

Minoxidil increases 17β -hydroxysteroid dehydrogenase and 5α -reductase activity of cultured human dermal papilla cells from balding scalp

Toshihiro Sato ^{a,*}, Taketsugu Tadokoro ^b, Tadashige Sonoda ^a, Yuji Asada ^a,
Satoshi Itami ^b, Susumu Takayasu ^a

^a Department of Dermatology, Oita Medical University, Hasama-machi, Oita 879-5593, Japan

^b Department of Dermatology, Osaka University Medical School, Osaka, Japan

Received 8 July 1998; received in revised form 10 August 1998; accepted 12 August 1998

Abstract

Minoxidil is known to induce hair growth in male pattern baldness, for which development androgen plays a central role. We studied the effect of minoxidil on testosterone metabolism by cultured dermal papilla cells from balding or nonbalding scalp and dermal fibroblasts. In all three groups, 17β -hydroxysteroid dehydrogenase activity was much higher than 5α -reductase activity. Minoxidil increased 17β -hydroxysteroid dehydrogenase activity by nearly 40% ($P < 0.001$) in dermal papilla cells of balding scalp, whereas the effect was less marked in dermal papilla cells from nonbalding scalp and dermal fibroblasts. 5α -Reductase activity was also slightly increased by minoxidil in dermal papilla cells from balding scalp. Again, the effect on 5α -reductase activity was insignificant in the other two groups of cells. Whether such modification of testosterone metabolism in dermal papilla cells of balding scalp by minoxidil is related to its therapeutic effect remains unknown. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Minoxidil; Dermal papilla cells; Androgen; 17β -HSD

1. Introduction

Minoxidil is a pyrimidine derivative (2,4-diamino-6-piperidinopyrimidine-3-oxide) initially

developed as a potent antihypertensive agent [1]. In addition to non-virilizing hypertrichosis, the drug was found to induce hair growth in male pattern baldness [2,3]. However, the mechanisms of action of minoxidil on hair growth are poorly understood. Androgen is prerequisite for the development of male pattern baldness [4]. More-

* Corresponding author. Tel.: +81 97 5865882; fax: +81 97 5865889.

over, male pattern baldness is not observed in male pseudohermaphrodites due to 5α -reductase deficiency [5]. Thus, 5α -reduction of testosterone may play an important role in the pathogenesis of this type of baldness.

Dermal papilla cells (DPC) of beard follicles are considered to play a key role in the anabolic action of androgen [6]. Therefore, in the present paper, we studied whether minoxidil affects metabolism of testosterone by DPC of balding scalp. The results were compared with its effects on DPC of other hair follicles and dermal fibroblasts.

2. Materials and methods

Skin specimens were obtained at plastic surgery. Dermal papillae were isolated according to the method of Messenger [7]. Five samples from balding scalp (aged 27–44 years), six from non-balding scalp (aged 27–63 years) and five dermal fibroblasts (aged 18–69 years) were used for experiments. DPC were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS), penicillin (50 U/ml), streptomycin (5 μ g/ml), glutamine (0.6 mg/ml) and non-essential amino acids at 37°C in a humidified atmosphere of 95% O₂ and 5% CO₂. After several passages of subculture, cells were once stocked in liquid nitrogen. For experiment, cells in three to seven passages were plated on 24-well culture dishes at a density of 5.3×10^4 cells/cm² (1.0×10^5 cells/well) in the culture medium described above. After 24 h, the medium was changed to DMEM with various additives except for serum in the presence or absence of 0.5 mM of minoxidil. On the fourth day, the medium was changed to DMEM containing 25 nM [³H]testosterone and the cells were incubated for 2 h. After the incubation, 10 μ g of each carrier steroid was added. Metabolites in the medium were extracted by a 4-fold volume of methanol–chloroform (1:2, v/v) [8]. The cells were lysed with 0.5 M NaOH and the protein content was measured by Bio-Rad protein assay kit [9]. Steroids were analyzed by thin-layer chromatography (TLC), using a solvent system of 99% chloroform:1% methanol [10]. The purity of dihydrotestosterone (DHT) and androstenedione

was confirmed by high-performance liquid chromatography (HPLC), using a Gilson gradient system [11]. The parameters for HPLC were: injection volume, 20 μ l; run time, 35 min; flow rate, 1.1 ml/min; aqueous methanol gradient, 55–100%; retention time, 22 min for DHT and 18 min for androstenedione. The radioactivity of each product was measured by a liquid scintillation spectrometer. Activities of 5α -reductase and 17β -hydroxysteroid dehydrogenase (17β -HSD) were expressed as the sum of the DHT and androstenedione formed and the sum of androstenedione and androstenedione formed, respectively.

