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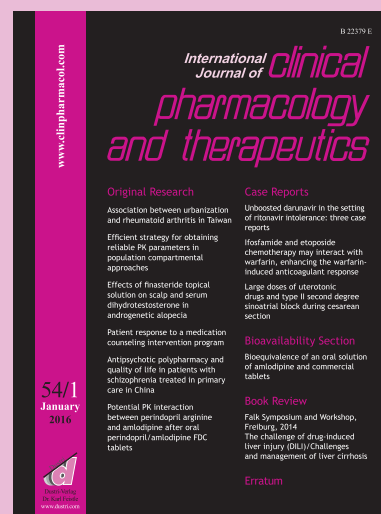
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Effects of a novel finasteride 0.25% topical solution on scalp and serum dihydrotestosterone in healthy men with androgenetic alopecia

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Key words

androgenetic alopecia – finasteride – topical treatment – type 2 5 α -reductase – dihydrotestosterone

Abstract. Objective: The effects on scalp and serum dihydrotestosterone (DHT) of different doses of a novel topical solution of 0.25% finasteride (P-3074), a type 2 5 α -reductase, were investigated in men with androgenetic alopecia. Methods: Two randomized, parallel-group studies were conducted. Study I: 18 men received 1 mL (2.275 mg) P-3074, applied to the scalp once a day (o.d.) or twice a day (b.i.d.), or 1 mg oral tablet o.d. for 1 week. Study II: 32 men received P-3074 at the dose of 100 (0.2275 mg), 200 (0.455 mg), 300 (0.6285 mg), or 400 (0.91 mg) μ L or the vehicle o.d. for 1 week. Scalp and serum DHT and serum testosterone were evaluated at baseline and treatment end. Results: Change from baseline in scalp DHT was –70% for P-3074 o.d. and approx. –50% for P-3074 b.i.d. and the tablet. Serum DHT decreased by 60–70%. The doses of 100 and 200 μ L P-3074 resulted in a –47/–52% scalp DHT reduction, similar to the 300 and 400 μ L doses (i.e., –37/–54%). A –5.6% inhibition was observed for the vehicle. Serum DHT was reduced by only –24/–26% with 100 and 200 μ L P-3074 and by –44/–48% with 300 and 400 μ L P-3074. No relevant changes occurred for serum testosterone. Conclusions: The novel finasteride 0.25% solution applied o.d. at the doses of 100 and 200 μ L results in an appropriate inhibition of scalp DHT potentially minimizing the untoward sexual side-effects linked to a systemic DHT reduction.

androgen insensitivity syndromes [1] and type 2 5 α -reductase deficiencies [4] do not experience androgenetic alopecia: these findings suggested that androgenetic alopecia is induced by activation of follicular androgen receptors by DHT [5].

Finasteride is an effective drug for the treatment of mild to moderate male androgenetic alopecia and is presently authorized and marketed as a 1-mg oral tablet in the USA and Europe [6, 7]. Finasteride selectively inhibits the type 2 5 α -reductase isoenzyme, blocking the conversion of testosterone to DHT, and, administered at the daily oral doses of 0.05–5 mg for 42 days, results in a 60–70% reduction in serum/plasma, prostate, and scalp DHT levels [8].

Finasteride was proven to be effective in the treatment of male pattern baldness in terms of stabilization of hair loss and promotion of the conversion of hair follicles into the actively growing phase [9, 10, 11]. A systematic review by Mella et al. [12] concluded that daily use of oral finasteride (1-mg tablet) increases hair count and improves patient and investigator assessment of hair appearance.

Generally, 1-mg oral finasteride is well tolerated with long-term use, although evidence from preclinical and clinical studies points to significant adverse effects of 5 α -reductase inhibitors on health and overall quality of life. Adverse sexual effects have consistently been reported [1, 13, 14]. In multiple double-blind randomized controlled trials, 1-mg oral finasteride has been associated with a significant number of sexual dysfunctions, including decreased libido (1.8%), erectile dysfunction (1.3%), ejaculation dis-

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Introduction

Androgenetic alopecia, or male pattern baldness, depends on genetic predisposition [1, 2] and on the local presence of the androgen dihydrotestosterone (DHT) [3], which is formed by testosterone reduction through the action of 5 α -reductases. Patients with

orders (0.8 – 1.2%), and orgasm disorders (0.4%) [12, 14, 15, 16]. It is likely that a lack of plasmatic DHT or another 5 α -reduced hormone is responsible for the reported decrease in libido and/or orgasm [17]. Recently, the use of 1 mg oral finasteride for the treatment of male pattern hair loss has been the focus of media and internet attention for potential irreversible sexual dysfunction and severe depression, which raises concerns about the safety of 1-mg oral finasteride [18].

