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Transdermal delivery of tetrahydrocannabinol

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Summary

The skin permeation behavior of a tetrahydrocannabinol (THC) is described for the first time. In the design of transdermal delivery systems, Δ^8-THC was chosen over Δ^9-THC due to the former's better stability and its lower psychotropic potency. Skin permeation kinetic parameters were established through rat and human skin in vitro. The rat skin was found to be about 13-fold more permeable to Δ^8-THC than the human skin. Autoradiographs showed that 24 h after application the drug was concentrated in the stratum corneum, in the upper epidermis, and around the hair follicles, indicating that THC penetrates the skin through the lipophilic pathways. A transdermal formulation containing oleic acid as a permeation enhancer was tested in rats. A serum level of about 50 ng/ml cross-reacting cannabinoids (THC + metabolites) was maintained for about 24 h, indicating a sustained delivery of THC to the bloodstream. The transdermal preparation presented in this study is designed for future clinical studies.

Introduction

Conventional antiemetics employed to overcome nausea and vomiting that results from the administration of cancer chemotherapeutics are not always effective. Moreover, their action is frequently observed only at high doses which may cause dangerous side-effects.

Δ^9-Tetrahydrocannabinol (Δ^9-THC) is a recent addition to standard antiemetic therapy marketed in the U.S. under the generic name "dronabinol". Its efficacy has been established in a number of comparative clinical studies when tested versus placebo and standard antiemetic drugs (Hart and Lopez, 1986). A comprehensive review on clinical trials and mechanisms of cannabinoids in cancer chemotherapy was recently published by Levitt (1986). Although the efficacy of THC has been proven in numerous cancer treatments, CNS side-effects such as sedation, confusion or anxiety seem to be major drawbacks to current therapy.

Since it was reported that the occurrence of side-effects is lowered by administration of low doses of THC at frequent intervals when the patients were maintained at minimum effective drug levels (Lucas and Lazlo, 1980), our idea was to prepare sustained release dosage forms which may successfully replace conventional treatment.

With the final goal being the design of a transdermal delivery system, in the present work the

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use of the skin as a non-invasive portal for THC sustained delivery was investigated for the first time. Transdermal administration permits low dosages directly to the blood stream without liver first-pass effect. Feasibility studies were undertaken to establish the route of drug penetration, to determine the permeation coefficient in vitro, and to measure blood concentration time patterns in vivo for a formulation containing THC.

Materials and Methods

Materials

We chose to use Δ^8-THC rather than Δ^9-THC for several reasons. (a) The latter is quite unstable, giving the inactive cannabinol on oxidation. In transdermal administration, which by its very nature takes place over relatively long periods of time, oxidation processes have to be taken into account. (b) Δ^8-THC is somewhat less cannabimimetic than Δ^9-THC. (c) In all pharmacological tests reported so far, Δ^8-THC exibited the same profile as Δ^9-THC. (d) As an antiemetic agent, Δ^8-THC is at least as potent as Δ^9-THC (Mechoulam and Avramov, unpublished data).

Δ^8-THC was synthesized and purified according to methods described previously (Mechoulam, 1973). Non-specific tritiated Δ^8-THC with a specific activity of 14.8 Ci/mmol and a concentration of 1 mCi/ml was prepared by Kamag-Dimona, Israel. Oleic acid was purchased from Sigma and conformed to BP requirements.

Two formulations containing THC were prepared. Formulation A contained 10% w/w oleic acid in a carrier comprised of propylene glycol, polyethylene glycol 4000, and ethanol. Formulation B was composed of the same carrier without oleic acid.

Animals

The animals used in this investigation were male, Sabra-strain rats (Hebrew University of Jerusalem) weighing 250–270 g.

Autoradiographic studies

50 μl (50 μCi) of $[^3H]\Delta^8$-THC in ethanol solution was applied to the dorsal region of rats 24 h after hair clipping (Oster, U.S.A.). Following the rapid evaporation of the solvent, the site was covered with a Hill Top cell (Hill Co., U.S.A.) and left in place for 24 h. The rat was then sacrificed; the skin area of interest was removed by means of a biopsy punch (diameter 3 mm), immersed in Tissue-Tek OCT458 (Miles Laboratories), and frozen immediately at −30 °C. The embedded skin was sectioned transversely at −30 °C using a cryostat microtome model CTI (International Equipment, U.S.A.), and the sections were fixed on slides. The slides were prepared for autoradiography by gelatinization and were exposed to Kodak Nuclear Emulsion type NTB-2 (Eastman Kodak) for 3 weeks. The autoradiographs were then developed and stained with haematoxylin and eosin (Baker et al., 1977).

