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The use of liposomes in the topical application of steroids

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The effect of topical application of the androgen 5α-dihydrotestosterone (DHT), both encapsulated in liposomes and solved in acetone, has been evaluated using the female hamster flank organ as a model system. Systemic absorption of DHT was significant from the acetone solution, but negligible from the liposome system. The topical biological effect is, however, proportionally diminished when the liposome system is used. Under the experimental conditions used, the liposome system had no advantages over application in acetone in this model.

Liposomes have been used as carriers for the topical application of triamcinolone acetonide (Mezei & Gulasekharam 1980, 1982). After 5 days of application these authors obtained a drug concentration in the epidermis and dermis that was 4 times higher than that obtained using a control ointment, whereas urinary excretion was diminished. Therefore their results indicated that the use of liposomes diminished the percutaneous absorption of the drug. They claimed a sustained (gradual) release of the drug as a consequence of the direct interaction of the drug-releasing vesicles with cells at the target site. This effect may be similar to the one that has been well documented for systemic applied liposomes (Patel & Ryman 1981). In the case of intact skin the above claim implies the passage of 0·2–1·0 μm particles through the densely packed, fully keratinized, horny layer, with barrier functions responsible for preventing the diffusion of many locally applied drugs into the depth of the skin.

To find further evidence that liposomes have a selective drug delivering potential for cutaneous application, we have applied 5α-dihydrotestosterone to hamster flank organs which are sebaceous structures located one on each flank. These organs, like the sebaceous gland in man and other species, are androgen dependent. In the mature male hamster the organ measures approximately 6 mm, is heavily pigmented and is covered with coarse dark hairs. In the female it is about 2 mm in diameter, lightly pigmented, with few dark hairs. The active androgen is 5α-dihydrotestosterone and its application to the flank organ of female hamsters induces an increase in its diameter and size (Takayasu & Adachi 1972; Vermorken et al 1980). The system allows verification of the biological effect of androgens and can be used to assess the merits of drugs encapsulated in liposomes in comparison with application in traditional formulations. Assessment of the biological effects might be expected to be a more accurate means for the evaluation of the efficiency of the dosage forms than dermal concentration measurements.

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Materials and methods

Preparation of liposomes. Liposomes were prepared from DL-α-palmatoyl-phosphatidylcholine and cholesterol (Sigma Chem. Co., St Louis, MO, USA) with a molar ratio of 1·1 : 0·5 in the same way as described by Mezei & Gusalekharam (1980).

In a separate experiment, using [^{3}H]DHT (Amersham, U.K.) this washing procedure was proven to be sufficient to remove all of the unincorporated DHT. The diameter of the liposomes was about 1 μm. 20 μl of the liposomal suspension, prepared as described above, contained 40 μg DHT. A liposomal suspension containing 20 μg DHT was prepared by diluting the original suspension. To achieve a DHT-concentration of 4 μg DHT in 20 μl liposomal suspension, new liposomes were prepared. For control experiments liposomes without DHT were also prepared. Each tenth day the liposome suspensions were freshly prepared.

Flank organ test. The hamster flank organ test was carried out as described earlier (Voigt & Hsia 1973; Vermorken et al 1980). The female hamsters were separated into six groups of five animals and treated according to the following scheme: Group I: 4 μg DHT (dissolved in acetone); Group II: 20 μg DHT (dissolved in acetone); Group III: 40 μg DHT (dissolved in acetone); Group IV: 4 μg DHT (encapsulated in liposomes); Group V: 20 μg DHT (encapsulated in liposomes); Group VI: 40 μg DH (encapsulated in liposomes). The animals were treated once daily (five days a week). After 28 days of treatment the flank organs were excised for morphometrical and histochemical examination according to the method of Goos et al (1982). Results are reported as mean ± s.e. Data were analysed by using Student's *t*-test to determine the difference between two means for paired observations on the basis of a P value less than 0·05 ($\alpha = 0{\cdot}05$).

Results and discussion

After topical application of 4 μg 5α-dihydrotestosterone (DHT) to the left flank organ of female hamsters, the diameter of the pigmented spot doubled to a value of

6 mm (Fig. 1). This effect is obtained by application of 4 μg DHT and the diameter did not increase with higher doses. The form of application (a solution in acetone or a suspension in the form of liposomes) did not influence this phenomenon.