Given the efficacy and a concerning side-effect profile of oral finasteride, there is potential for the development of a better-tolerated formulation for male pattern baldness.

A topical formulation of finasteride 0.25% solution (namely P-3074), in hydroxypropyl chitosan (HPCH) film-forming technology, allows finasteride to act on the scalp skin follicular portion of the bulb [19] and to promote a cutaneous depot of finasteride in the region of hair bulbs, thus minimizing systemic absorption even after repeated treatments [20]. A recent study in 24 healthy men with androgenetic alopecia [21] showed that 1-week treatment with 1 mL P-3074 applied twice daily (b.i.d.) to the scalp and with finasteride 1-mg tablet orally administered once daily (o.d.) resulted in a similar plasma DHT reduction: plasma DHT was in fact reduced by 68 – 75% with P-3074 and by 62 – 72% with the reference tablet. As expected, no relevant changes in plasma testosterone occurred with either treatment. Notably, plasma finasteride rate and extent of systemic absorption were ~ 10 – 15 times lower for the topical solution than for the tablet.

On the basis of the results of the previous study, the present set of studies aimed at further investigating the systemic and local (scalp) DHT suppressive effects of P-3074, topically applied at different dose regimens (1 mL o.d. or b.i.d., study I) and at different o.d. doses (study II) in men with androgenetic alopecia.

Primary objective of study I was to investigate whether multiple applications of 1 mL P-3074 o.d. resulted in an inhibition of scalp and serum DHT similar to that obtained with b.i.d. applications and with the 1-mg oral tablet. Study II was designed to evaluate whether lower doses of P-3074 applied to the scalp of men with androgenetic alopecia could achieve the same inhibitory effect on

scalp DHT levels, minimizing at the same time the systemic effects on serum DHT. Four doses of P-3074, as 4 different volumes (i.e., 100 μ L (0.2275 mg), 200 μ L (0.455 mg), 300 μ L (0.6825 mg), and 400 μ L (0.91 mg)) were thus investigated in terms of their effects on scalp DHT levels. In both studies, serum testosterone was also evaluated. The final aim was to optimize finasteride delivery from the new topical formulation, reducing finasteride systemic absorption and minimizing its systemic effects.

Subjects and methods

Subjects

In study I and II, 18 – 65 year-old healthy men with recession of the frontal hairline and hair loss over the frontal and vertex scalp regions (at least stage II of the Hamilton-Norwood classification scale [22]) were enrolled. All men were in good physical health, as assessed at study entry by medical history and physical examination, including electrocardiogram (ECG) recording, vital signs measurement, and routine laboratory blood and urine assays. Men with damaged scalp skin, such as abrasions, hyperkeratosis, or inflammatory disorders, were excluded. Subjects were not enrolled if they had participated in other clinical trials or donated blood in the previous 2 months, or if they had been on any medications in the 2 weeks preceding the study. All the subjects were given a detailed description of the study, and all of them gave their written informed consent before enrollment. The studies were approved by an independent Ethics Committee, Canton Ticino, Switzerland, and were performed at CROSS Research S.A., Switzerland, in accordance with the Declaration of Helsinki and the harmonized European standards of Good Clinical Practice (ICH E6 1.24).

Study design and dose regimens

Test product was finasteride 0.25% topical solution (P-3074; 2.275 mg/mL, Polichem, S.A., Switzerland). The study design was consistent across the two trials: both studies were single center, randomized,

parallel-group, pharmacodynamic studies. The randomization lists for the two studies were computer generated by CROSS Metrics S.A., Switzerland, using the PLAN procedure of the validated SAS for Windows Version 9.1.3, Service Pack 4.

