

ORIGINAL ARTICLE

Topical valproic acid increases the hair count in male patients with androgenetic alopecia: A randomized, comparative, clinical feasibility study using phototrichogram analysis

Seong Jin JO,^{1,2} Hyoseung SHIN,^{1,2} Young Woon PARK,^{1,2} Seung Hwan PAIK,^{1,2} Won Seok PARK,³ Yeon Su JEONG,³ Hong Ju SHIN,³ Ohsang KWON^{1,2}

¹Department of Dermatology, Seoul National University College of Medicine, ²Institute of Human-Environment Interface Biology, Medical Research Center, Seoul National University, and ³Advanced Hair Research Laboratory, R&D Center, AmorePacific, Gyeonggi-do, Korea

ABSTRACT

Valproic acid (VPA), a widely used anticonvulsant, inhibits glycogen synthase kinase 3 β and activates the Wnt/ β -catenin pathway, which is associated with hair growth cycle and anagen induction. To assess the efficacy of topical VPA for treating androgenetic alopecia (AGA), we performed a randomized, double-blind, placebo-controlled clinical trial. Male patients with moderate AGA underwent treatment with either VPA (sodium valproate, 8.3%) or placebo spray for 24 weeks. The primary end-point for efficacy was the change in hair count during treatment, which was assessed by phototrichogram analysis. Of the 40 patients enrolled in the study, 27 ($n = 15$, VPA group; $n = 12$, placebo group) completed the entire protocol with good compliance. No statistical differences in age, hair loss duration and total hair count at baseline were found between the groups. The mean change in total hair count was significantly higher in the VPA group than in the placebo group ($P = 0.047$). Both groups experienced mostly mild and self-limited adverse events, but their differences in prevalence rates were similar between the two groups ($P = 0.72$). A subject treated with topical VPA developed ventricular tachycardia, but it did not seem to be related to the VPA spray. Topical VPA increased the total hair counts of our patients; therefore, it is a potential treatment option for AGA.

Key words: androgenetic alopecia, β -catenin, glycogen synthase kinase 3, hair, valproic acid, Wnt.

INTRODUCTION

Androgenetic alopecia (AGA) is a common condition that involves progressive hair loss. Terminal scalp hairs are replaced by smaller vellus hairs in patients with AGA. The frequency of AGA increases with age, and approximately 80% of white men are known to develop AGA by the age of 70 years,¹ many of whom experience distress because of diminished body images of themselves.² However, the available treatments for AGA are quite limited.

Development of AGA is related to androgen hormones. Dermal papilla cells (DPC) from balding hair follicles (HF) have higher androgen receptor levels than those from non-balding scalp.³ 5 α -Reductase inhibitors such as finasteride and dutasteride were found to improve hair loss in AGA patients.⁴ However, the molecular mechanism of AGA is still unclear.

Several recent studies have suggested a relationship between Wnt/ β -catenin signaling and AGA.^{5–7}

Wnt/ β -catenin signaling is critical for the development⁸ and inductive potential of DPC for HF regeneration and hair shaft growth.⁹ In the absence of Wnt signals, cytoplasmic β -catenin is phosphorylated by glycogen synthase kinase 3 (GSK-3), resulting in the degradation of β -catenin. A previous study showed that Dickkopf 1, a potent Wnt antagonist, was upregulated in the dermal papilla of HF in AGA patients.⁵ In addition, decreased β -catenin expression and upregulation of GSK-3 β activity were observed when DPC from AGA patients were treated with androgen.^{6,7} Therefore, increment of β -catenin by inhibition of GSK-3 may be a treatment option for AGA. In fact, the treatment of human DPC with (2'Z,3'E)-6-bromindirubin-3-oxime (BIO), a well-known GSK-3 β inhibitor, was reported to increase the activity and expression of indicators of hair inductivity *in vitro*.¹⁰ How-

Correspondence: Ohsang Kwon, M.D., Department of Dermatology, Seoul National University College of Medicine, 101 Daehak-ro, Chongno-Gu, Seoul 110-744, Korea. Email: oskwon@snu.ac.kr

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ever, BIO is considered unsuitable for clinical use owing to toxicity concerns.