THC transdermal administration in vivo

Two formulations containing 26.5 mg/g Δ^8-THC were tested in rats. 32 mg THC was applied to each rat over a total surface area of 6.5 cm^2 on both sides of the dorsal region 24 h after hair removal. Treated skin surfaces were covered with Hill Top cells which were held in place by porous tape (Kendall Co., Boston, U.S.A.) for 48 h. Blood samples were collected from the tail vein at various time intervals following topical application, and total THC derivatives (drug + metabolites) was measured by a radioimmunoassay described further. The experiments were replicated.

Skin for in vitro permeation studies

Both rat and human full thickness skin were tested. The human skin was obtained from the forearms of human volunteers using a biopsy punch with a diameter of 1 cm. The skin was immediately frozen at −20 °C before use. The rat skin used in these experiments was freshly excised from the back region of rats, 24 h after the removal of hair using a clipper (Oster, U.S.A.).

In vitro skin permeation experiments

Skin permeation of THC from preparations containing 26.5 mg drug/g final formulation was measured on human and rat skin in vertical diffusion cells (Franz cell assembly) supplied by Crown Glass Co. (New Jersey, U.S.A.). The top of the

Franz cells was covered with parafilm and aluminum foil.

The skin was mounted in the cells, and the experiments were performed according to a method described previously (Touitou, 1986). The exposed skin surface area was 1.77 cm^2 for the rat skin and 0.64 cm^2 for the human skin, and the receiver volumes were in the range of 3.0–9.5 ml. The experiments were run for 48 h at 37°C (receiver temperature). 100 μl aliquots were taken from the receiver compartment at determined intervals, mixed with Instagel scintillation cocktail (Packard, U.S.A.), and assayed in the Kontron Betamatic scintillation counter (Lumitron Scientific Instr.). The results are presented as a mean of 3 experiments.

A computer program designed for determination of kinetic parameters (lag time, flux and permeability coefficient) in permeation experiments was used to manipulate the data (Touitou, 1986; Touitou and Wartenfeld, 1987).

Radioimmunoassay for cannabinoids

The RIA used in this study was a modification of a previously published method (Cais et al., 1983) for measurement of cannabinoids. The minimum detectable concentration of THC is 2 ng/ml, and the within-day CV is 7.5%. A tritiated radioligand with a specific activity of 12.2 Ci/mmol, and a 0.1 M phosphate saline buffer, pH 7.4, containing 0.2% PVP, 0.1% Triton X-405 and 0.1% NaN_3 were used.

Aliquots of 100 μl sera were treated with 200 μl methanol to precipitate the proteins. 50 μl of supernatant, 200 μl antiserum and 200 pg/tube [^{3}H]THC were mixed and incubated for 60 min at room temperature. 100 μl from a continuously stirred suspension of dextran-coated charcoal was added to all tubes and incubated again for 10 min, and then centrifuged at 3000 *g* for 20 min. The supernatant was decanted and transferred to plastic minivials containing 4 ml scintillation liquid and counted on a liquid scintillation spectrometer for 1 min.

Results and Discussion

The autoradiographs of rat skin after topical application of [^{3}H]Δ^8-THC presented in Fig. 1 and 2 show that at the end of 24 h the most dense labelling was concentrated in the stratum corneum,