In study I, which was conducted from August 3 to September 7, 2012, 18 men with androgenetic alopecia were randomly allocated to 3 treatment groups in a 1 : 1 : 1 ratio to receive, in open-label fashion, 1 mL topical solution twice daily (P-3074 b.i.d.) or once daily (P-3074 o.d.), or finasteride 1-mg oral tablet o.d. (Propecia[®], MSD S.A., Switzerland) for 1 week. Before the first application of P-3074, subjects had their scalp completely shaven. For each application, 1 mL (i.e., 2.275 mg finasteride) of the solution was homogeneously sprayed by the investigator on the whole scalp, for a total of either 7 applications (P-3074 o.d. group) or 14 applications (P-3074 b.i.d. group). The reference tablet was orally administered with 150 mL of still mineral water. 4-mm punch scalp biopsies for DHT and testosterone analysis were performed at baseline (day -17) and 6 ± 2 hours after the last multiple dose. Before each biopsy, the scalp skin was cleansed with betadine solution, and the scalp area was anaesthetized by injection of lidocaine 1%/epinephrine 10 µg/mL and sodium bicarbonate 8.4%. Punch-biopsied sites were disinfected with betadine and sutured. Blood samples for serum DHT and testosterone determinations were collected before the first application (baseline) and after 1-week treatment just before (predose, 0 hour) and at 6 and 12 hours after the last dose.

In study II (13 March – 22 April, 2014), 4 active doses of P-3074, i.e. 100 µL (0.2275 mg), 200 µL (0.455 mg), 300 µL (0.6825 mg), or 400 µL (0.91 mg), or the vehicle solution, were administered to 4 subject cohorts. Each cohort was composed of 8 subjects for a total of 32 subjects enrolled in the study. According to a computer-generated randomization list, within each cohort 6 subjects received 1 of the 4 P-3074 dose regimens, and 2 subjects received the vehicle in double blind fashion. The topical solution (P-3074 or the vehicle) was applied to the subjects' shaven scalp o.d., in the morning, for 7 days. The solution was sprayed from a distance of 8 – 10 cm, without overlapping subsequent

puffs, in order to homogeneously cover the scalp skin area. Scalp and serum concentrations of DHT and serum testosterone were determined at baseline and 6 ± 2 hours after the last application of P-3074 or vehicle topical solution. Punch scalp biopsies were collected as described for study I. Plasma finasteride was analyzed at baseline and 6 ± 2 hours after the last dose of P-3074.

The subjects were confined to the clinical center from the day of the first application or the day of the last administration (reference tablet group only, study I) up to at least 12 hours after the last dose. Final assessment was performed after completion of all study procedures.

Analytical methods

DHT concentrations in scalp/serum, testosterone concentrations in serum, and finasteride concentrations in plasma (study II only) were determined at ABL, Analytisch Biochemisch Laboratorium, Assen, The Netherlands, using fully validated LC-MS/MS methods [21, 23]. Lower quantification limits (LQL) were 0.05 ng/mL for serum DHT and testosterone, 0.25 ng/g for scalp DHT, and 0.10 ng/mL for plasma finasteride. All the analyses were conducted under blind conditions.

Pharmacodynamic and pharmacokinetic analyses

DHT concentrations in scalp and serum, testosterone concentrations in serum, and finasteride concentrations in plasma were summarized by descriptive statistics. Changes from baseline in DHT scalp levels and in DHT and testosterone serum levels after multiple doses were calculated and presented as percentage of inhibition. Individual and mean DHT scalp/serum ratios of the percentage of change were calculated for all active treatment groups (both studies). Ratios equal (or very close) to 1.0 indicate that DHT changed equally in scalp and serum, ratios above 1.0 indicate that DHT was inhibited more in scalp than in serum, and ratios below 1 indicate that DHT was inhibited less in scalp than in serum.

Table 1. Serum DHT concentrations (mean \pm SD) and changes from baseline (%) after 1 week of treatment.

