ELSEVIER

International Journal of Pharmaceutics 194 (2000) 125–134

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

New topical antiandrogenic formulations can stimulate hair growth in human bald scalp grafted onto mice

Amnon Sintov [a,*], Sima Serafimovich [b], Amos Gilhar [b]

[a] *Ben-Gurion University of the Negev, The Institutes for Applied Research, PO Box 653, Beer-Sheva 84105, Israel*
[b] *Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 31096, Israel*

Received 8 May 1999; accepted 18 October 1999

Abstract

The purpose of this study was to test the ability of topical formulations of finasteride and flutamide to re-enlarge hair follicles in male-pattern baldness. This was evaluated by an experimental model of human scalp skin graft transplanted onto SCID mice. A comparison was made between formulations containing finasteride and flutamide, and a vehicle formulation in terms of the mean hairs per graft, length, diameter of the shafts, and structures of the growth stages of the hair. Flutamide and finasteride had a significantly higher effect ($P < 0.05$) than the placebo in all the tested parameters, but flutamide demonstrated more hair per graft and longer hair shafts than finasteride ($P < 0.05$). The number of hairs per graft for flutamide and finasteride groups were 1.22 ± 0.47 and 0.88 ± 0.95 hairs/0.5 mm2 graft, respectively, versus 0.35 ± 0.6 hairs/graft for vehicle-treated graft. Similarly, hair lengths for flutamide and finasteride were 5.82 ± 0.50 and 4.50 ± 0.32 mm, respectively, versus 2.83 ± 0.18 mm for the vehicle-treated grafts. An in vitro diffusion study of flutamide gel using hairless mouse skin demonstrated the beneficial effect of the vehicle composition in comparison with a hydroalcoholic solution or a gel containing no penetration enhancer. It is therefore suggested that this topical composition containing flutamide or finasteride may effectively result in regression of male-pattern baldness.

Keywords: Androgenetic alopecia; Flutamide; Finasteride; Topical drug delivery; Skin permeation; Mice

1. Introduction

Testosterone metabolites exert a significant hormonal influence on hair growth by interacting with receptors at the follicular papilla. It has long been known that an increased susceptibility of scalp follicles to these androgens is the main cause of androgenetic alopecia (or male-pattern baldness) in genetically predisposed individuals (Imperato-McGinley et al., 1974; Ebling et al., 1991). In this type of alopecia, scalp follicles exhibit increased levels and activity of scalp 5α-reductase isoenzyme, which converts testosterone (T) to dihydrotestosterone (DHT) (Bingham and Shaw, 1973; Schweikert and Wilson, 1974). Taken together, increased conversion of T to DHT and

* Corresponding author. Tel.: +972-7-6472709; fax: +972-7-6472960.
E-mail address: asintov@bgumail.bgu.ac.il (A. Sintov)

0378-5173/00/$ - see front matter
PII: S0378-5173(99)00359-2

increased DHT binding capacity in bald scalp as compared to hairy scalp (Sawaya et al., 1989) provide a mechanistic explanation for androgenetic alopecia. DHT shortens the hair cycle and progressively miniaturizes scalp follicles. The miniaturized follicles all remain present and thus the possibility of reversal by re-enlargement exists. It is reasonable, therefore, to suppose that by administration of 5α-reductase inhibitors and/or non-steroidal antiandrogens, this reversal should occur.

Finasteride, a 4-azasteroid inhibitor of 5α-reductase, was introduced by Merck in 1989. Finasteride is known to inhibit the prostate 5α-reductase isoenzyme type 2 more effectively than type 1 isoenzyme predominantly found in the skin of the scalp. However, while type 1 isoenzyme is located in the sebaceous glands, there is still significant activity of type 2 isoenzyme in the hair follicles (Sawaya and Price, 1997). This is, therefore, the reason why finasteride decreased the level of DHT in bald scalps after a long-term oral administration (Diani et al., 1992; Dallob et al., 1994); it also provides the justification for the topical mode of delivery. It should be emphasized that oral finasteride has already been introduced as an effective hair growth treatment, with only minor systemic adverse effects. Nevertheless, systemic therapy for a disorder such as male-pattern baldness is obviously not the treatment of choice if the option of topical delivery is available option.

