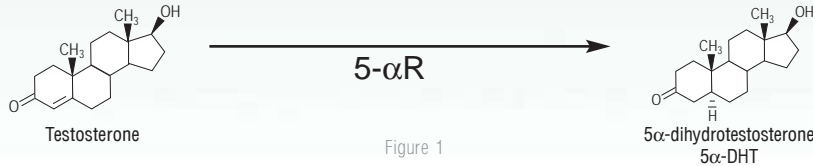


# PHARMACOLOGICAL AND CLINICAL ACTIVITIES OF 5- $\alpha$ AVOCUTA<sup>®</sup>. APPLICATION FOR THE MANAGEMENT OF ANDROGENIC DISORDERS.

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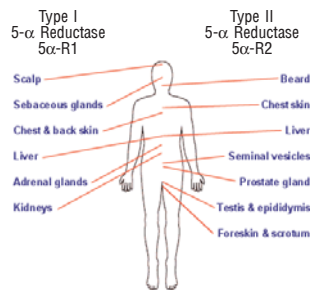
## Introduction

Androgen-dependent disorders, such as seborrhoea, acne and alopecia are among the most common diseases encountered by dermatologists in daily practice. These pathologies are in part related to the hyper-activity of the steroid 5-alpha reductase (5- $\alpha$ R). This enzyme metabolises testosterone into dihydrotestosterone (DHT) the potent androgen (Figure 1).



Two different isotypes have been characterized (5- $\alpha$ -R1 and 5- $\alpha$ -R2) and differ especially by their tissue expression patterns (Figure 2). 5- $\alpha$ -R2 is mainly found in the prostate and in genital skin, but also in hair follicle. 5- $\alpha$ -R1 is principally localized in the skin and in the hair follicle. Within the skin, 5- $\alpha$ -R1 activity predominates in sebaceous gland where it may be involved in sebum production.

Figure 2 : Expression pattern of 5- $\alpha$  reductase type 1 and 2 (from www. keratin.com)



The development of new and original 5-alpha reductase, especially type 1, inhibitors is thus of outmost importance for the treatment of androgen-dependent skin disorders. The purpose of this work was first to select among different fatty esters the most potent inhibitor of 5- $\alpha$ -R1. The selected inhibitor, 5- $\alpha$  Avocuta<sup>®</sup>, was first formulated in a shampoo in order to test its efficiency for treating scalp hyper-seborrhoea and greasy hair. Secondly, it was incorporated in a day cream and tested in a clinical trial implying volunteers having face hyper-seborrhoea.

## Protocole

### 1- IN VITRO EVALUATION OF THE INHIBITION OF 5- $\alpha$ -R1 BY FREE FATTY ESTERS

- Cells**  
Human skin fibroblasts were obtained from excess plastic surgery.
- Treatment**  
Tested products were dissolved in DMSO and pre-incubated with the cells during 2h before the addition of radio-labelled testosterone. The tested products were chosen among free fatty esters varying in the length and functionality of the fatty chain, and the nature of the alkoxy group.
- Evaluation of 5-alpha reductase activity**  
The formation of DHT from testosterone is directly correlated to 5- $\alpha$ -R activity. The different androgens were separated by thin layer chromatography and DHT was quantitated using a radioactivity analyser.
- Statistics**  
Experiments were done in triplicate and data were analysed by ANOVA followed by a Dunett test.

### 2- IN VIVO EVALUATIONS

The clinical trials, described in this work, were both designed to mimic the normal conditions of use of the products (shampoo & skin care).

- Scalp hyper-seborrhoea and greasy hair**  
Firstly, we sought to determine whether a shampoo containing 1% of the selected 5- $\alpha$ -R1 inhibitor, was able to improve scalp hyper-seborrhoea and greasy hair.
- Subjects**  
27 volunteers (16 females and 11 males, mean age 33.4) having hyper-seborrhoea and greasy hair conditions were enrolled by 6 dermatologists. The main exclusion criteria were scalp seborrheic dermatitis, folliculitis, or psoriasis.
- Treatment**  
Subjects were placed on 1% 5- $\alpha$  Avocuta<sup>®</sup> containing shampoo for 3 weeks (1 application each 2 days).
- Methods of evaluation**  
A clinical evaluation was performed by the dermatologist at T0 and T4 weeks using a 10 points scale according to the following criteria : scalp and hair seborrhoea, stinging, pruritus, scalp erythema, dandruff. Scalp sebum secretion (Sebufix<sup>®</sup> F16, a white foil which gets black spots when coming in contact with sebum) was analysed by visual scoring (Figure 3) and by image analysis on 13 subjects (Skin Visiometer<sup>®</sup>, SV600, CK, Germany) at T0 and T4 weeks. The efficiency of the shampoo was also evaluated by the subject itself at the end of the study.