| Study | Treatment group | Baseline ng/g | 1 week – last dose | | | | | |
|----------|--------------------------|------------------|--------------------|--------|-----------------|--------|-----------------|--------|
| | | | Predose | | 6 h postdose | | 12 h postdose | |
| | | | ng/g | % | ng/g | % | ng/g | % |
| Study I | Tablet, 1 mg o.d. | 0.40 \pm 0.11 | 0.12 \pm 0.05 | – 69.7 | 0.10 \pm 0.06 | – 75.9 | 0.10 \pm 0.03 | – 76.1 |
| | P-3074, 1 mL b.i.d. | 0.53 \pm 0.34 | 0.16 \pm 0.02 | – 69.3 | 0.14 \pm 0.03 | – 74.0 | 0.14 \pm 0.02 | – 73.8 |
| | P-3074, 1 mL o.d. | 0.32 \pm 0.09 | 0.10 \pm 0.02 | – 67.6 | 0.07 \pm 0.01 | – 76.4 | 0.06 \pm 0.03 | – 80.4 |
| Study II | P-3074, 400 μ L o.d. | 0.52 \pm 0.14 | NA | | 0.27 \pm 0.09 | – 47.7 | NA | |
| | P-3074, 300 μ L o.d. | 0.50 \pm 0.19 | NA | | 0.28 \pm 0.08 | – 44.1 | NA | |
| | P-3074, 200 μ L o.d. | 0.39 \pm 0.07 | NA | | 0.29 \pm 0.04 | – 26.2 | NA | |
| | P-3074, 100 μ L o.d. | 0.65 \pm 0.27 | NA | | 0.49 \pm 0.16 | – 24.4 | NA | |
| | Vehicle o.d. | 0.49 \pm 0.12 | NA | | 0.46 \pm 0.14 | – 7.1 | NA | |

P-3074: finasteride 0.25% topical solution; P-3074 1 mL = 2.275 mg; P-3074 400 μ L = 0.91 mg; P-3074 300 μ L = 0.6825 mg; P-3074 200 μ L = 0.455 mg; P-3074 100 μ L = 0.2275 mg. Tablet, 1 mg: Reference tablet, Propecia®, MSD S.A. DHT

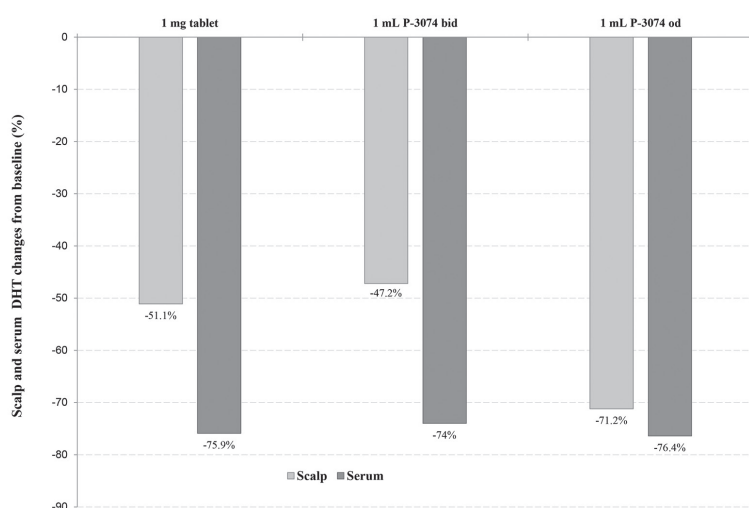


Figure 1. Mean changes from baseline (%) in scalp and serum DHT concentrations after 1 week of treatment with 1 mL P-3074 b.i.d., 1 mL P-3074 o.d., and 1-mg oral tablet o.d. – study I.

Safety profile

Safety and tolerability were assessed by physical examinations, ECG, vital signs check, routine laboratory tests, and adverse events monitoring throughout the study. Adverse events were coded by system organ class and preferred term using the medical dictionary for regulatory activities version 15.1 (study I) or 17.0 (study II).

Sample size and statistical methods

For the two studies, no formal sample size power calculation was performed. The number of 18 volunteers (N = 6/treat-

ment group) for study I and the number of 32 volunteers (N = 8 (6 active product + 2 vehicle)/cohort) for study II were regarded as being sufficient for the purposes of the studies. Baseline-corrected (i.e., changes from baseline) DHT and testosterone serum and DHT scalp concentrations at 6 \pm 2 hours after the last dose were compared between dose groups using an independent sample t-test. Individual DHT scalp/serum percentage of change ratios were compared between the active treatment groups, both within and between the two studies, using the nonparametric Wilcoxon Mann Whitney test.

Results

All the randomized subjects, i.e., 18 in study I (N = 6/treatment group) and 32 in study II (N = 6/active dose group; N = 8/vehicle group), completed the study per protocol and were included in the pharmacodynamic, pharmacokinetic (study II only), and safety analyses.

