in the presence of octanoic acid at pH 6.0.

3.4. Release of apomorphine from thickened microemulsions through double membrane

The experiments were monitored for 8 h; a pseudo-zero-order kinetics was achieved for both microemulsions.

After 8 h microemulsion, no. 1 released 15.52% of apomorphine and microemulsion no. 2 released 14.49% of apomorphine.

3.5. Release of apomorphine from thickened microemulsions through hairless mouse skin

The fluxes of apomorphine through dorsal hairless mouse skin at steady-state were:

- 100 µg h^{-1} cm^{-2} (lag time 2 h) for microemulsion no. 1;
- 88 µg h^{-1} cm^{-2} (lag time 3 h) for microemulsion no. 2.

The experiments were monitored for 24 h. A pseudo-zero-order kinetics was seen (Fig. 1).

4. Discussion

Apomorphine has to date most frequently been administered by the parenteral route, normally subcutaneously. First-pass effect is high and bioavailability is low when the drug is administered by the oral route (Gancher et al., 1991).

This study aimed to evaluate the capability of the internal phase of water-in-oil microemulsions to act as a reservoir, thus achieving transport of apomorphine in vitro. Preliminary experiments were done to obtain microemulsions from biocompatible and safe components: the two microemulsions studied differ only for the presence or absence of hexanoate and for the biliar salt used as cosurfactant. Antioxidants were added to both the oil phase and the aqueous phase to avoid oxidation of the drug.

An important requirement for transdermal therapy is that a drug carried by a vehicle be able to reach the skin surface at an adequate rate and in sufficient amounts. To pass through the hairless mouse skin, the lipophilicity of apomorphine was increased by forming ion pairs between the drug and octanoate; some preliminary experiments were done in order to evaluate how the pH (between 5.5 and 6.2) of the aqueous phase affects the partition of apomorphine in octanol. The highest partition of apomorphine, in the presence of a fixed amount of octanoate, was found at pH 6.0; consequently this pH was chosen for the aqueous phase of the two microemulsions. Table 1 shows the increasing lipophilicity of apomorphine in the presence of increasing amounts of octanoic acid, at pH 6.0. Indeed log *P* of apomorphine increased from 0.30 in the absence of octanoic acid to 2.77 in the presence of octanoic acid (at an apomorphine:octanoic acid molar ratio of 1:2.5).

Microemulsions no. 1 and 2 both contained an apomorphine:octanoic acid molar ratio of 1:2.5: the two thickened microemulsions contained the maximum amount of solubilised apomorphine. The formation of an ion pair is presumably the main reason for the facilitated transport of apomorphine through hairless mouse skin; indeed the amounts of apomorphine released from microemulsions diminished when the amounts of octanoate in the microemulsions were lowered.

The steady-state fluxes of apomorphine from thickened microemulsions no. 1 and 2 through dorsal hairless mouse skin were 100 and 88 $\mu g\ h^{-1}\ cm^{-2}$, respectively: the kinetics, monitored for 24 h, was of pseudo-zero-order in both cases.

A first approximation of the feasibility of the transdermal administration of microemulsions in man can be made by estimating the C_{ss} concentration of apomorphine in plasma. The approximation can be made from the flux of the drug at steady-state through hairless mouse skin plus two other parameters: the volume of distribution and the systemic elimination kinetics (Guy and Hadgraft, 1985).

From the distribution volume ($1.61\,kg^{-1}$; van der Geest et al., 1998), the clearance (40.4 ml min^{-1} kg^{-1}; van Laar et al., 1998), a steady-state flux of 88 $\mu g\ h^{-1}\ cm^{-2}$ and assuming an application area of 10 cm^2, a steady-state concentration (C_{ss}) of 11 ng ml^{-1} plasma was obtained. Even if the flux through intact human skin were different from that through hairless mouse skin, the concentration of apomorphine in the plasma might become sufficient to have a therapeutic effect on increasing the application area.

In conclusion, thickened water-in-oil microemulsions were prepared with rather interesting fluxes in vitro. Further research is in progress to investigate their application in vivo.

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