3. Results

The 17β -HSD activity was significantly higher in DPC of balding scalp hairs than in those of non-balding scalp hairs and dermal fibroblasts ($P < 0.05$ and < 0.001 , respectively) (Fig. 1). 5α -Reductase activity was much lower than 17β -HSD in all three groups (Fig. 2). Minoxidil augmented both 17β -HSD and 5α -reductase activities of DPC from balding scalp, whereas the effects of minoxidil on the enzyme activities were

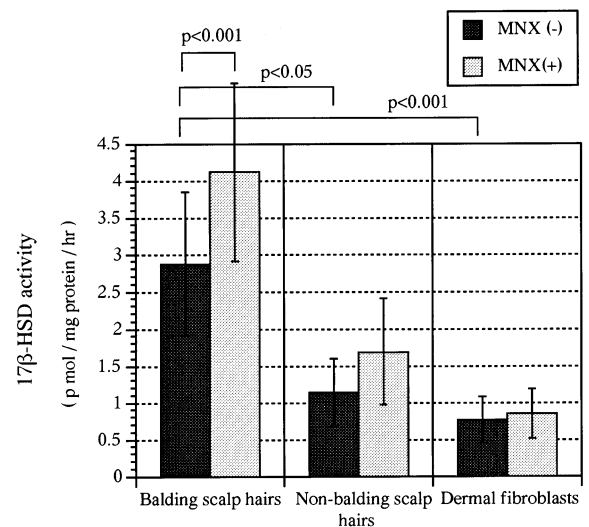


Fig. 1. Effect of minoxidil on 17β -HSD activity of cultured dermal papilla cells. Cells were treated with 0.5 mM minoxidil for 3 days in the serum-free medium and were then incubated with 25 nM ³H-testosterone for 2 h. The mean \pm SEM of three determinations is shown.

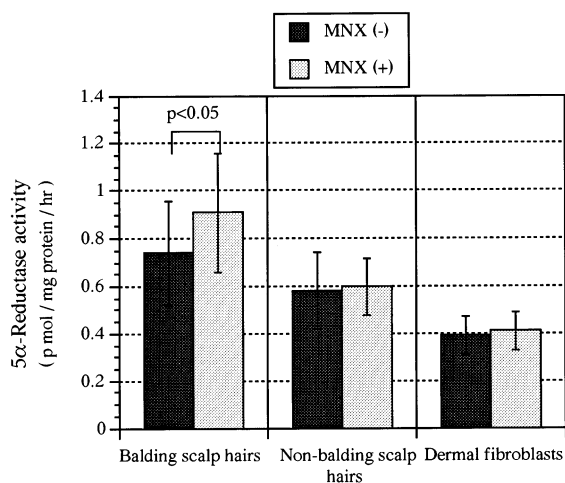


Fig. 2. Effect of minoxidil on 5 α -reductase activity of cultured dermal papilla cells. Cells were treated with 0.5 nM minoxidil for 3 days in the serum free medium and then were incubated with 3H-testosterone for 2 h. The mean \pm SEM of three determinations is shown.

less marked in those from non-balding scalp and dermal fibroblasts.

4. Discussion

DPC play a fundamental role in the induction of hair follicle differentiation [12] and define the androgen sensitivity of hair follicles [6]. In the present study, 17 β -HSD activity was increased about 40% by minoxidil in DPC of balding scalp, while it was less affected in those from non-balding scalp and dermal fibroblasts. The result suggests that the treatment with minoxidil accelerates the conversion of testosterone to less active androgen and thereby protects hair follicles of balding scalp from excessive androgen action. 5 α -Reductase activity was also slightly increased by minoxidil in these DPC; however, the activity of 5 α -reductase of these cells was much lower than that of beard DPC [13] in all three groups of cells in the present study. Further characterization of 5 α -reductase is required to clarify its role in male pattern baldness, in spite of suggestive role of this enzyme in the development of male pattern baldness [5].

Whether the effect of minoxidil on testosterone metabolism shown in the present study is related to

its therapeutic effect remains unknown. Thus far, minoxidil has not been shown to have any antiandrogenic effects either locally [14] or systemically [3].

Acknowledgements

This report was partially supported by grants from Taisho Pharmacological Corporation.

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