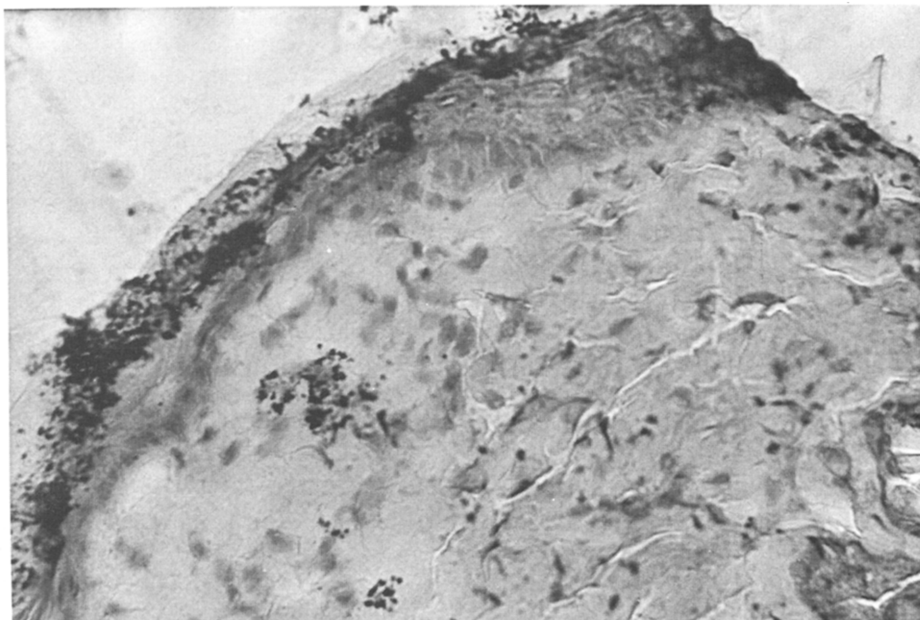

Fig. 1. Autoradiograph of rat skin section, 24 h after the application of [^{3}H]THC.

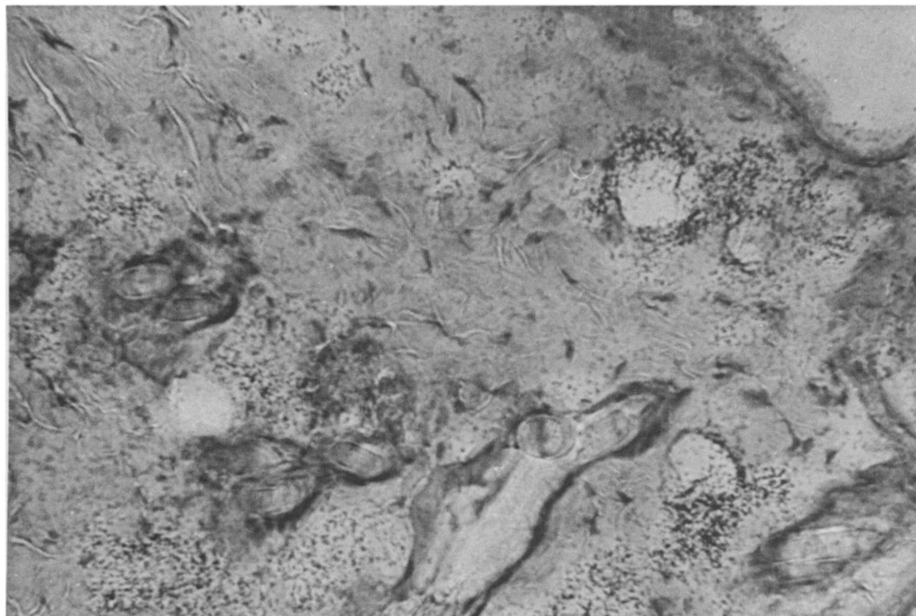

Fig. 2. Autoradiograph of rat skin section, 24 h after the application of [^{3}H]THC. Close-up of hair follicles.

in the upper epidermis and over the shaft and epithelium of hair follicles, with only a few grains overlying the corium.

This lipophilic pathway for THC is not surprising when the physicochemical properties of the drug are considered, THC being a lipophilic molecule with a partition coefficient (octanol/water) in the order of 6000 (Mechoulam, 1981). The drug penetrates the skin through the more lipophilic regions, while the hydrophilic epidermis and dermis generate a barrier for drug diffusion.