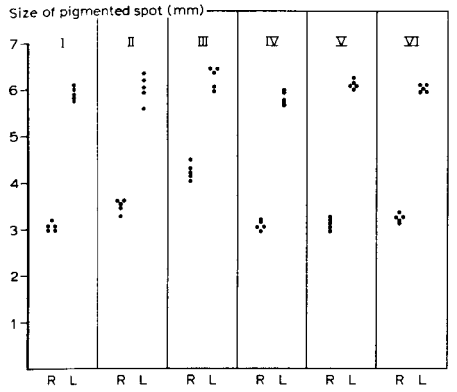

FIG. 1. Diameter of the left (L, treated) and right (R, control) pigmented spot of groups of 5 female hamsters in relation to the concentration of 5α-dihydrotestosterone (DHT) and to the vehicle used. The groups are treated as described in Materials and methods.

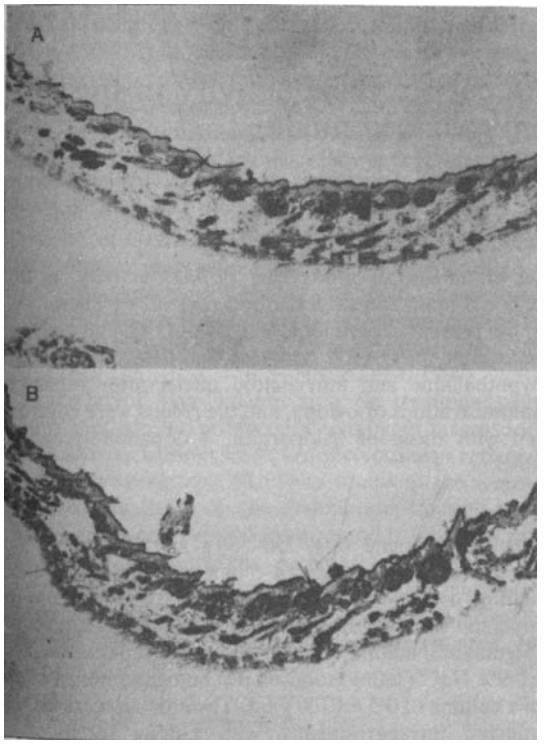

FIG. 2. Effect of the application of 5α-dihydrotestosterone (DHT) to the contralateral flank organ. A, Application of 40 μg DHT in liposomes. B, Application of 40 μg DHT in acetone.

The application of 20 μg DHT and especially 40 μg DHT in acetone resulted in an increase in diameter of the pigmented spot of the contralateral organ (Fig. 1). This effect did not occur when the DHT was encapsulated in liposomes.

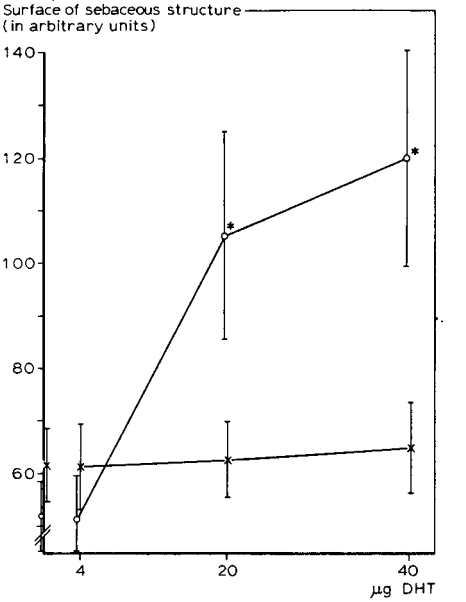

FIG. 3. Effect of increasing concentrations of 5α-dihydrotestosterone (DHT) on the size of the untreated control flank organ in relation to the various vehicles used: ○ — ○, acetone (results are expressed as mean ± s.d.); × — ×, liposomes; * = significant difference on the basis of $\alpha = 0{\cdot}05$.

The increase in size of the total sebaceous gland structure is a more specific indication of the stimulatory properties of androgens than the increase of the pigmented spot alone (Goos et al 1982). Under the influence of DHT the female flank organ increases in size to resemble the male flank organ. The effect can be quantified by measuring the area of the sebaceous structure, and the extent of the increase will depend on the amount of the active agent reaching the target site.