Another agent with a hair growth potential is the nonsteroidal anti-androgen flutamide. This drug, produced by Schering-Plough, was introduced as a new potent compound for treatment of prostatic carcinoma (Martindale, 1993). The systemic administration of flutamide causes several unwanted side effects, such as reducing libido and impairing spermatogenesis in men and feminizing male fetuses in pregnant women. Topical administration, therefore, is an important goal for such a drug, especially if indicated for skin disorders. In a comparative study, Chen et al. (1995) showed that topical administration of finasteride (in ethanol/propylene glycol vehicle) caused local inhibition of androgen-controlled sebaceous gland growth in hamster flank organ and that had a similar action to that of the same doses of flutamide. To date, clinical studies have not been performed for testing the efficacy of topical flutamide in male-pattern baldness. It is likely that the success (i.e. effective with minimal systemic exposure) of this drug would be dependent on a well-designed vehicle that would increase skin accumulation and decrease percutaneous absorption.

In this paper, we present a new topical base formulation for finasteride and flutamide (representing two anti-DHT categories). We studied the effect of the topical preparations of these two compounds on the growth of human hair in a murine transplantation model. The effect was monitored in scalp skin biopsies taken from bald subjects before plastic surgery procedures. This model which has been described previously by Gilhar et al. (1988), Van Neste (1996) and De Brouwer et al. (1997), is specific to male-pattern baldness, in which hairs of the bald skin graft do not re-enlarge after transplantation, while the hair of grafts taken from patients with alopecia areata (an auto-immune problem) begin to grow shortly after transplantation (Gilhar and Krueger, 1987).

To correlate the pharmacological efficacy of the new drug-vehicle system with its cutaneous penetration properties, topical preparations containing flutamide were tested in vitro using excised hairless mouse skin.

2. Materials and methods

2.1. Formulation

Gel preparations containing 1% of flutamide (Eulexin, Schering-Plough Lab., Belgium) or finasteride (Proscar®, Merck Sharp & Dohme, UK) were produced as follows. The drug was dissolved in ethyl alcohol (30% w/w in the final gel for flutamide, and 58% w/w in the final gel for finasteride); then 1% glyceryl oleate (as an enhancer) and distilled water were added gradually with mixing. The solutions were finally gelled by adding 4% hydroxypropyl methylcellulose (for flutamide) or ethylcellulose (for finasteride). A vehicle corresponding to the flutamide formula-

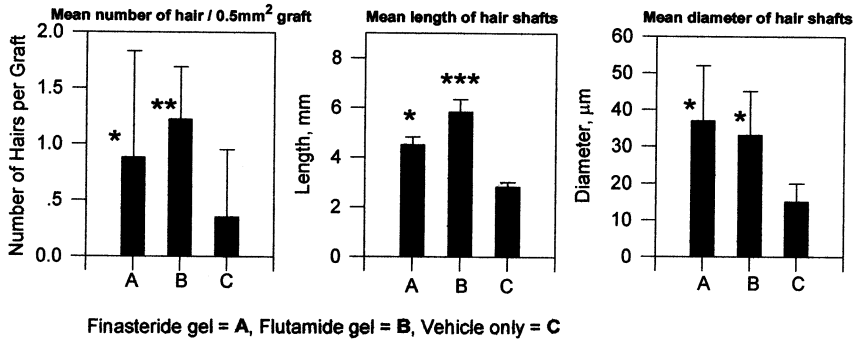

Fig. 1. The density of hair in human grafts, the length and the diameter of hair shafts after 60 days of treatment with topical finasteride, flutamide and a vehicle. $n = 16$ (Finasteride); $n = 9$ (flutamide); $n = 17$ (vehicle). *, $P < 0.05$ versus vehicle; **, $P < 0.01$ versus vehicle; ***, $P < 0.001$ versus vehicle.

tion but containing no drugs was prepared for the purpose of in vivo comparison. In addition, a 1% flutamide formulation without enhancer was prepared and tested in vitro together with the formulation containing the enhancer (as described above), and a hydroalcoholic formulation (1:1 ethanol–water).

2.2. Animals

Severe combined immune deficient mice (male Prkdc SCID-Charles River, UK), 2–3 months of age, were used in this study. The mice were grown in a pathogen-free animal facility.

2.3. Skin grafting

Punch grafts, 0.5mm^2, obtained from scalp skin of five bald men were used for transplantation to the SCID mice (three grafts per mouse). The transplantation procedure was performed as previously described (Gilhar et al., 1988). Each graft was inserted, through an incision in the skin, into the subcutaneous tissue over the lateral thoracic cage of each mouse, and covered with a standard band aid dressing. The dressing was removed on day 7, and the grafts, which were located at the surface, were treated from day 8 for 60 days as described below. The procedure protocol related to animals was reviewed and approved by the Institutional Animal Care and Use Committee.

2.4. Treatment

Specimens of each topical preparation, 20–30 mg, were spread gently over each transplanted

Table 2
Serum T/DHT levels (in nmol/l)[a]

Group	T	DHT
Finasteride	7.2 ± 6.9	0.91 ± 0.57
Flutamide	5.8 ± 3.1	1.06 ± 1.62
Vehicle (control)	8.9 ± 7.4	1.10 ± 1.02

[a] No difference between groups was found for T or DHT ($P > 0.05$).