Pharmacodynamics and pharmacokinetics

Study I

After multiple-dose treatment for 1 week, a clear and similar suppressive effect of the 3 finasteride treatments on serum DHT levels was evident at all postdose assessment times: serum DHT was, in fact, reduced by ~ 69 – 74% with P-3074 b.i.d., 68 – 80%

Table 2. Scalp DHT concentrations (mean \pm SD; range) and changes from baseline (%) after 1 week of treatment.

| Study | Treatment group | Scalp DHT | | |
|----------|--------------------------|-----------------|-----------------|--------------------------|
| | | Baseline ng/g | 1 week ng/g | Change from baseline (%) |
| Study I | Tablet, 1 mg o.d. | 1.39 \pm 0.25 | 0.68 \pm 0.34 | -51.1 |
| | P-3074, 1 mL b.i.d. | 1.91 \pm 0.54 | 1.01 \pm 0.39 | -47.2 |
| | P-3074, 1 mL o.d. | 1.52 \pm 0.41 | 0.44 \pm 0.08 | -71.2 |
| Study II | P-3074, 400 μ L o.d. | 1.03 \pm 0.28 | 0.47 \pm 0.10 | -54.3 |
| | P-3074, 300 μ L o.d. | 1.03 \pm 0.14 | 0.65 \pm 0.32 | -37.2 |
| | P-3074, 200 μ L o.d. | 1.22 \pm 0.52 | 0.65 \pm 0.31 | -46.8 |
| | P-3074, 100 μ L o.d. | 1.24 \pm 0.14 | 0.59 \pm 0.10 | -52.3 |
| | Vehicle o.d. | 1.03 \pm 0.19 | 0.97 \pm 0.27 | -5.6 |

P-3074: finasteride 0.25% topical solution; P-3074 1 mL=2.275 mg; P-3074 400 μ L=0.91 mg; P-3074 300 μ L=0.6825 mg; P-3074 200 μ L=0.455 mg; P-3074 100 μ L=0.2275 mg. Tablet, 1 mg: Reference tablet, Propecia[®], MSD S.A. DHT = dihydrotestosterone.

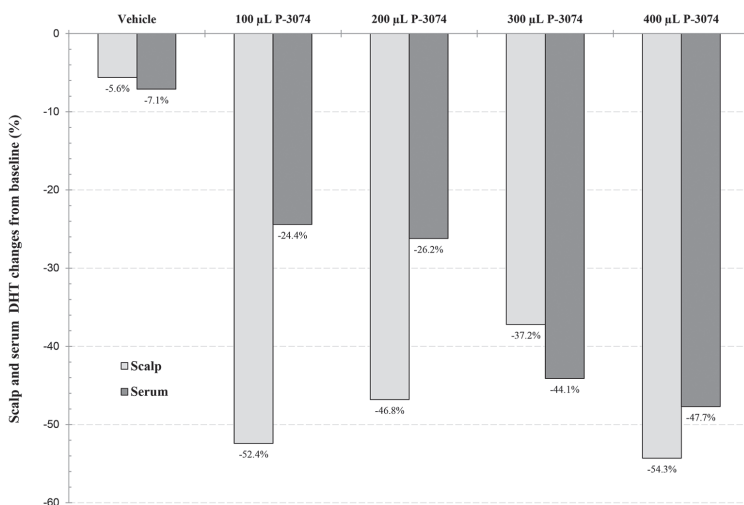


Figure 2. Mean changes from baseline (%) in scalp and serum DHT concentrations after 1 week of treatment with 100 μ L (0.2275 mg), 200 μ L (0.455 mg), 300 μ L (0.6825 mg), and 400 μ L (0.2275 mg) P-3074 o.d. and vehicle o.d. – study II.

with P-3074 o.d., and 70 – 76% with the reference tablet (Table 1) (Figure 1).

Reduction from baseline in scalp DHT was similar for P-3074 b.i.d and the oral tablet, corresponding to -47% and -51%, respectively, and more marked for P-3074 o.d. (i.e., -71%) (Table 2) (Figure 1). Notably, at the end of the 1-week treatment, scalp DHT concentrations were similar for all the subjects in the P-3074 o.d. treatment group (range 0.36 to 0.57 ng/g, coefficient of variation (CV): 18.4%) despite the quite different baseline levels (range 1.03 – 2.20 ng/g, CV: 26.8%), and ranged from 0.44 to 1.56 ng/g (CV: 39.1%) for P-3074 b.i.d. For the oral

tablet, scalp DHT concentrations at the end of the study ranged from 0.41 to 1.29 ng/g (CV: 50.6%).

