BIOalternatives
State-of-the-art laboratory.

Proposal n°: AD070315C-2

Study n°: AD070315

# EFFECTS OF CLEAROGEN ACNE LOTION AND REVIVOGEN SCALP THERAPY ON TESTOSTERONE METABOLISM IN RECONSTRUCTED HUMAN EPIDERMIS

**STUDY REPORT AD070315**


**Study client:** **ADVANCED SKIN AND HAIR Inc.**
Dr. Alan SHARGANI
12121 Wilshire Blvd
Suite 1012
LOS ANGELES CA 90025
USA
E-mail: alan@clearogen.com


Date: July 6th, 2007

The investigators and the author of this report hereby certify the validity of the data presented and attest their full agreement with the conclusions presented at the end of the report.

Certified by:

Name: Franck JUCHAUX

Position: Study director

Date: July 6th, 2007

Signature

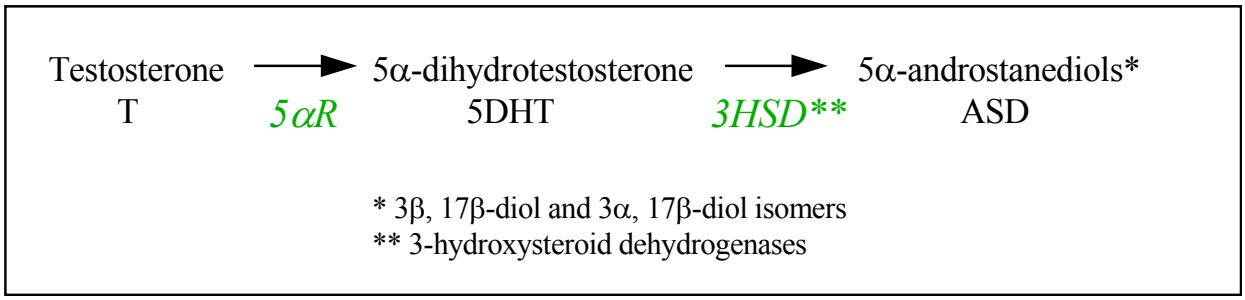
## TABLE OF CONTENT

# 1 - INTRODUCTION

**ADVANCED SKIN AND HAIR, INC.** has developed the compounds **Clearogen Acne Lotion** for acne and **Revivogen Scalp Therapy** for hair loss. Acne is a multiparametric skin disorder where sebum hypersecretion induced by circulating testosterone is involved. Testosterone is not the active form of this hormone. It is activated by 5α reductase which produces dihydrotestosterone, the efficient steroid. Inhibition of 5α reductase activity is known to reduce acne in human skin. The metabolism of testosterone is also involved in hair loss since inhibitors of 5α reductase have been demonstrated to reduce alopecia.

**BIO***alternatives* performed this study in order to assess the effects of the test compounds on the metabolism of testosterone in reconstructed human epidermis. This model has been shown to be useful for the evaluation of inhibitors of this metabolism *(Bernard et al. 2000; Int. J. Cosm. Sci.,22, 397-407)*.

The steroid 5α-reductase isoenzymes (5αR) transform testosterone (T) into 17β-hydroxy-5α-androstan-3-one (5-dihydrotestosterone, DHT). This reaction is crucial in the action of androgens.




# 2 - MATERIALS AND METHODS

## 2.1 Biological model

**Reconstructed Human Epidermis (RHE)**

- Tissues: 18 RHE (0.50 cm², 10 days), batch n° 01015-31
- Culture: at 37°C and 5 % $CO_2$
- Culture medium: differentiation medium

## 2.2 Test compounds and references

| Test compound | Stock-solution | Dilution | Application |
|---|---|---|---|
| **Clearogen Acne Lotion** batch 0K085B (AD070315/1) | Cream supplied by the study promoter and stored at room temperature. | - | **topical at 3 µl/RHE** |
| **Revivogen Scalp Therapy** batch 07-06 (AD070315/2) | Liquid supplied by the study promoter and stored at room temperature. | - | **topical at 50 µl/RHE** |

| Reference | Stock-solution | Dilution | Application |
|---|---|---|---|
| **Finasteride** batch 231664 | $10^{-2}$ M in ethanol | In water | **topical at $10^{-5}$ M/RHE** |
| **Avodart®** batch 053721A | $10^{-3}$ M in ethanol | In water | **topical at $10^{-6}$ and $10^{-5}$ M/RHE** |

## 2.3 Testosterone

*Testosterone:* [4-$^{14}$C] testosterone (Amersham B76, 54 mCi/mmole, 2.35 nmole/epidermis). [4-$^{14}$C] testosterone stock-solution was dissolved in ethanol and diluted in sterile water (1% ethanol final).