From the autoradiograph it seems that the main route of THC entry into the rat skin is the pilosebaceous system. Based on the above findings, small fluxes of THC through the skin were expected. Preliminary studies undertaken in vitro using hairless mouse skin showed that oleic acid boosts THC penetration through the skin (Touitou and Fabin, 1987). Based on these findings an enhancing formulation containing 10% w/w oleic acid (Formulation A) was designed (Touitou, 1987). For comparison, a "control" formulation, without oleic acid, was also prepared (Formulation B). The formulations were tested in rats. Fig. 3 shows the time–concentration profile of cross-reacting cannabinoids (THC + metabolites) in rat serum (mean of 12 rats) after skin application of Formulation A. From the plot in Fig. 3, it can be seen that THC was delivered systemically for 48 h from Formulation A. It is interesting to observe that the steady-state levels of cannibinoids were achieved after a period of about 17 h and remained relatively stable for about 24 h. Following the steady-state phase, the concentration slowly declined with time; moreover, relatively high levels were found in the serum until the end of the experiment. The high inter-rat variation shown in Fig. 3 may be ascribed to the binding properties of the lipoprotein fraction of the blood, its value varying among individual animals (Wahlquist et al., 1970), and possibly to differences in skin permeability.

Inconsistent absorption of the drug followed the application of the "control" formulation (B) on the skin of 5 rats; cannabinoids were detected only in the serum of 3 rats as follows. After 9 h, serum levels were 10 and 12 ng/ml in rats 3 and 4, respectively; and after 24 hours, the levels were 20, 6 and 5 ng/ml in rats 2, 3 and 4, respectively. Apart from this, no detectable drug concentrations were found with Formulation B.

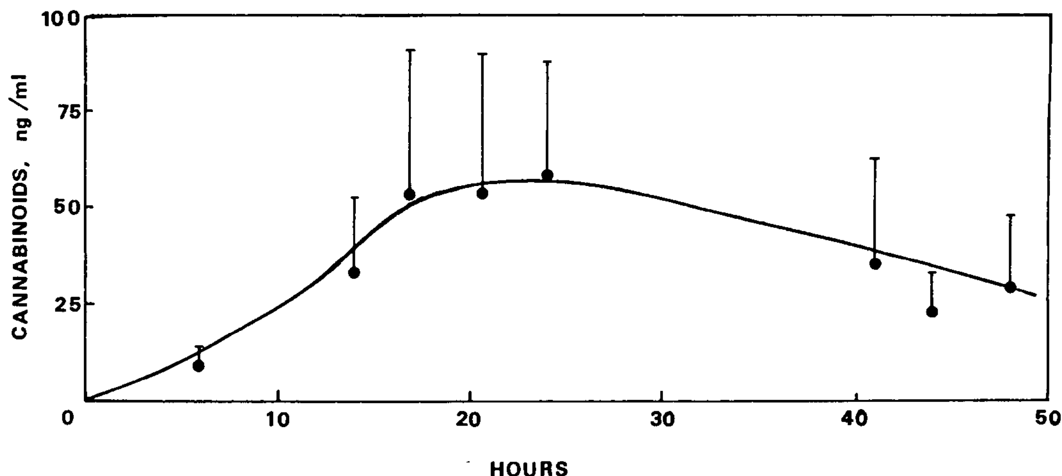

Fig. 3. Cross-reacting cannabinoid (THC + metabolites) serum levels in rats following application of Formulation A containing 26.5 mg/g Δ^8-THC (Mean ± S.D.; $n = 12$).

The permeability of THC from the enhancing formulation was further tested in vitro through human and rat skin by carrying out experiments in Franz cells. Human skin was selected for in vitro evaluation to further enable the prediction of in vivo blood levels in humans. The data presented by Bronaugh et al. (1982) and Scott et al. (1986) show that the permeability of the rat skin, respective to the human skin, varies by factors of 0.6–40 depending on the drug tested.

The skin permeation profiles and the respective kinetic parameters are given by computer outputs (Figs. 4 and 5). It is interesting to note that although classic patterns of permeation were observed for both skins, the kinetics are different. The rat skin was found to be 12.8-fold more permeable than the the human skin when tested in vitro under the same conditions, the permeability coefficient of THC through human skin having a value of 1.3×10^{-4} cm · h^{-1}. This value is two orders of magnitude smaller than that of the lipophilic molecule, octanol e.g. 5.2×10^{-2} cm · h^{-1} (Poulsen and Flynn, 1985). The differing lag times, 11.5 vs 8.5 h for the rat and human skin, respectively, point toward different diffusion coefficients and diffusional pathlengths of the drug in its pathway through the skin. Moreover, different in vivo absorption patterns are expected as a result of the differing pharmacokinetic parameters in rats and in humans.