Table 1
Distribution of the histological hair structures in the treated grafts

	Anagen (%)	Catagen (%)	Telogen (%)	T+C (%)
Before treatment	0	35.7	64.2	100%
Finasteride	30.4	22.8	46.8	69.6
Flutamide	47.0	26.5	26.5	53.0
Vehicle (control)	10.5	24.6	64.9	85.5

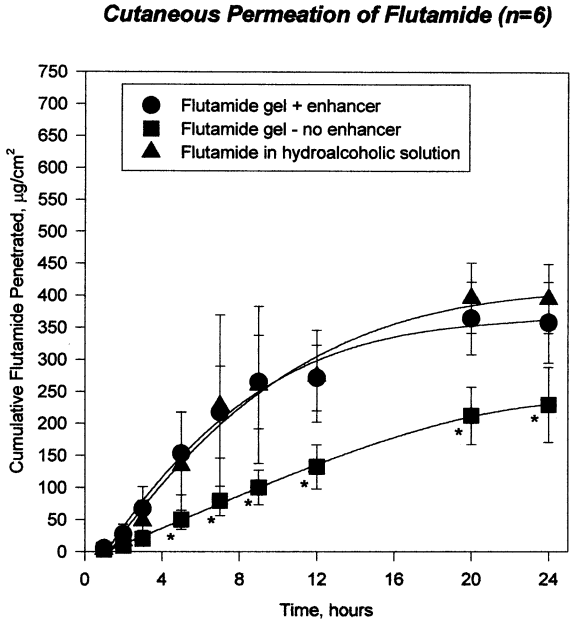

Fig. 2. In vitro skin penetration of flutamide across hairless mouse skin after topical application of 50 mg/1.767cm^2 of a flutamide gel with or without the enhancer, glyceryl oleate, and a flutamide hydroalcoholic solution. *, $P < 0.05$ versus all other treatments.

graft (not more than a total of 100 mg per mouse). A total of 42 grafts were treated, 17 with the vehicle, 16 with finasteride gel, and nine with flutamide gel. The application was performed twice daily.

2.5. Histology

Biopsies were taken from the bald grafts before and at the end of the study. Tissue for routine histological sections was fixed in 10% neutral buffered formaldehyde solution. The biopsies were sectioned horizontally at the sebaceous glands layer. The sections of these biopsies were stained with hematoxylin and eosin, and examined under light microscopy.

2.6. Measurement of hair growth

The number of hairs per graft and the hair lengths were determined under $\times 5$ magnification. The hair shaft diameters were measured microscopically in the horizontal sections with the aid of a calibrated ocular micrometer. Hair structures in the histological specimens were counted.

2.7. In vitro permeation testing

The in vitro diffusion of a topical drug through skin (in which the flux of the drug molecules through human cadaver or animal skin is determined) was performed basically according to the FDA guidelines (Skelly et al., 1987). Based on the large number of penetration experiments that have been carried out with fresh skin excised from hairless mice, this model skin is conveniently accepted for skin penetration studies although its relevance to percutaneous penetration in man is controversial (Simon and Maibach, 1998).

In the present study, we used flutamide as the model drug to evaluate the properties of the vehicle composition to penetrate drugs (see Formulation). Flutamide permeability through hairless mouse skin was measured in-vitro with a Franz diffusion cell system (Crown Bioscientific, Clinton, NJ, USA). The diffusion area was 1.767 cm^2 (15 mm diameter orifice), and the receptor compartment volumes varied between 11.1 and 12.1 ml. The solutions on the receiver side were stirred by externally driven, Teflon-coated magnetic bars. Sections of full-thickness hairless mouse (CD1, male, 6–7 weeks old, Weizmann Institute, Rehovot) abdominal skin were excised from the fresh carcasses of animals killed with ethyl ether. Subcutaneous fat was removed with a scalpel, and the skin sections were mounted in the diffusion cells. The skin was placed with the stratum corneum facing up on the receiver chambers, and then the donor chambers were clamped in place. The excess skin was trimmed off, and the receiver chamber, defined as the side facing the dermis, was filled with phosphate buffer (4 mM, pH = 7.4). After 30 min of skin washing performed at 37°C, the buffer was removed from the cells. Gel specimens (each 45–55 mg) were carefully spread over the skin with a gloved finger, and the receiver chambers were filled with phosphate buffer (4 mM, pH 7.4)-ethyl alcohol (analytical grade) (1:1). Samples (2 ml) were withdrawn from the receiver solution at predetermined time intervals,