Valproic acid (VPA) has been used as an anticonvulsant for a long time and is now used for various psychiatric disorders including bipolar disorder, mania and migraine headaches.^{11,12} The inhibitory effect of VPA on GSK-3 β was recently observed in neuronal cells in an *in vitro* study,^{13–16} implicating its promoting effects on hair growth via β -catenin stabilization. Actually, VPA promoted human hair growth in an *in vitro* culture model.¹⁷ Furthermore, Lee *et al.*¹⁸ recently reported that topical application of VPA in C3H mice notably stimulated hair growth and increased β -catenin expression in the skin. However, no clinical trial investigating the effect of VPA on hair growth has been performed. To assess the effect of topical VPA in AGA patients, we conducted a randomized, double-blind, placebo-controlled study. The patients underwent scalp treatment with either a VPA or a placebo spray for 24 weeks.

METHODS

Study population

Male patients aged 19–45 years who had moderate androgenetic alopecia (types IIIv, IV and V according to Hamilton–Norwood classification) were recruited from the Department of Dermatology, Seoul National University Hospital (SNUH), Seoul, Korea. Patients who had significantly abnormal physical or laboratory evaluation results, severe medical problems such as renal and heart diseases, a history of hair transplantation, topical minoxidil within 3 months, or any systemic treatment such as finasteride and dutasteride within 6 months were excluded. This study was approved by the institutional review board (IRB) of SNUH (no. H-1003-067-313) and the Korean Food and Drug Administration (KFDA). Written informed consent was obtained from each subject before participating in this study.

Study agent

A colorless tonic spray containing 8.3% sodium valproate was prepared as the study agent, using a 27% ethanol solution as the vehicle. The placebo was a colorless tonic spray, but it did not contain sodium valproate. The agents were provided to the patients in identical containers with a corresponding randomization number.

Study design

This pilot study was a randomized, double-blind, placebo-controlled clinical trial. The blocked randomization (block size of 2) schedule was generated by a computer, and the investigator in charge of the randomization process was not involved in the other aspects of the study. According to the randomization schedule, we sequentially assigned the subjects to either a VPA or a placebo treatment group. The clinical investigators, clinical monitors, technicians, subjects and all the other study personnel were blinded to the treatment assignment of each subject. The subjects were instructed to use either the VPA (sodium valproate, 8.3%) or placebo spray twice daily, with seven or eight pumps (~0.8 mL) for each dose. They visited the clinic every 8 weeks for supplementation of the study agent

and evaluation of adverse events. The total treatment duration was 24 weeks.

Compliance evaluation

We supplied the subjects with a new spray (150 mL) at baseline, and 8 and 16 weeks during the treatment period (for a total of 450 mL) and collected the used spray every follow-up visit. According to the spray usage instruction (twice daily with seven or eight pumps), the subjects who used less than 250 mL of the spray for 24 weeks were excluded from the efficacy assessment owing to their poor compliance of the protocol approved by the IRB of SNUH and KFDA.

Efficacy assessment

For the objective efficacy assessment of topical VPA, a phototrichogram analysis was performed as described previously.⁴ Macrophotographs of a 1-cm² circular scalp area, 2-cm apart from the vertex, were taken using a camera system developed by Canfield Scientific (Fairfield, NJ, USA). Before the first macrophotograph was obtained, the hairs in the area were clipped and the center of the circle was marked with a small tattoo using a transparent template. Hair counts, the primary end-point of this study, was measured at baseline and 24 weeks after the treatment by converting the macrophotographs into dot maps using a computer imaging system. The secondary end-points were hair diameter and hair growth rate, which were also measured at baseline and 24 weeks after the treatment. To evaluate hair growth rate, we obtained macrophotographs at an interval of 3 days, matched each hair and measured the difference in hair length.