## 2.4 Treatment

The RHE were topically treated (or not, control) with the test compounds or the references. Three RHE were used for each experimental condition.

After **24h of treatment**, the RHE were topically re-treated and incubated for **5 hours**. After incubation, the test compounds and references were removed from the top of the RHE and 100 µl of the labelled testosterone solution were loaded on the *stratum corneum* of each RHE (127 nCi/epidermis).

After a 24-hour incubation period, the media underneath the RHE were collected for sterols analysis. The RHE viability, at the end of the experiment, was assessed by MTT reduction.

## 2.5 Extractions and analysis

*Transepidermal diffusion assessment:* the amount of testosterone that passed through the epidermal tissues was measured by liquid scintillation counting (LKB 1211 Rackbeta counter) of a fraction of culture medium.

*Metabolism analysis:* the steroid molecules from culture media were extracted by 2 volumes of chloroform/methanol (98:2) and dried. The various molecular species (testosterone metabolites) were separated by thin layer chromatography (**TLC**) on silica plates (RE/Silice, Whatman) in a solvent system containing dichloromethane, ethylacetate and methanol (85:15:3).
The plates were autoradiographed and testosterone metabolites were quantified using a phosphorImager and specific software (Packard instrument).

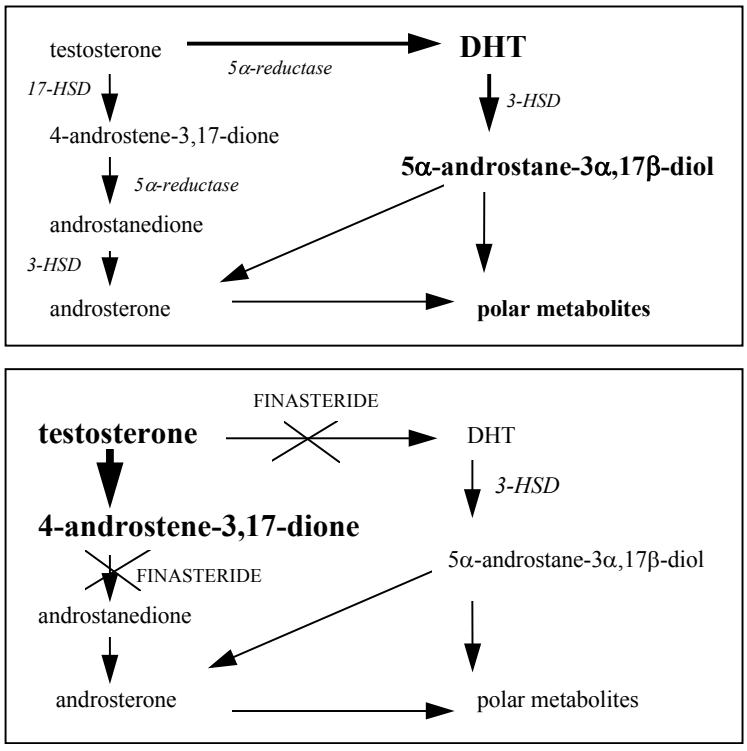
# 3 - RESULTS AND CONCLUSION

## 3.1 RHE viability

**Table 1**

**Clearogen and Revivogen** or the reference compounds did not induce any cytotoxicity effects and did not clearly modify transepidermal diffusion.

## 3.2 Testosterone metabolism




Schematic simplified pathway for testosterone metabolism. Effects of finasteride
(from Bernard F-X *et al*., Int. J. Cosmetic Science, **22** 397-407 (2000))

**Tables 1 and 2**

***Untreated control:***
After 24h of culture, the rate of testosterone metabolism was very high.
Dihydrotestosterone (DHT) was clearly identified in the steroid profile. DHT was the major metabolite in the control epidermis. After 24h, about 74% of the deposited testosterone was converted into DHT. Other important metabolites were androstane-diols (e) and 4-androstene-3,17-dione (b).

***Effects of finasteride:***
**Finasteride** at $10^{-5}$ M strongly inhibited the transformation of testosterone into DHT (67% inhibition compared to the control). Furthermore, as expected, finasteride decreased the amount of androstane-diols (e) and induced a strong accumulation of 4-androstene-3,17-dione (b) (Figure 1).