and the cells were replenished to their marked volumes with fresh buffer–ethanol solution. The samples were kept at 4°C until analyzed by HPLC. After 24 h of diffusion, the exposed skin was carefully cut, and the remaining gel was rinsed with buffer. The skin sections were extracted into 1 ml ethyl alcohol at 50°C for 1 h, and the extracts were analyzed for flutamide by HPLC. As a result of the sampling of large volumes from the receiver solution-and the replacement of these amounts with equal volumes of buffer-the receiver solution was constantly being diluted. The cumulative drug permeation (Q_t) was therefore calculated from the following equation:

$$Q_t = V_r C_r + \sum_{i=0}^{t-1} V_s C_i$$

where C_t is the drug concentration of the receiver solution at each sampling time, C_i is the drug concentration of the ith sample, and V_r and V_s are volumes of the receiver solution and the sample, respectively. Data were expressed as the cumulative flutamide permeation per unit of skin surface area, Q_t/S ($S = 1.767$ cm^2).

2.8. HPLC analysis of flutamide in the receiver medium

Aliquots of 10 μl from each vial were injected into the HPLC system, equipped with a prepacked C18 column (Lichrosphere 60 RP-select B, 5 μm, 125 × 4 mm). The detection of flutamide was carried out at 210 nm. The samples were chromatographed using an isocratic mobile phase consisting of 80:20 acetonitrile–water. A flow rate of 1 ml/min was used. A calibration

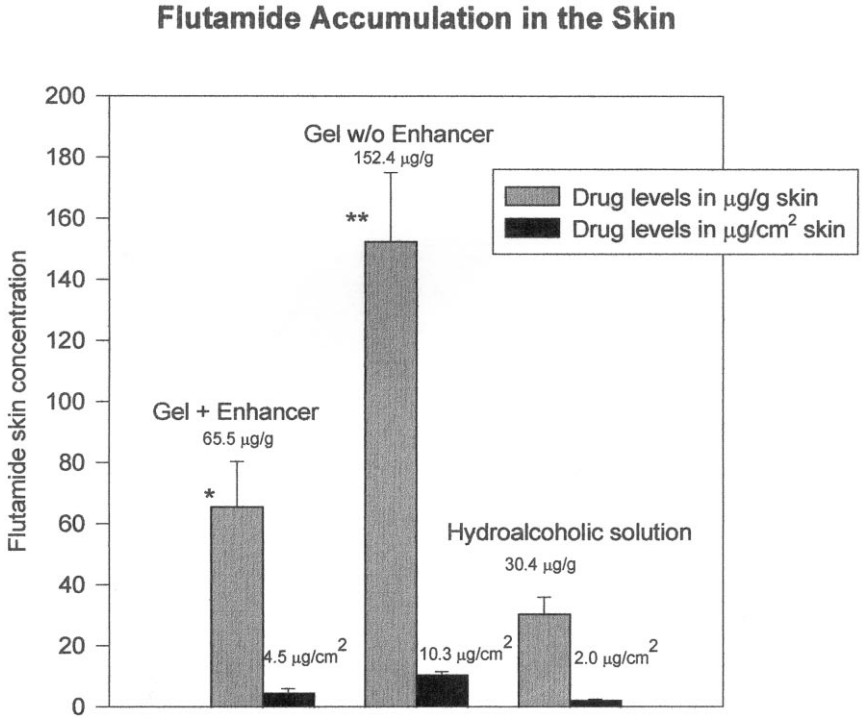

Fig. 3. Accumulation of flutamide in the mouse skin after 24 h from application of flutamide gels (with and without the enhancer, glyceryl oleate) and a hydroalcoholic solution. The values are presented in μg drug per g skin tissue and in μg drug per cm^2 skin surface area. As shown, a correlation exists between the values calculated as a function of the penetration area and the tissue weight. *, $P < 0.05$ versus all other treatments; **, $P < 0.01$ versus all other treatments.