Safety assessment

The subjects visited the clinic at 8, 16 and 24 weeks for safety assessment by physical examination and self-reporting of adverse events. Adverse event severity was classified as follows: mild (no disruption in daily life), moderate (little disruption in daily life), severe (hospitalization required) and life-threatening. The severe and life-threatening adverse events that occurred were reported to the IRB.

Serum VPA concentration

Because of the concern about the systemic effect of topical VPA, we measured the serum VPA concentrations at 8 weeks after the treatment. The investigator who was in charge of the randomization process kept the data undisclosed until the end of the study, except when a severe or life-threatening adverse effect occurred.

Statistical analysis

The efficacy analysis was performed on a modified intention-to-treat basis. Only the data of the subjects who completed the study schedules with good compliance were included in the efficacy assessment, but the data of all the patients who used the spray treatment at least once were included in the safety assessment. The Mann–Whitney *U*-test and χ^2 -test were performed using the Statistical Package for the Social Science

version 19.0 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered significant.

RESULTS

The stages of the study with the number of subjects are summarized in the study flowchart (Fig. 1). A total of 40 male patients with moderate AGA were recruited from late October to early December 2011 and randomly assigned to either the VPA ($n = 20$) or placebo group ($n = 20$). Four subjects dropped out of the study because of withdrawal of consent ($n = 3$) and severe adverse event ($n = 1$). The data of the other nine subjects were excluded from the efficacy assessment because of poor compliance.

Demographic characteristics

The demographic characteristics of the subjects are presented in Table 1. At baseline, the median ages were 38 and 40 years in the VPA and placebo groups and the median hair loss durations were 5 and 7 years in the VPA and placebo groups,

respectively. However, the differences between the groups were not statistically significant.

Efficacy assessment

The representative clinical photographs and macrophotographs are presented at baseline and after 24 weeks of treatment (Fig. 2). The total hair count increased after 24 weeks of treatment in the VPA group; the median hair counts were 181/cm² (range, 125–241) at baseline and 192/cm² (range, 153–271) at 24 weeks. However, the total hair count did not change in the placebo group; the median hair counts were 194/cm² (range, 155–244) at baseline and 197/cm² (range, 132–253) at 24 weeks (Fig. 3a). The median change in total hair count from the baseline was 23/cm² (range, –17 to 39) in the VPA group and –1/cm² (range, –68 to 70) in the placebo group, and the difference between groups was statistically significant ($P = 0.047$; Fig. 3b).

The decrease in hair diameter was greater in the placebo group than in the VPA group, but the differences were not statistically significant ($P = 0.167$, Fig. 3c,d). The changes in linear