The application of 20 and 40 μg DHT in acetone results in an increase in size of the sebaceous glands of the contralateral flank (Figs 2, 3). This increase is significant ($\alpha = 0{\cdot}05$), both in comparison with the totally untreated flank organ (DHT-concentration = 0) and those of animals treated with 4 μg DHT in acetone or 20 or 40 μg DHT in liposomes. Since such an increase cannot be the result of a direct diffusion of the androgen from the skin surface to the sebaceous gland it must be indirect and it is highly likely it is acetone that allows DHT to reach the lower layers of the skin where it is absorbed in sufficient amount to affect the untreated flank organ since the effect does not occur when DHT is applied in liposomes (Fig. 3). When DHT is applied

directly to the flank organ, the sebaceous structures increased in size, the effect of 20 and 40 μg DHT solved in acetone being greater than that obtained with the same amount of DHT encapsulated in liposomes (α = 0·05).

Mezei & Gulasekharam (1980, 1982) held that the use of cutaneously applied liposomes would diminish systemic side-effects of encapsulated drugs. This effect, however, can only be partly affirmed by our experiments since although the stimulation of the untreated flank organ after androgen application occurs only after application of DHT in acetone, the drug in liposomes had a smaller effect than that applied in acetone. Yet, in the experiments of Mezei & Gulasekharam (1980, 1982), a four times higher concentration of the tested substance was found when liposomes were used. Several factors might cause the discrepancy between the work of Mezei and Gulasekharam and the present report: (i) different steroids were used; (ii) Mezei & Gulasekharam measured the concentration of the test substance in the skin whereas we measured the biological effect; (iii) different species of animals were used and large differences in percutaneous absorption do occur between different species (Bartek & LaBudde 1975); (iv) the schemes of application were different.

Since our results indicate that a liposome formulation shows a diminished systemic absorption in parallel with a reduced biological effect, we may conclude that DHT when applied in a liposome formulation in our model shows no percutaneous advantages in comparison with more conventional delivery systems.

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Effect of L-dopa on glutamate decarboxylase activity in the hypothalamic and amygdaloid nuclei

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Repeated administration of L-dopa methylester produced a significant increase in glutamate decarboxylase (GAD) activity without pyridoxal-5'-phosphate in the lateral hypothalamic area and medial amygdaloid nucleus. The effect of L-dopa on GAD activity was opposite to that of haloperidol in the lateral hypothalamic area.

L-Dopa is widely used in the treatment of Parkinson's disease, in which there is a deficiency of GABAergic neurons as well as dopaminergic neurons (Lloyd et al 1976). Moreover, many experiments suggest that a strict functional relationship exists between GABAergic and dopaminergic neurons in mammalian central nervous systems. The interaction between the GABAergic and dopaminergic systems, however, has been studied mainly in the extrapyramidal system. Although the hypothalamic and amygdaloid nuclei contain GABAergic neurons and dopaminergic terminals, limited investigation has been carried out on the possible GABA-dopamine interaction in these areas. In addition, L-dopa treatment has produced a different effect on glutamate decarboxylase (GAD) activity in the regional brain areas (Di Giorgio et al 1979). Therefore, in the present experiment, activity of GAD, an enzyme reponsible for GABA synthesis, was determined in the hypothalamic and amygdaloid nuclei after repeated administrations of L-dopa, and the results were contrasted with those of haloperidol, a dopamine-receptor blocker.

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Methods

Wistar-King male rats, 250–330 g at the start of the experiment, were housed 4–5 in a cage under standard lighting conditions and maintained with free access to food and water in the home cages. L-Dopa methylester (Sigma) and haloperidol (Dainippon) were dissolved in a 0·9% NaCl (saline) solution just before administration in a volume of 0·5 ml/100 g rat. The animals were either injected intraperitoneally with L-dopa methylester (100 mg kg^{-1}) or with haloperidol (1·5 mg kg^{-1}) twice daily at 8.00 and 20.00 h for 10 consecutive days. The control group was injected with a corresponding amount of saline. The rats were decapitated 60 min after