No clinically relevant changes occurred for serum testosterone during the study.

Study II

After multiple-dose treatment for 1 week, serum DHT was reduced by -24.4, -26.2, -44.1, and -47.7% with 100, 200, 300, and 400 μ L P-3074 o.d., respectively (Table 1) (Figure 2). Change from baseline with the vehicle solution was -7.1%.

Percentage of scalp DHT inhibition was similar in the 4 finasteride dose groups, with values ranging from -37 to -54%. In comparison, a reduction of -5.6% was observed in the vehicle group (Table 2) (Figure 2). Differences in scalp DHT were statistically significant between each dose group and the vehicle ($p \leq 0.0249$), whereas no significant differences between P-3074 dose groups were observed.

No clinically relevant changes occurred for serum testosterone during study II.

After 1-week treatment with P-3074 at the 4 investigational doses, plasma finasteride was below the lower quantification limit for all subjects, with the exception of 1 subject in the 300 μ L dose group, who showed a low but detectable concentration (i.e., 0.13 ng/mL) at 6 hours postdose.

Studies I and II – DHT scalp/serum percentage of change ratios

Figure 3 reports the mean scalp DHT/serum DHT percentage of change from baseline ratios: The higher the reported ratio value, the better the expected safety profile due to a lower serum DHT inhibition.

Mean scalp/serum DHT reduction percentage ratios were 2.3 and 1.7 for 100 and 200 μ L P-3074, and 0.6 – 1.1 for the oral and higher topical P-3074 dose groups.

In particular, scalp DHT reduction was similar in all tested oral and topical P-3074 formulations, indicating a potentially similar efficacy profile. On the contrary, the inhibition of serum DHT differed between the lower topical (i.e., 100 and 200 μ L P-3074 o.d.) and the oral and higher topical tested doses

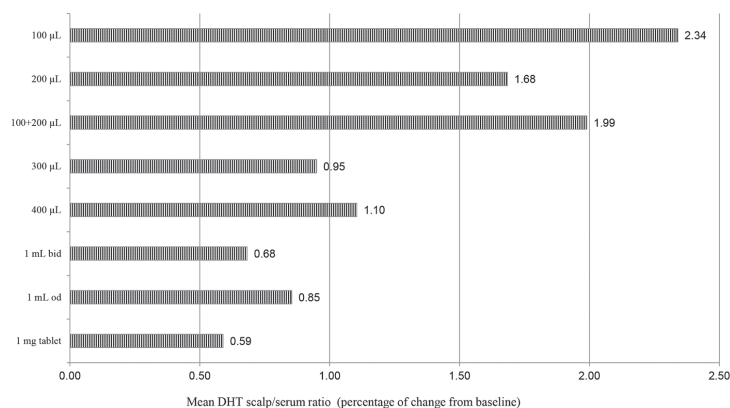


Figure 3. Mean DHT scalp/serum percentage of change from baseline ratios – study I and study II.

(i.e., 1-mg tablet, 300 µL, 400 µL, 1 mL o.d., 1 mL b.i.d. P-3074).

In order to evaluate which was the lead P-3074 dosage, able to separate the effect on scalp from the effect on serum, pair-wise comparisons among all P-3074 dose groups and the oral tested dose were performed on the efficacy/safety ratio parameter using the Wilcoxon Mann Whitney test (Table 3). The P-3074 dosages up to 200 µL were statistically different from the other P-3074 dose groups and the oral finasteride. In contrast, all the other dose group comparisons were not statistically different.

These results confirm that low doses of the topical solution (100 and 200 µL P-3074 o.d.) have a stronger inhibitory effect on scalp than on serum DHT, as opposed to the higher doses of the same topical solution (1 mL P-3074 b.i.d. and o.d.) and to the reference oral tablet (1 mg), which exhibited a stronger effect on serum DHT.

Safety

Tolerability of the topical formulation was excellent, with no signs or symptoms detected at the scalp application site throughout the studies. In study I, 3 adverse events were reported by 2 (11.1%) subjects with P-3074 (b.i.d. or o.d.), i.e., increased alanine aminotransferase, pollakiuria, and testicular pain. In study II, 5 (15.6%) subjects suffered from adverse events, namely presyncope, conjunctivitis, headache, and oropharyngeal pain. All the reported TEAEs were of mild intensity, resolved by the end of the study, and did not give rise to any safety concern.

