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LETTER TO THE EDITOR

Reversal of androgenetic alopecia by topical ketoconzole: Relevance of anti-androgenic activity

KEYWORDS
Androgenetic alopecia; Ketoconazole; Androgen; Reporter assay; Open trial

Ketoconazole (KCZ), an imidazole anti-fungal agent, is known to be effective for the treatment of seborrheic dermatitis and dandruff. In addition, 2% KCZ shampoo was found to improve hair density and the size and proportion of anagen follicles in androgenetic alopecia (AGA) [1] and, in combination with finasteride, to have an additive effect for AGA [2]. Recently, it has been reported that topical application of KCZ stimulates hair growth in C_3H/HeN mice [3]. However, whether topical KCZ is effective enough to improve the clinical appearance of AGA is not yet clear. We therefore carried out an open trial of topical 2% KCZ lotion (Nizoral®) in combination with shampoos. Furthermore, to identify the mechanism, which can explain the clinical effect of KCZ on AGA, we performed transient transfection assays using CV-1 cells transfected with androgen receptors (AR).

The six Japanese males from 23 to 51 years old were enrolled in this study with their written informed consent. They presented with grade II vertex to IVa AGA according to the Hamilton—Norwood classification [4]. The subjects applied topical 2% KCZ lotion (Nizoral®) almost every day during or immediately after hair washing with their own unmedicated shampoos. When they revisited our clinic every several months, clinical pictures were obtained to determine the efficacy of the treatment. Two of the men, one 23 years old with grade II vertex and the other 25 years old with Va AGA, showed remarkable hair regrowth after 6 and 10 months, respectively (Fig. 1). The 23-year-old male stopped using KCZ and 3 months later hair loss recurrence on the vertex was noted (Fig. 1c). When he started using KCZ again during shampooing, hairs on the vertex grew again after 3 months (Fig. 1d). These findings constitute evidence of the clinical efficacy of KCZ for AGA. A 41-year-old male showed a slight increase in vertex hair growth after 1 year. Other three of the men, 31, 38 and 51 years old did not show significant improvement. These findings suggest that topical KCZ with shampoo can be effective for some males with AGA.

To identify the mechanism, which can explain the effect of KCZ on AGA, we performed transient transfection assays using an androgen-responsive synthetic promoter for CV-1 cells transfected with AR. At 50—70% confluency in a 24-well plate, the CV-1 cells, maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, were transfected with the transfection reagent Fugene-6 (Roche Diagnostics Corp., Indianapolis, IN, USA) according to the manufacturer's instructions. For luciferase assays, we transfected 0.1 μg of the reporter plasmid, pGL2-GRE3-bG-luc [5], 12.5 ng of pCI-neo-BamX-AR(Gly23) [5] and 0.2 μg of the pRL-CMV vector, the *Renilla* luciferase control reporter vector driven by the CMV immediate-early enhancer/promoter, as the internal controls. At 24 h after transfection, we added fresh DMEM supplemented with 10% charcoal-treated fetal bovine serum with methyltrienolone (R1881, a stable synthetic androgen) or ethanol as a mock vehicle. The cells were also treated with KCZ (Janssen, L.P., Titusville, NJ) or ethanol as a mock vehicle. At 48 h after transfection, the cells were harvested for luciferase assays. Luciferase activities were measured with a luminometer using the Dual-Luciferase™ reporter assay system (Promega, Madison, WI). The results were summarized from three independent sets of transfections and presented as mean ± S.D.; statistical significance was tested with Student's *t*-test. The results demonstrated that 10 or 20 μg/ml KCZ reduced luciferase activity to 67.5% ($p < 0.01$) or 49.9% ($p < 0.03$), respectively, reflecting its suppressive action on AR (Fig. 2). This finding suggests that KCZ improves AGA through the suppression of AR activity.