Figure 3 : Qualitative scale (five levels) for sebum evaluation

- Face hyper-seborrhoea**  
The goal of this second trial was to determine the efficiency of 5- $\alpha$  Avocuta<sup>®</sup> in the management of face hyper-seborrhoea.
- Subjects**  
23 volunteers (female, mean age 30.8) having face hyper-seborrhoea were enrolled by 6 dermatologists. 87% of the volunteers have reported acne antecedents. The main exclusion criteria were active acne, acne treatment stopped less than 15 days before the beginning of the study, use of cosmetics containing vitamin C, retinol or AHA in the past 15 days.
- Treatment**  
Subjects have to applied the day cream containing 2% of 5- $\alpha$  Avocuta<sup>®</sup>, twice a day during 3 weeks.
- Methods of evaluation**  
A clinical evaluation was performed by the dermatologist at T0 and T4 weeks using a 10 points scale. Fronthed sebum secretion (Sebufix<sup>®</sup> F16) was analysed by visual scoring (Figure 3) at T0 and T4 weeks. The efficiency of the product was also evaluated by the subject itself at the end of the study.

## Conclusion

Using an *in vitro* model, which was accurate to screen the relation between structure and activity of potential 5- $\alpha$ R inhibitors, we have selected 5- $\alpha$  Avocuta<sup>®</sup> (butyl avocadate) has the most potent inhibitor of 5- $\alpha$ R1. This inhibitor was then tested for its ability to treat scalp hyper-seborrhoea and greasy hair conditions. After 3 weeks, clinical evaluation by the dermatologists, auto-evaluation made by the volunteers as well as the objective evaluation of sebum production were in accordance and allow us to conclude that 5- $\alpha$  Avocuta<sup>®</sup> is effective in the management of scalp disorders. The second clinical trial has demonstrated that 5- $\alpha$  Avocuta<sup>®</sup> was also adapted to face hyper-seborrhoea, with an unambiguous decline of sebum production as attested by clinical evaluation, SEBUFIX<sup>®</sup> F16 analysis and the auto-evaluation performed by the volunteers. The efficiency of the formulation is also underlined by an improvement of the quality of life of the volunteers (data not shown). Moreover, the good tolerance of 5- $\alpha$  Avocuta<sup>®</sup> was confirmed by these clinical trials. In conclusion, 5- $\alpha$  Avocuta<sup>®</sup>, a specific 5- $\alpha$  reductase inhibitor obtained from virgin avocado oil through a biotechnological process, has demonstrated its usefulness in the management of skin disorders related to hyper-seborrhoea.

## Results

### 1- IN VITRO SELECTION OF 5- $\alpha$ -R1 MOST POTENT INHIBITOR

Under our experimental conditions, where the products were tested at 1, 10 and 100  $\mu$ g/ml, it was possible to select the most potent inhibitor : butyl avocadate or 5- $\alpha$  Avocuta<sup>®</sup>. The inhibitory activity is dose-dependent and comprised between 21 and 49% (Figure 4).

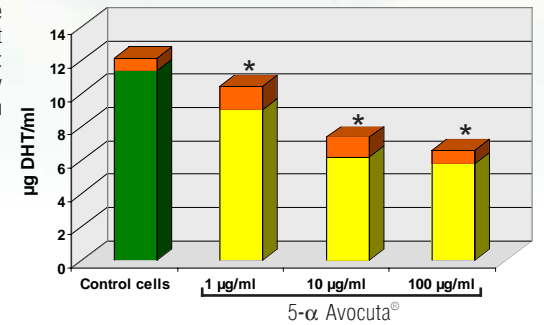


Figure 4 : Inhibition of 5-alpha reductase type 1 by 5- $\alpha$  Avocuta<sup>®</sup>. \* difference statically significant.

5- $\alpha$  Avocuta<sup>®</sup> is a specific lipid, specially designed to inhibit 5-alpha reductase type 1. This active ingredient is composed by a complex blend of fatty acid butyl esters, obtained from a cold-pressed avocado oil, according to a patented process. This process includes a first purification step of virgin avocado oil by short path distillation and a trans-esterification reaction in presence of butanol and enzyme as catalyst (biotechnological process). In a final step, butyl esters are purified by molecular distillation to give 5- $\alpha$  Avocuta<sup>®</sup>.