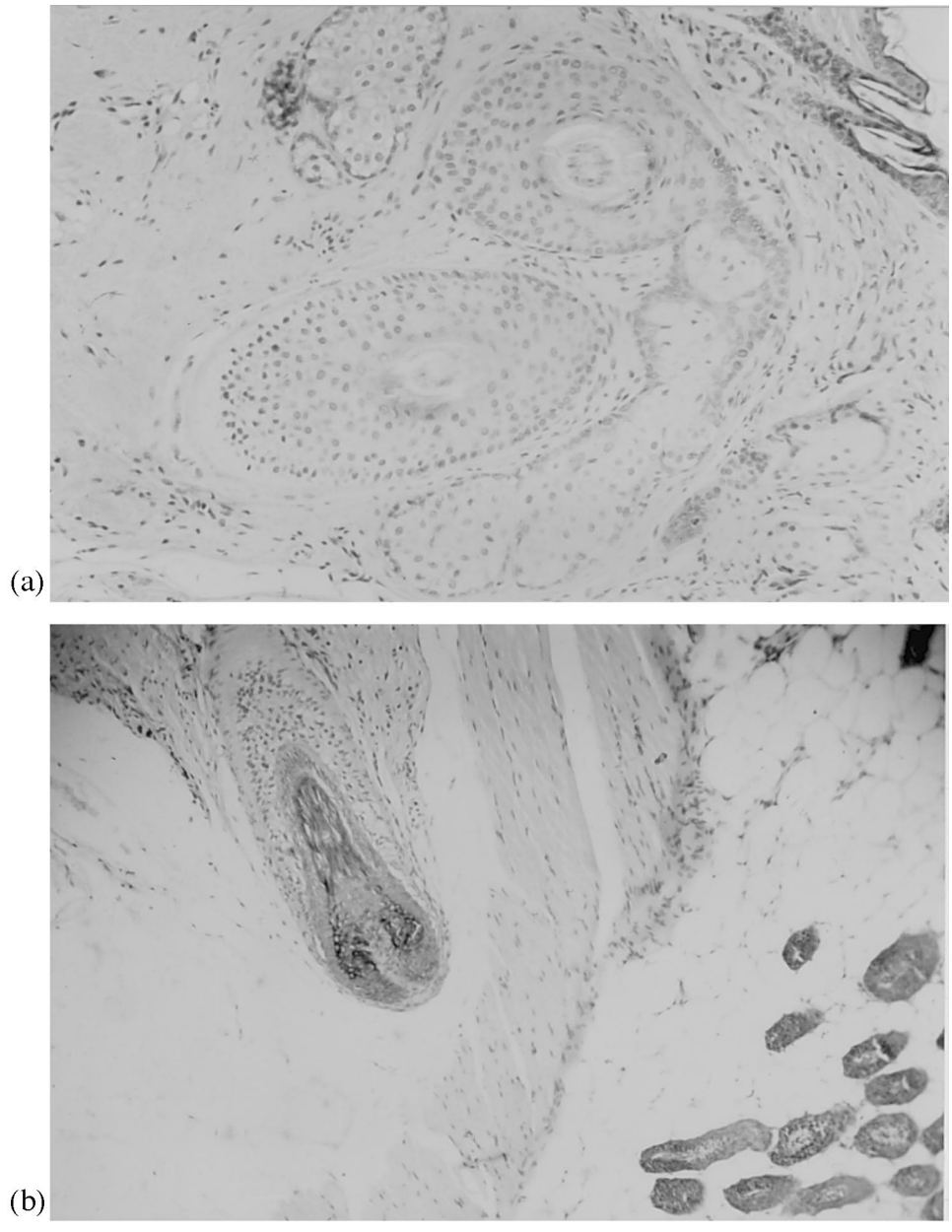

Fig. 4. Photomicrographs of horizontal sections of skin grafts on mice, (a) treated with finasteride, demonstrating human anagen follicles and sebaceous glands (HE x128); (b) and (c) treated with flutamide, demonstrating normal anagen follicles alongside mouse follicles (HE x80-B, x20-C); (d) treated with vehicle, showing telogen follicles. Normal anagen follicles were not found (HE x800).

curve (peak area vs. drug concentration) was constructed by running working standard solutions in buffer (pH 7.4)–ethanol for every series of chromatographed samples. Calibration curves over the range of 0.5–50 μg/ml (0.5, 1, 3, 5, 7, 10, 15, 20, 50 μg/ml) were linear.