By using the above permeability coefficient, calculated from the in vitro experiments on human skin (Fig. 5), blood levels of cannabinoids in humans following application of the transdermal formulation were predicted. Steady-state concentrations (C_{ss}) were calculated by means of Eqn. 1 (Guy and Hadgraft, 1985):

$$C_{ss} = A \cdot C \cdot K_p / V_d \cdot K_e \tag{1}$$

where A is the area of application to the skin; C is

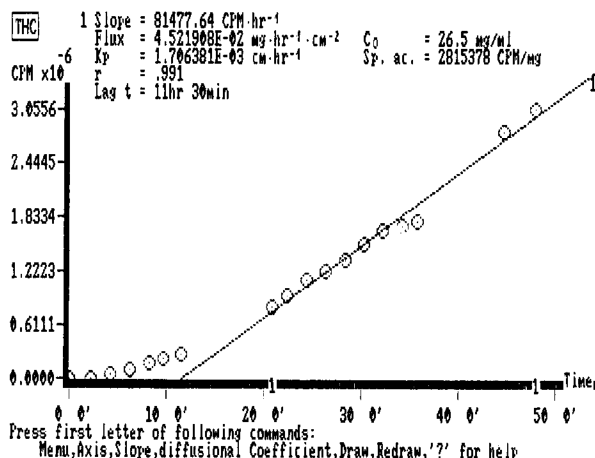

Fig. 4. Skin permeation profile and kinetic parameters of Δ^8-THC through rat skin in vitro.

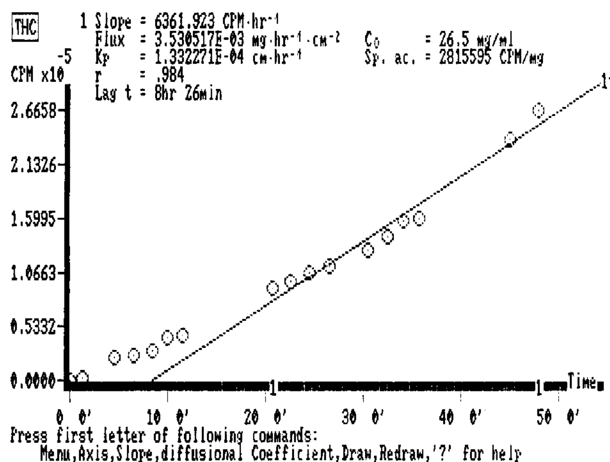

Fig. 5. Skin permeation profile and kinetic parameters of Δ^8-THC through human skin in vitro.

the initial drug concentration in the transdermal formulation; K_p is the permeability coefficient; V_d is the volume of distribution; and K_e is the elimination constant. V_d and K_e values of THC, 734 liters and 2.0×10^{-2} h^{-1}, respectively, for i.v. administration were reported by Wall et al. (1984).

In studies of the antiemetic effect of THC in cancer patients receiving high dose methotrexate, it was shown that the effect is correlated with THC plasma concentration (Chang et al., 1979). When compared with placebo, the incidence of vomiting and nausea was reduced to one-third at THC plasma concentrations of 5–10 ng/ml and was reduced to one-tenth when THC levels exceeded 10 ng/ml.

Theoretically, the transdermal flux can be directly increased by increasing the area of the transdermal system applied to the skin. Thus, for a formulation delivering THC and having a permeability coefficient of 1.3×10^{-4} $cm \cdot h^{-1}$, a blood concentration of 12 ng/ml is predicted for a system designed to have an effective area of 50 cm^2 and a drug reservoir concentration of 26.5 mg/g. This is only a coarse estimation because: (1) we are assuming that the kinetic parameters for Δ^8-THC and Δ^9-THC are the same; and (2) biotransformation of THC in its passage through the skin is not known.

The blood level profile shown following the transdermal delivery is significantly different from that obtained after oral administration of THC in man; by the oral route, a t_{max} value of 2 h and a 50% decrease in cannabinoid blood level was measured over a period of 6 h (Wall, 1984), implying a multidose administration schedule where the THC is given every 3 h.

In further clinical studies, this transdermal formulation should be applied the night before chemotherapy so that THC will have its maximum effect during the peak period of emesis.

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