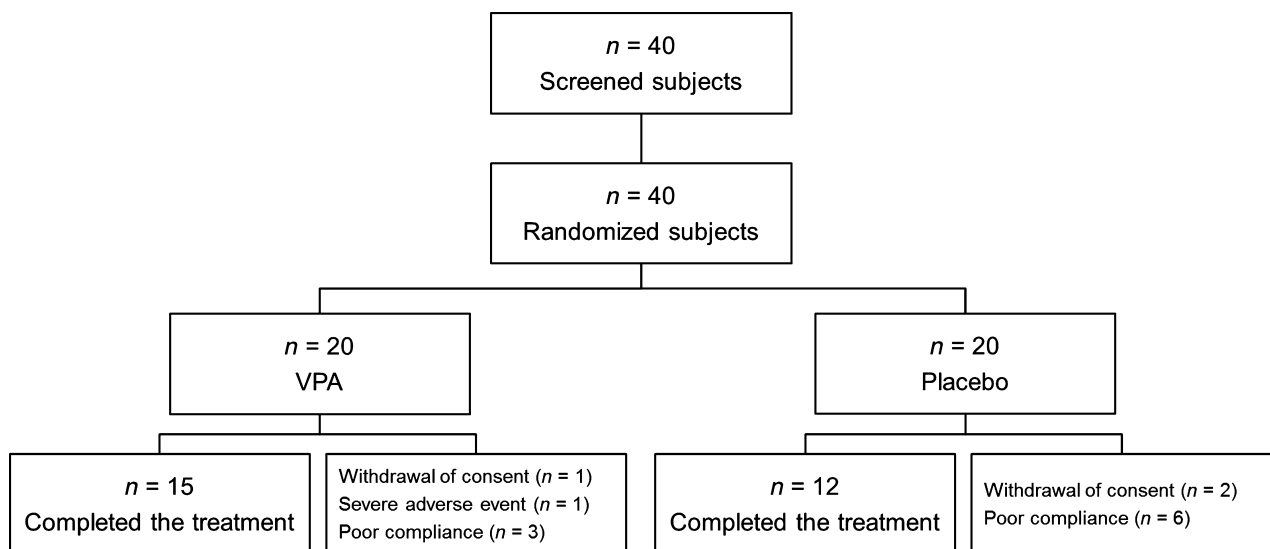


Figure 1. Flowchart of the clinical trial and subject disposition. VPA, valproic acid.

Table 1. Patients' demographic characteristics at baseline

	VPA ($n = 15$)	Placebo ($n = 12$)	
	Mean \pm SD (median, range)		P
Age (years)	37.1 \pm 5.5 (38, 27–45)	38.3 \pm 5.5 (40, 27–44)	0.54
Duration of hair loss (years)	6.7 \pm 4.9 (5, 1–17)	8.0 \pm 5.0 (7, 1–18)	0.42
Total hair count (/cm ²)	182.4 \pm 36.5 (181, 125–241)	195.8 \pm 24.5 (194, 155–244)	0.26
Hamilton–Norwood classification, n			
III/IV/V	5/6/4	2/7/3	0.55
Bodyweight (kg)	73.5 \pm 14.6 (71, 37.5–95.0)	73.3 \pm 13.3 (72, 55.0–102.0)	0.92
Height (cm)	173.8 \pm 4.7 (174, 163–180)	170.3 \pm 6.4 (170, 158–181)	0.15

SD, standard deviation; VPA, valproic acid.

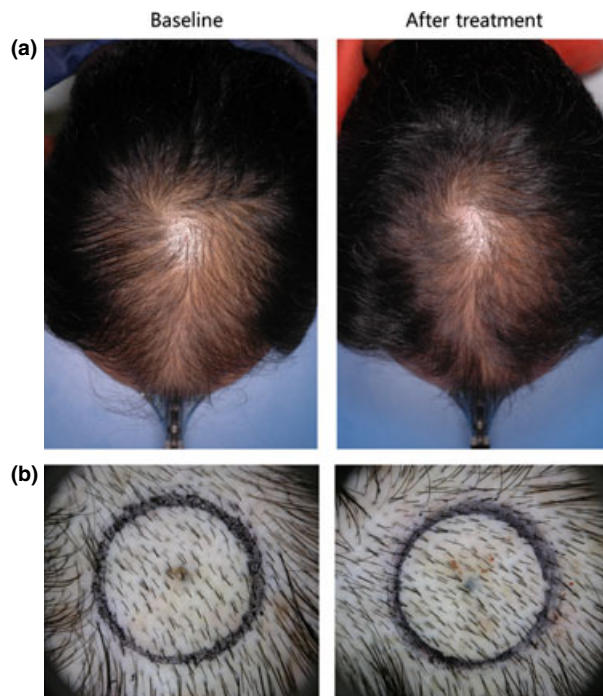


Figure 2. Photographs at baseline and after 24 weeks of treatment in the same patient. Clinical photographs of the vertex (a). Macrophotographs of 1 cm² circular scalp area, 2 cm apart from the vertex (b).

hair growth rate after the treatment were also not different between groups ($P = 0.829$, Fig. 3e,f).