***Effects of dutasteride:***
**Dutasteride** at $10^{-6}$ M and $10^{-5}$ M strongly inhibited dose dependently the transformation of testosterone into DHT (respectively 80% and 86% of inhibition of the DHT production compared to the control). Furthermore, as expected, dutasteride decreased the amount of androstane-diols (e) and induced a strong accumulation of 4-androstene-3,17-dione (b) (Figure 1).

***Effects of Clearogen Acne Lotion:***
**Clearogen Acne Lotion** (5 mg/cm²) reduced the transformation of testosterone into DHT (49% of inhibition of the DHT production compared to the control) and of DHT into androstane-diols (e). Surprisingly the accumulation of 4-androstene-3,17-dione (b) was not visible.

***Effects of Revivogen Scalp Therapy:***
**Revivogen Scalp Therapy** (5 mg/cm²) strongly reduced the production of DHT (90% of inhibition of the DHT production compared to the control).

**To conclude, Clearogen Acne Lotion and Revivogen Scalp Therapy clearly decreased the production of DHT and therefore could be used to treat acne and hair loss.**

# 4 - TABLES AND FIGURES

**Table 1:** Diffusion of [$^{14}$C]-testosterone (and metabolites) through RHE.

**Trans-epidermal diffusion (24h)**

| Treatment | Conc. | cpm | sd | n | % control | nmol steroid | *Viability* |
|---|---|---|---|---|---|---|---|
| *Total testosterone* | - | ***326060*** | **-** | *1* | **-** | *2.35* | - |
| **Untreated control** | - | **125307** | 14977 | 3 | **100** | 0.9 | *100* |
| **Finasteride** | $10^{-5}$ M | **147747** | 6335 | 3 | **118** | 1.1 | 101 |
| **Dutasteride** | $10^{-5}$ M | **150160** | 10809 | 3 | **120** | 1.1 | 63 |
| | $10^{-6}$ M | **159213** | 20653 | 3 | **127** | 1.1 | 98 |
| **Clearogen Acne Lotion** | 5 mg/cm² | **140640** | 14980 | 3 | **112** | 1.0 | 131 |
| **Revivogen Scalp Therapy** | 5 mg/cm² | **30026** | 1181 | 3 | **99** | 0.2 | 96 |

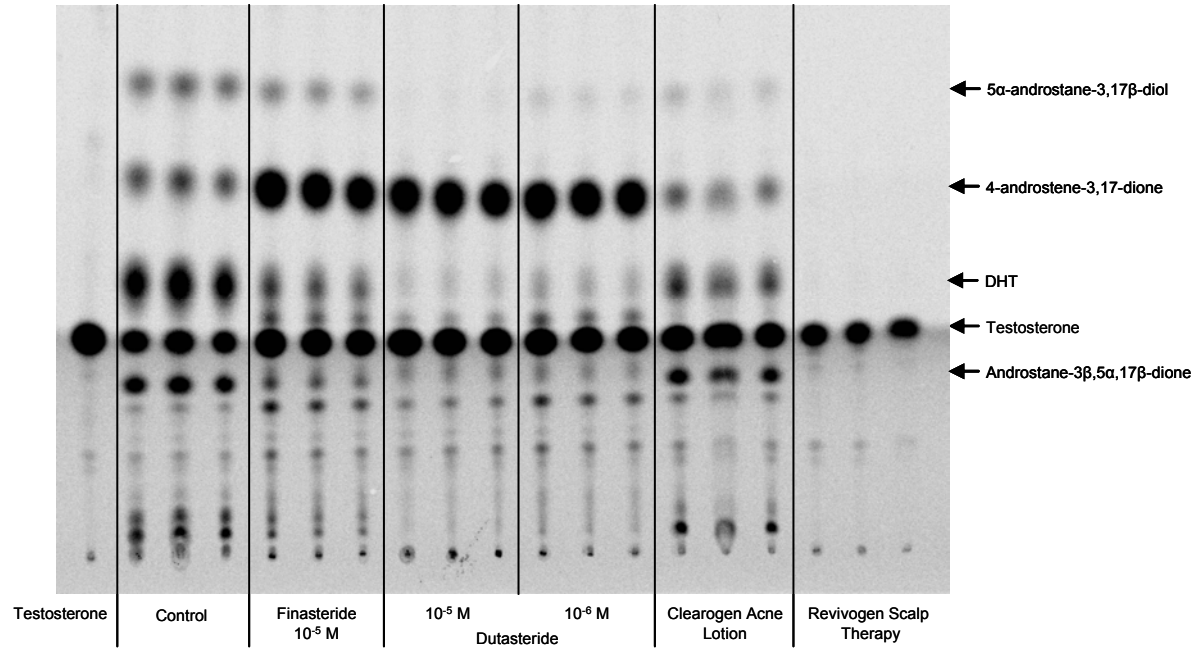



**Figure 1:** Thin layer chromatography and autoradiography of [$^{14}$C]-testosterone and metabolites after transepidermal diffusion (24h).