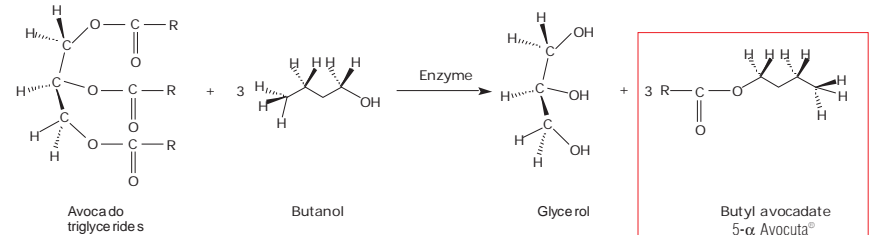


Figure 5 : Synthesis process of 5- $\alpha$  Avocuta<sup>®</sup>

### 2- IN VIVO EVALUATION

#### • Scalp hyper-seborrhoea and greasy hair

Clinical investigations performed by the dermatologist have shown that the shampoo clearly improved greasy hair aspect, and was able to reduce itching and pruritus, as well as dandruffs (Figure 6).

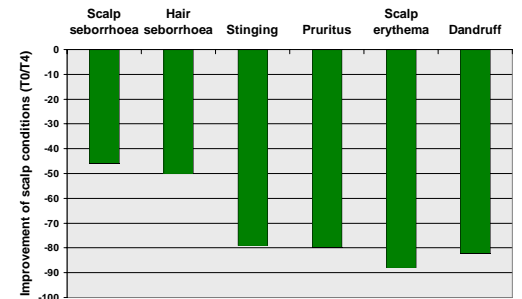


Figure 6 : Improvement of scalp disorders following 3 weeks of treatment with a shampoo containing 1% of 5- $\alpha$  Avocuta<sup>®</sup>

Comparison of the visual scoring, of SEBUFIX<sup>®</sup> F16, at T0 and T4 weeks, showed a decrease of 34% of the sebum secretion, decrease which was confirmed by image analysis (Table I and Figure 7)

	Mean for 13 subjects (T0/T4)
Lipidic area/total area (%)	-7.8 pts
Cumulative total lipidic area (mm <sup>2</sup> )	-69%
Number of lipidic spots	-137

Table I : Results of sebum secretion as recorded by image analysis

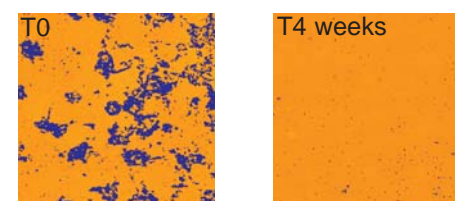


Figure 7 : Illustration of the decrease of scalp sebum production (case n°12).

The auto-evaluation by the volunteers have confirmed these data (Table II).

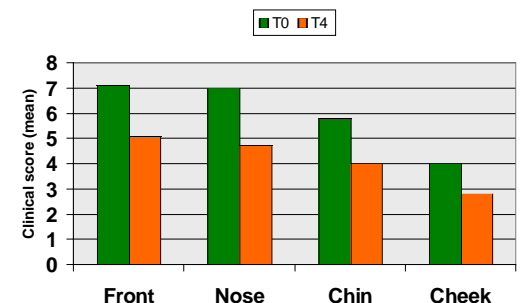
	% of good opinions
Decrease of greasy hair	78
Decrease of scalp seborrhoea	74
Improvement of hair tightness	81
Decrease of dandruff	67
Decrease of scalp itching	59
Decrease of scalp irritation	63

Table II : Results of the auto-evaluation by the volunteers (% of good opinions)

#### • Face hyper-seborrhoea

As shown in Figure 8, the regular application (twice a day, during 3 weeks) of 5- $\alpha$  Avocuta<sup>®</sup> led to a clear decrease of sebum secretion on the face.

Figure 8 : Improvement of face hyper-seborrhoea following 3 weeks of treatment with a cream containing 2% of 5- $\alpha$  Avocuta<sup>®</sup>



Sebum secretion was also evaluated using SEBUFIX<sup>®</sup> F16. The analyse of the patch by comparison with a qualitative scale confirmed the clinical scores with a decrease of 30% of sebum production.

The efficiency of the formulation was also approved by the volunteers (Figure 9). The anti-seborrheic effect of the formulation reported by the panellists is in correlation with the clinical evaluation performed by the dermatologists and with the lipidic score measured with the SEBUFIX<sup>®</sup> F16. Moreover, 61% of the volunteers pointed out that the product acted quickly, with a persistent effect 3 weeks after the end of the study.

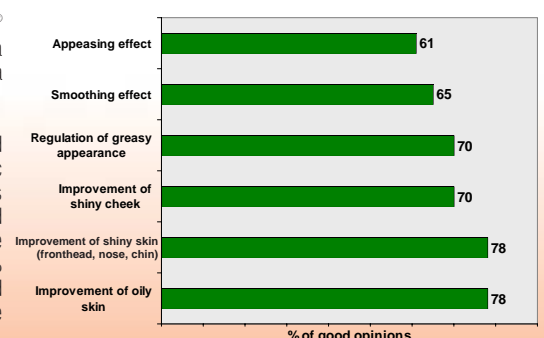


Figure 9 : Auto-evaluation by the volunteers