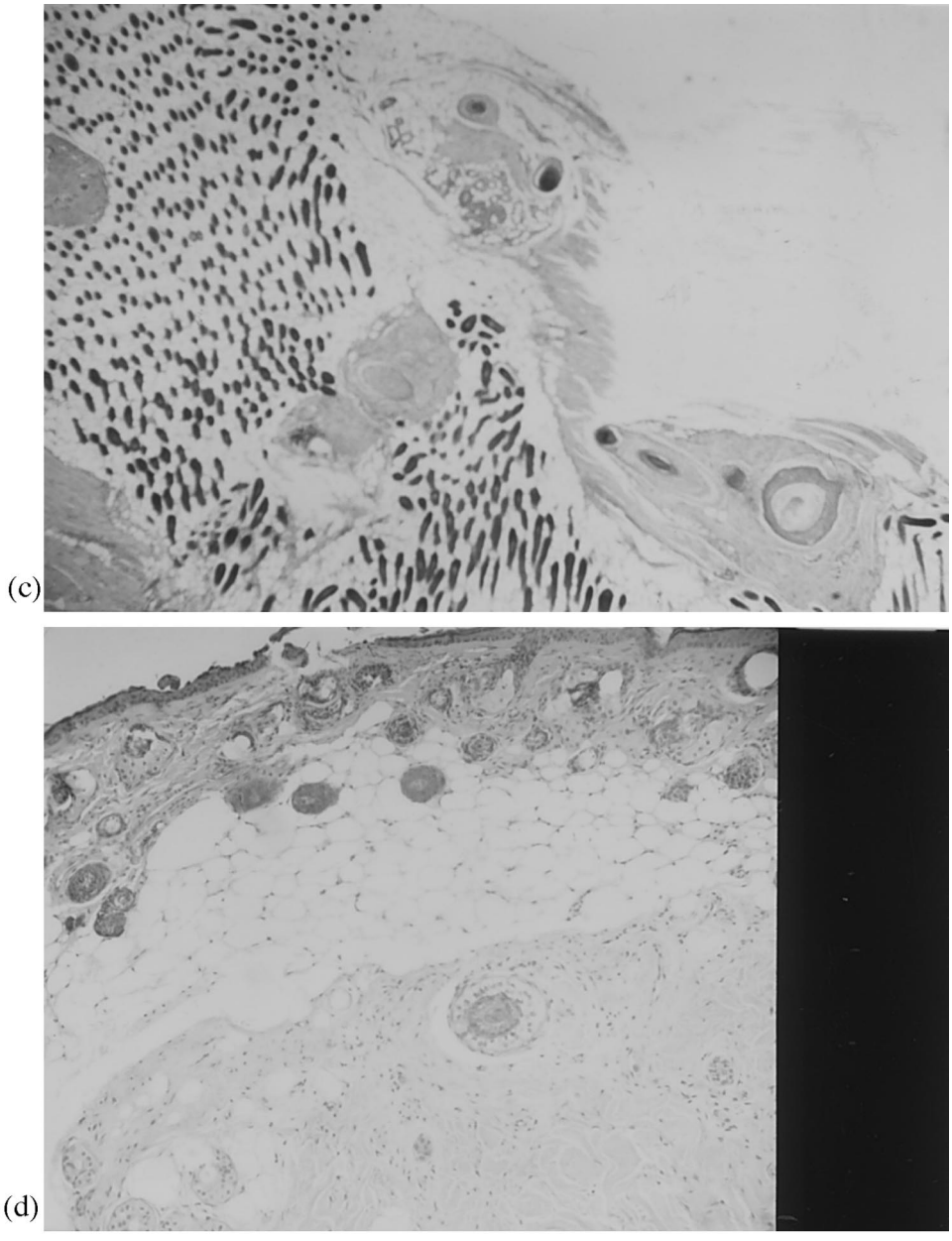

Fig. 4. (*Continued*)

2.9. Testosterone/dihydrotestosterone plasma monitoring

The determination of the steroids was performed in plasma using T/DHT[^{3}H] Biotrak radioimmunoassay system (Amersham, England).

2.10. Statistical analysis

The one-way analysis of variance (ANOVA) was used to test the variation among the three groups, i.e. two drug-treated groups and one vehicle-treated group. Bartlett's test for the homo-

geneity of variances was also applied. The Student–Newman–Keuls multiple comparison test was used for comparing the two drug-treatment groups, finasteride-treated versus flutamide-treated group, and each drug-treatment group versus the vehicle-treated group. ($P < 0.05$).

3. Results

3.1. Efficacy tests in human scalp-grafted mice

The two topical formulations, finasteride and flutamide gels, showed significantly greater efficacy ($P < 0.05$) than the vehicle formulation in enlarging hair length and diameter as well as in increasing the number of hairs per graft. Fig. 1 presents the results after 60 days of graft treatment with the two antiandrogenic formulations and with the vehicle. For the grafts treated with flutamide and finasteride gels, the number of hairs were 1.22 ± 0.47 and 0.88 ± 0.95 hairs/0.5 mm^2 graft, respectively, versus 0.35 ± 0.6 hairs/graft for the vehicle-treated graft. Hair lengths were 5.82 ± 0.50 and 4.50 ± 0.32 mm for the flutamide and finasteride groups, respectively, compared to 2.83 ± 0.18 mm for the vehicle-treated grafts. The diameter of the hair shafts for the two drug-treatment groups was approximately twice that for grafts treated with the vehicle alone. It should be noted that hair growth in the two treatment groups started as early as after 36 days post transplantation. It can been seen in Fig. 1 that the topical application of flutamide, representing the anti-androgenic mechanism of action, resulted in more hairs per graft and longer hair shafts than the topical finasteride (5α-reductase inhibition). The difference was statistically significant ($P < 0.05$).