**Table 2:** Effects of **Clearogen Acne Lotion**, **Revivogen Scalp Therapy** and the reference compounds on the production of testosterone metabolites. Instant Imager analysis of TLC in figure 2 (direct radioactivity measurement).

| | | | Total | | Testo | | | DHT | | | DHT/testo | % viabilité |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Traitement** | **Conc.** | # lane | radio. units | average | radio. units | % total | average (%) | radio. units | % total | average (%) | ratio | |
| **Control** | - | 1 | 67533836 | | 19608834 | 29.0 | | 19548847 | 28.9 | | | |
| | | 2 | 66111927 | **67757438** | 15474027 | 23.4 | **26.0** | 20991700 | 31.8 | **29.7** | **1.14** | **100** |
| | | 3 | 69626551 | | 17879331 | 25.7 | | 19852895 | 28.5 | | | |
| **Finasteride** | $10^{-5}$M | 4 | 63319493 | | 23577062 | 37.2 | | 5938803 | 9.4 | | | |
| | | 5 | 68117243 | **69558063** | 22823245 | 33.5 | **34.7** | 6816384 | 10.0 | **9.8** | **0.28** | **101** |
| | | 6 | 77237453 | | 25816879 | 33.4 | | 7650690 | 9.9 | | | |
| **Dutasteride** | $10^{-5}$M | 7 | 65796070 | | 29420454 | 44.7 | | 2613421 | 4.0 | | | |
| | | 8 | 63118793 | **64087078** | 27525535 | 43.6 | **44.3** | 2919610 | 4.6 | **4.2** | **0.09** | **63** |
| | | 9 | 63346370 | | 28330573 | 44.7 | | 2508143 | 4.0 | | | |
| | $10^{-6}$M | 10 | 68156904 | | 24334912 | 35.7 | | 4166042 | 6.1 | | | |
| | | 11 | 63825642 | **71667199** | 22557438 | 35.3 | **35.4** | 3762372 | 5.9 | **6.0** | **0.17** | **98** |
| | | 12 | 83019052 | | 29272407 | 35.3 | | 4885796 | 5.9 | | | |
| **Clearogen Acne Lotion** | 5 mg/cm$^2$ | 13 | 36823292 | | 13233882 | 35.9 | | 7875463 | 21.4 | | | |
| | | 14 | 63324273 | **48563401** | 23345746 | 36.9 | **47.9** | 11925017 | 18.8 | **15.1** | **0.31** | **131** |
| | | 15 | 45542638 | | 32314124 | 71.0 | | 2284742 | 5.0 | | | |
| **Revivogen Sclap therapy** | 5 mg/cm$^2$ | 16 | 4003068 | | 8453714 | 211.2 | | 179542 | 4.5 | | | |
| | | 17 | 4094100 | **3796260** | 10244949 | 250.2 | **224.1** | 111193 | 2.7 | **3.0** | **0.01** | **96** |
| | | 18 | 3291611 | | 6938151 | 210.8 | | 62873 | 1.9 | | | |

AU: Arbitrary Unit for radioactivity quantification

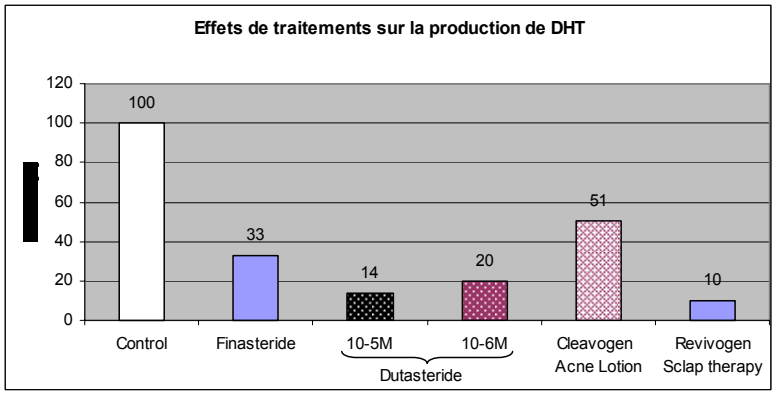



**Figure 2**: Effects of **Clearogen Acne Lotion**, **Revivogen Scalp Therapy** and the reference compounds on DHT metabolism.