Discussion

In men with androgenetic alopecia, after 1-week treatment (study I), the reduction in scalp DHT levels was more marked for P-3074 o.d. (approx. –70%) than for P-3074 b.i.d. and for the oral tablet (approx. –50% each). A marked decrease in serum DHT levels was found at all time-points in the 3 treatment groups, in good agreement with the data of the previous study [21] and with literature data [12, 24], which reported 60–70% reduction in serum DHT levels following treatment with different finasteride oral doses.

Moreover, application of 1 mL P-3074 o.d. for 1 week resulted in a more constant response in terms of scalp DHT inhibition, than the 1-mg reference tablet administered o.d., taking into consideration the possible intersubject variability derived from various factors, such as the different grade of baldness in treated subjects, differences in

Table 3. Comparison between treatment groups - DHT scalp/serum percentage of change ratios.

| Scalp/serum DHT | 100 µL | 200 µL | 100 + 200 µL | 300 µL | 400 µL | 1 mL b.i.d. | 1 mL o.d. | 1-mg tablet |
|-----------------|--------|--------|--------------|--------|--------|-------------|-----------|-------------|
| 100 µL | NA | n.s. | n.s. | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 |
| 200 µL | n.s. | NA | n.s. | n.s. | n.s. | < 0.05 | < 0.05 | < 0.05 |
| 100+200 µL | n.s. | n.s. | NA | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 |
| 300 µL | < 0.05 | n.s. | < 0.05 | NA | n.s. | n.s. | n.s. | n.s. |
| 400 µL | < 0.05 | n.s. | < 0.05 | n.s. | NA | n.s. | n.s. | < 0.05 |
| 1 mL b.i.d. | < 0.05 | < 0.05 | < 0.05 | n.s. | n.s. | NA | n.s. | n.s. |
| 1 mL o.d. | < 0.05 | < 0.05 | < 0.05 | n.s. | n.s. | n.s. | NA | n.s. |
| 1 mg tablet | < 0.05 | < 0.05 | < 0.05 | n.s. | < 0.05 | n.s. | n.s. | NA |

< 0.05 = statistically significant (p-value below 0.05); n.s. = not statistically significant (p-value above 0.05); NA = not applicable; DHT = dihydrotestosterone.

density of sebaceous glands, hair follicles, and blood vessels as well as differences in desquamating part of the stratum corneum, intercellular lipid matrix, and hydration level [25]. The difference in response between the o.d. and b.i.d. P-3074 dose regimens with respect to scalp DHT reduction is most likely to be ascribed to the nature of the topical formulation, containing HPCH, which forms an elastic, smooth, and almost invisible film on the scalp surface after homogeneous application, and to the finasteride release pattern from the film. Previous nonclinical studies in hairless rat skin showed that after application of the film-forming P-3074 solution, finasteride permeated through the rat skin, with total permeated drug corresponding to ~ 3.7% of the applied dose [19]. Permeated finasteride was detectable starting from 8 to 12 hours after application, and transdermal penetration lasted up to at least 24 hours postdose. This permeation pattern could be explained by a very slow release of finasteride entrapped in the HPCH matrix and/or by a slow degradation of the film and subsequent release of the drug. A similar finasteride release profile from the film was observed in the previous clinical study performed in 12 healthy volunteers with androgenetic alopecia administered P-3074 b.i.d. for 1 week [21]. In the few subjects for whom finasteride plasma concentrations were above the quantification limit of the assay, in fact, low finasteride levels were detectable starting from 16 hours after single dose application. After the last multiple dose, on average slightly higher levels were observed at 10 – 12 hours postdose. Considering finasteride-release pattern from the film and the two dose regimens of the test formulation of study I, we can deduce that the P-3074 b.i.d. application does not improve the response and might somehow impair the release of the drug from the film.

From these findings and the considerations reported above, it seems that the o.d. application of P-3074 is preferable to the b.i.d. one, leading to a better response in terms of inhibition of DHT formation in the scalp. In addition, topical o.d. application of P-3074 overcomes the limits of the oral administration, which for its action is limited by the concentration of blood vessels in the scalp skin dermis, which varies among individuals.