Histological examination of the grafts treated with finasteride and flutamide (Table 1) demonstrated relatively more hairs in the growth (anagen) phase than in regression (catagen) and rest (telogen) phases (see also photographs a–c of Fig. 4), with some superiority of the topical flutamide over finasteride. In the two drug groups, before the treatment and in the vehicle group most if not all the hairs were in the catagen and the telogen phases (100 and 85.5%, respectively) (Fig. 4, photograph d). These findings, as shown in Table 1, support the above-described clinical results for hair growth, as presented in Fig. 1.

Table 2 presents the plasma monitoring of T and DHT. The measurements show that there was no systemic effect that might change the androgenic balance as a consequence of the topical applications of either flutamide or finasteride.

3.2. In vitro skin permeation of flutamide from the gel

Fig. 2 shows the skin permeation kinetics of the new gel formulation as compared to the same gel without glyceryl oleate and to a regular hydroalcoholic solution. Although viscosity of the gel formulations is higher than that of the solution, the gel with the enhancer provided a similar rate of flutamide transport through the skin. The transdermal properties of the gel are apparently due to the presence of the enhancer in the preparation, since the corresponding gel without enhancer exhibited significantly reduced permeability for the drug. In accordance with the cutaneous penetration kinetics of the three tested formulations, the retention of flutamide in the skin was much higher with the enhancer-free gel (152.4 ± 22.6 μg/g or 10.3 ± 1.0 μg/cm^2). In contrast, both the enhancer-containing gel and the hydroalcoholic solution showed a relatively reduced skin retention of flutamide (Fig. 3). However, while providing a similar rate of drug penetration through the skin as that provided by the hydroalcoholic solution, the enhancer-containing gel increased the retention of the drug in the skin. Thus, the gel facilitated in a twofold higher concentration of drug retained in the skin as compared to the hydroalcoholic solution (65.5 ± 14.9 μg/g or 4.5 ± 1.4 μg/cm^2 vs. 30.4 ± 5.5 μg/g or 2.0 ± 0.4 μg/cm^2).

4. Discussion

We compared the ability of topical formulations of a 5α-reductase inhibitor (finasteride) and a non-steroid anti-androgenic agent (flutamide) to

re-enlarge hair follicles in an experimental model of human scalp skin transplanted to SCID mice. Both inhibitors were effective in increasing the number of hairs per graft as well as hair length and diameter, when applied in a gel formulation containing glyceryl oleate as an enhancer. It was also found that flutamide was superior to finasteride.

As known, the receptor antagonist flutamide exhibited higher systemic toxicity after oral administration than the enzyme inhibitor finasteride. Therefore, the attempt to deliver flutamide, in particular, to the target skin of the scalp with a minimum of systemic exposure is more critical compared to finasteride delivery. According to the present study, the effective topical delivery of flutamide and finasteride for alopecia is thus feasible, giving similar (if not better) results to those obtained with oral finasteride, i.e. with no systemic side effects, as demonstrated by T/DHT monitoring. It should be noted that, although the reversal of the vellus to terminal hair can occur in both the human scalp-grafted mouse and in man, it is still difficult to predict the degree of efficacy of this treatment in clinical studies or to compare it with oral finasteride trials.

Hair growth is a cyclic process consisting of an active growth phase known as anagen, a brief involutional (regression) phase known as catagen, and a resting phase known as telogen. The miniaturization of scalp hair in male-pattern baldness results from shortening the anagen phase, leaving more hair in the catagen and telogen phases. This phenomenon was illustrated in the bald scalp grafts before treatment and after treatment with vehicle alone (Table 1). Flutamide and finasteride in topical gel vehicles increased significantly the number of hairs in the anagen phase, demonstrating a reversal of alopecia. Apart from the increase in the number of anagen hairs, the diameter of the hair shafts also increased and reached the diameter of normal hairs in a non-balding skin, which is about 30–40 μm.

In order to optimize the basic formulation and to further understand the mechanism that governs the efficacy of the chosen vehicle formulations, we also examined flutamide-containing preparations in vitro. It was shown that the gel formulation that contained glyceryl oleate as an enhancer not only aided the penetration of the drug across the skin at a rate similar to that of the hydroalcoholic solution, but also delivered the drug significantly deeper into the skin. It was obvious that the gel that did not contain glyceryl oleate represented the extreme situation in which a low rate of drug penetration was combined with a very high level of drug accumulation in the skin, apparently in its upper layers. We therefore decided to compromise to obtain a higher penetration rate leading to deeper skin accumulation. This consideration was in accordance with the general rationale behind the use of topical drugs, i.e. to reduce the systemic effect of such drugs during chronic use and to increase the local efficacy on scalp follicles by increasing the drug level at the target site.