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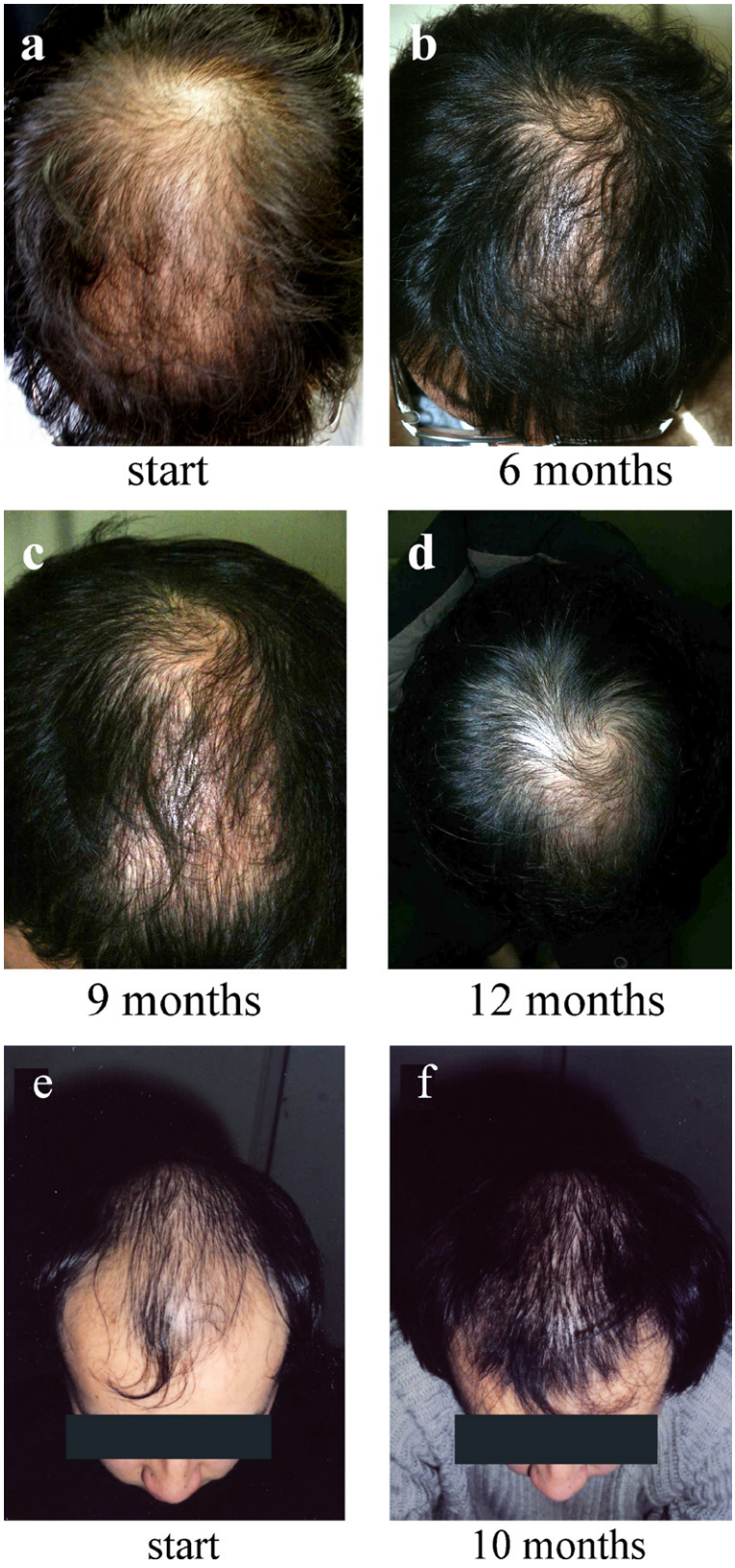

Fig. 1 A 23-year-old Japanese man who used 2% ketoconazole (KCZ) lotion during shampooing everyday. Six months later, hair regrowth was attained (b) in comparison with the pre-treatment condition (a). Suspension of use for 3 months, however, caused recurrent hair loss (c). Renewed use of KCZ induced renewed growth of vertex hair (d). A 25-year-old Japanese man with AGA (e) applied 2% KCZ lotion immediately after shampooing everyday. Ten months later, hair regrowth was apparent (f).

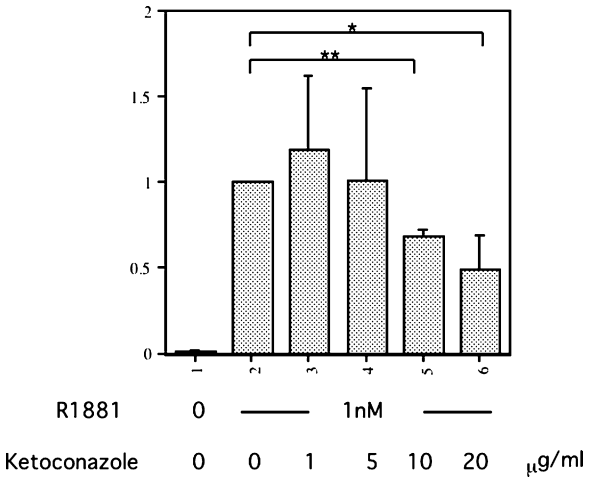

Fig. 2 KCZ effect on R1881-induced AR transcactivity in CV-1 cells transfected with AR. We used the transfection reagent Fugene-6 (Roche Diagnostics Corp., Indianapolis, IN, USA) to transfect 0.1 μg of the reporter plasmid, pGL2-GRE3-bG-luc, 12.5 ng of pCI-neo-BamX-AR(Gly23) and 0.2 μg of pRL-CMV vector (lanes 1—6) into CV-1 cells cultured at 50—70% confluency in a 24-well plate. The infected cells were treated with 10^{-9} M R1881 (lanes 2—6) or ethanol as a mock vehicle (lane 1). After overnight incubation, KCZ at the indicated concentration was added to the cultures. The activities of the various reporter genes were compared with the luciferase activity in the presence of R1881 and the absence of KCZ (lane 2).

Dermal papilla cells are the main targets for androgen in hair follicles, as evidenced by immunohistochemistry [6] and reporter assays [7] for the detection of AR. Deep penetration of KCZ into dermal papilla is therefore necessary to realize the suppressive action on androgen in vivo. The use of KCZ in combination with detergent containing shampoos in this study may enhance KCZ penetration. On the other hand, a recent study demonstrated that KCZ stimulates hair growth in mice [3], suggesting that its effect on hair is androgen independent. To summarize, KCZ may exert its effect on AGA in both an androgen-dependent and -independent manner.

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