The findings of study I clearly showed that o.d. applications of P-3074 for 1 week already exerted a maximal effect on scalp DHT concentrations (~ 70% inhibition), suggesting that the final dose of the topical solution applied to the scalp could be significantly lower than the previously used doses. Thus, study II was designed to evaluate whether lower doses of P-3074 applied to the scalp of men with androgenetic alopecia could achieve a consistent inhibitory effect on scalp DHT levels, minimizing at the same time the systemic effects on serum DHT. Results of the study showed that P-3074 applied o.d. at the lower investigated doses resulted in a more favorable scalp/serum DHT ratio (~ 3 times higher than for the reference 1-mg oral tablet) than when applied o.d. at the higher doses. In fact, doses of 100 (0.2275 mg) and 200 μ L (0.455 mg) P-3074 applied o.d. for 1 week resulted in a scalp DHT reduction of -47/-52%, i.e., similar to the one obtained with 300 (0.6825 mg) and 400 μ L (0.91 mg) (i.e., -37/-54%), and a reduction in serum DHT of only -24/-26%. In comparison, inhibition of DHT in serum at the higher doses of 300 and 400 μ L was -44/-48%.

Notably, in the 100 and 200 μ L dose groups, percentage of subjects with reductions greater than the maximal individual reduction observed in the vehicle group (-0.22 ng/g) was 100% and 83.3%, respectively. Considering the two dose groups together, subjects with a reduction in scalp DHT greater than for the vehicle corresponded to 96.7% (11/12). This result confirms the results of study I with topical applications of 1 mL P-3074 o.d. and b.i.d. for 1 week, in which 100% of the treated subjects showed a marked reduction in scalp DHT concentrations.

The results obtained by comparing the DHT scalp/serum percentage of change ratios between treatment groups (both studies) confirmed that low doses of the topical solution (100 and 200 μ L P-3074 o.d.) have a stronger inhibitory effect on scalp than on serum DHT, as opposed to the higher doses of the same topical solution (1 mL P-3074 b.i.d. and o.d.) and to the reference oral tablet (1 mg), which exhibited a stronger effect on serum DHT. Thus, the pharmacodynamic assessment showed that the candidates for further clinical development of P-3074 are the

100 (0.2275 mg) and the 200 μ L (0.455 mg) P-3074 doses.

No relevant changes in serum testosterone were observed in any of the studies reported in the literature [8, 12, 21, 24]. Similarly, for all P-3074 dosages tested in the two studies presented in this paper, no relevant changes occurred for serum testosterone, also considering the marked interindividual variation in testosterone levels both at baseline and at all postdose assessment times.

Study II results on finasteride plasma levels confirmed a negligible finasteride systemic exposure at the doses of 100 – 400 μ L P-3074 administered o.d. for 1 week.

The two studies also confirmed a favorable safety and tolerability profile of P-3074, applied to the scalp of healthy men with androgenetic alopecia b.i.d. or o.d. However, as also previously discussed [21], the safety profile of the study products could not be properly investigated, considering the short-term treatment of the present studies (i.e., 1 week) as opposed to the clinical practice long-term treatment of months or years.

Study I was open-label whereas study II was double-blind. For both studies, however, the end-points were based on objective assessments, i.e., the determination of DHT and testosterone levels in the study samples by blinded analysts. In addition, to increase the reliability of the study end-points, the subjects were confined in the clinical center under the supervision of clinical staff either during the entire treatment and evaluation period (all P-3074 treatment groups, both studies) or for blood sample and scalp biopsy collections (reference tablet group, study I).

In conclusion, results of the two studies suggest the further development of P-3074 applied o.d. at the lower doses of 100 (0.2275 mg) and 200 μ L (0.455 mg), which result in an appropriate inhibition of scalp DHT potentially minimizing the untoward sexual side-effects linked to a systemic DHT reduction.

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Conflict of interest

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the submitting author) and declare: M.C. and R.P. are employees of Polichem S.A.; M.R., C.L., and E.T. are employees of CROSS Alliance group. M.I. is a consultant in Dermatology. Polichem S.A., Switzerland, funded this study. The relationships between the Sponsor, M.I., and CROSS Alliance group were regulated by financial agreements.

R.P. and M.C. made substantial contributions to the conception and design of the study and reviewed and approved the final draft of the manuscript. M.R. was the Principal Investigator at the clinical site. C.L. is the author of the clinical study reports and of the manuscript. M.I. reviewed the final draft of the manuscript. E.T. was study I clinical coordinator.

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