In conclusion, it was shown that finasteride and flutamide gels had a significantly higher effect than the drug-free vehicle in all tested parameters, with flutamide (representing the anti-androgenic mechanism) giving significantly more hairs per graft and significantly longer hair shafts than finasteride (5α-reductase inhibition mechanism). Further work should be done to elucidate the penetration enhancing mechanism of glyceryl oleate in aiding the optimal delivery of anti-androgens to scalp follicles. Of course, human studies should be conducted to provide evidence of the clinical efficacy of these gel products.

References

Bingham, K.D., Shaw, D.A., 1973. The metabolism of testosterone by human male scalp skin. J. Endocrinol. 57, 111–121.

Chen, C., Puy, L.A., Simard, J., Li, X., Singh, S.M., Labrie, F., 1995. Local and systemic reduction by topical finasteride or flutamide of hamster flank organ size and enzyme activity. J. Invest. Dermatol. 105, 678–682.

Dallob, A.L., Sadick, N.S., Unger, W., Lipert, S., Geissler, L.A., Gregoire, S.L., et al., 1994. The effect of finasteride, a 5α-reductase inhibitor, on scalp skin testosterone and dihydrotestosterone concentrations in patients with male pattern baldness. J. Clin. Endocrinol. Metab. 79, 703–706.

De Brouwer, B., Tetelin, C., Leroy, T., Bonfils, A., Van Neste, D., 1997. A controlled study of the effects of RU58841, a non-steroidal antiandrogen, on human hair production by balding scalp grafts maintained on testosterone-conditioned nude mice. Br. J. Dermatol. 137, 699–702.

Diani, A.R., Mulholland, M.J., Shull, K.L., Kubicek, M.F., Johnson, G.A., Schostarez, H.J., et al., 1992. Hair growth effects of oral administration of finasteride, a steroid 5α-reductase inhibitor, alone and in combination with topical minoxidil in the balding stumptail macaque. J. Clin. Endocrinol. Metab. 74, 345–350.

Ebling, F.J.G., Hale, P.A., Randall, V.A., 1991. Hormones and hair growth. In: Goldsmith, L.A. (Ed.), Physiology, Biochemistry and Molecular Biology of the Skin. Oxford University Press, Oxford, pp. 660–696.

Gilhar, A., Krueger, G.G., 1987. Hair growth in scalp grafts from patients with alopecia areata and alopecia universalis grafted onto nude mice. Arch. Dermatol. 123, 44–50.

Gilhar, A., Pillar, T., Etzioni, A., 1988. The effect of topical cyclosporin on the immediate shedding of human scalp hair grafted onto nude mice. Br. J. Dermatol. 119, 767–770.

Imperato-McGinley, J., Guerrero, L., Gautier, T., Peterson, R.E., 1974. Steroid 5α-reductase deficiency in man: an inherited form of male pseudo-hermaphroditism. Science 186, 1213–1215.

Martindale, 1993. The Extra Pharmacopoeia, 30th Edition, The Pharmaceutical Press, London, p. 482.

Sawaya, M.E., Honig, L.S., Hsia, S.L., 1989. Increased androgen binding capacity in sebaceous glands in scalp of male-pattern baldness. J. Invest. Dermatol. 92, 91–95.

Sawaya, M.E., Price, V.H., 1997. Different levels of 5-α-reductase type I and II, aromatase, and androgen receptor in hair follicles of women and men with androgenetic alopecia. J. Invest. Dermatol. 109, 296–300.

Schweikert, H.U., Wilson, J.D., 1974. Regulation of human hair growth by steroid hormones. 1. Testosterone metabolism in isolated hairs. J. Clin. Endocrinol. Metab. 38, 811–819.

Simon, G.A., Maibach, H.I., 1998. Relevance of hairless mouse as an experimental model of percutaneous penetration in man. Skin Pharmacol. Appl. Skin Physiol. 11, 80–86.

Skelly, J.P., Shah, V.P., Maibach, H.I., Guy, R.H., Wester, R.C., Flynn, G., et al., 1987. FDA and AAPS report of the workshop on principles and practices of in vitro percutaneous studies: relevance to bioavailability and bioequivalence. Pharm. Res. 4, 265–267.

Van Neste, D., 1996. The use of scalp grafts onto nude mice as a model for human hair growth. In: Maibach, H.I. (Ed.), Dermatologic Research Techniques. CRC Press, Boca Raton, pp. 37–49.