Safety assessment

During the 24-week study period, 11 of the 40 subjects experienced adverse events, including local skin problems and insomnia (Table 2), most of which were mild and self-limited. However, a subject in the VPA group developed ventricular tachycardia, a serious adverse event that originated from the left anterior fascicle, and dropped out of the study. The prevalence rates of the adverse events were not significantly different between both groups ($P = 0.72$).

Serum VPA concentration

The serum VPA concentration was measured in 20 subjects in the VPA group and 19 subjects in the placebo group. The serum VPA level was detectable in only seven subjects in the VPA group (range, 0.4–2.3 µg/mL), whereas it was too low (<0.4 µg/mL) to be detected in the other 13 subjects in the VPA group and all of the subjects in the placebo group.

DISCUSSION

For several decades, VPA has been frequently prescribed for epilepsy because of its safety and effectiveness. The classic mechanism of its antiepileptic effects was considered to be related to the inhibition of succinic semialdehyde

dehydrogenase activity and increased γ -aminobutyric acid level in the brain. The various effects of VPA at the molecular level have been under investigation. VPA affects gene expression by inhibiting histone deacetylase activity^{19,20} and various signaling pathways, including the extracellular signal-regulated kinase, protein kinase C and Wnt/ β -catenin pathways.^{13–16,21}

An interesting case of fetal valproate syndrome with generalized hypertrichosis except the hands and feet, resulting from *in utero* exposure to VPA, was reported.²² Although this case suggests that VPA has some promoting effects on hair growth, this potential of VPA has not attracted the interest of investigators because of some reported cases of hair loss induced by VPA medication.^{23–25} However, VPA was not the direct cause of hair loss in these cases but the decreased biotinidase activity and biotin deficiency induced by VPA. Serum biotinidase levels were generally low and biotin supplementation was demonstrated to restore hair loss in these cases.^{26–28} The mechanism of biotin deficiency by VPA is not clear, but impaired liver function by VPA may result in low serum biotinidase activity or inhibit carboxylase activity, which is involved in the biotin cycle.^{26,29,30} In an animal study using mice, p.o. administration of VPA failed to promote hair growth, whereas topical VPA application significantly promoted hair growth.¹⁸ Therefore, we used VPA as a topical agent in this study to avoid the possible systemic effect of VPA on biotinidase or carboxylase activity. Because of the low molecular weight of VPA (144.2 g/mol), it can be easily absorbed into the skin. Furthermore, it is highly soluble in water and stable at room temperature.

As in many previous studies,^{4,31,32} the primary end-point of this study was hair growth based on hair count. In comparison with the placebo group, the hair count increased and the change in hair count was statistically significant in the patients treated with the VPA spray. However, hair diameter and hair growth rate decreased in both groups without significant differences between groups. The reason is unclear but may be partly related to seasonal changes in human hair growth.^{33,34} Most of the subjects in our study were enrolled during early winter and completed the study during early summer. A longer study duration of up to 1 year may be helpful to avoid the seasonal changes.

For epilepsy and other psychiatric diseases, VPA is usually administrated p.o. or by injection. Therefore, topical VPA is expected to be safe systemically and to present little risk of severe adverse events. In this study, the frequency of adverse events in the VPA group was similar to that in the placebo group and most of the adverse events were mild and transient, suggesting that topical VPA was generally well tolerated. A patient treated with VPA spray developed ventricular tachycardia and underwent radiofrequency catheter ablation. However, the ventricular tachycardia did not seem to be related to the application of the VPA spray because cardiac arrhythmia is a rare adverse event even when VPA is administrated systemically and known to be related to VPA intoxication.³⁵ In our patient with ventricular tachycardia, the serum VPA concentration was 2.3 µg/mL, which is much lower than the therapeutic range for seizure control (50–100 µg/mL).³⁶

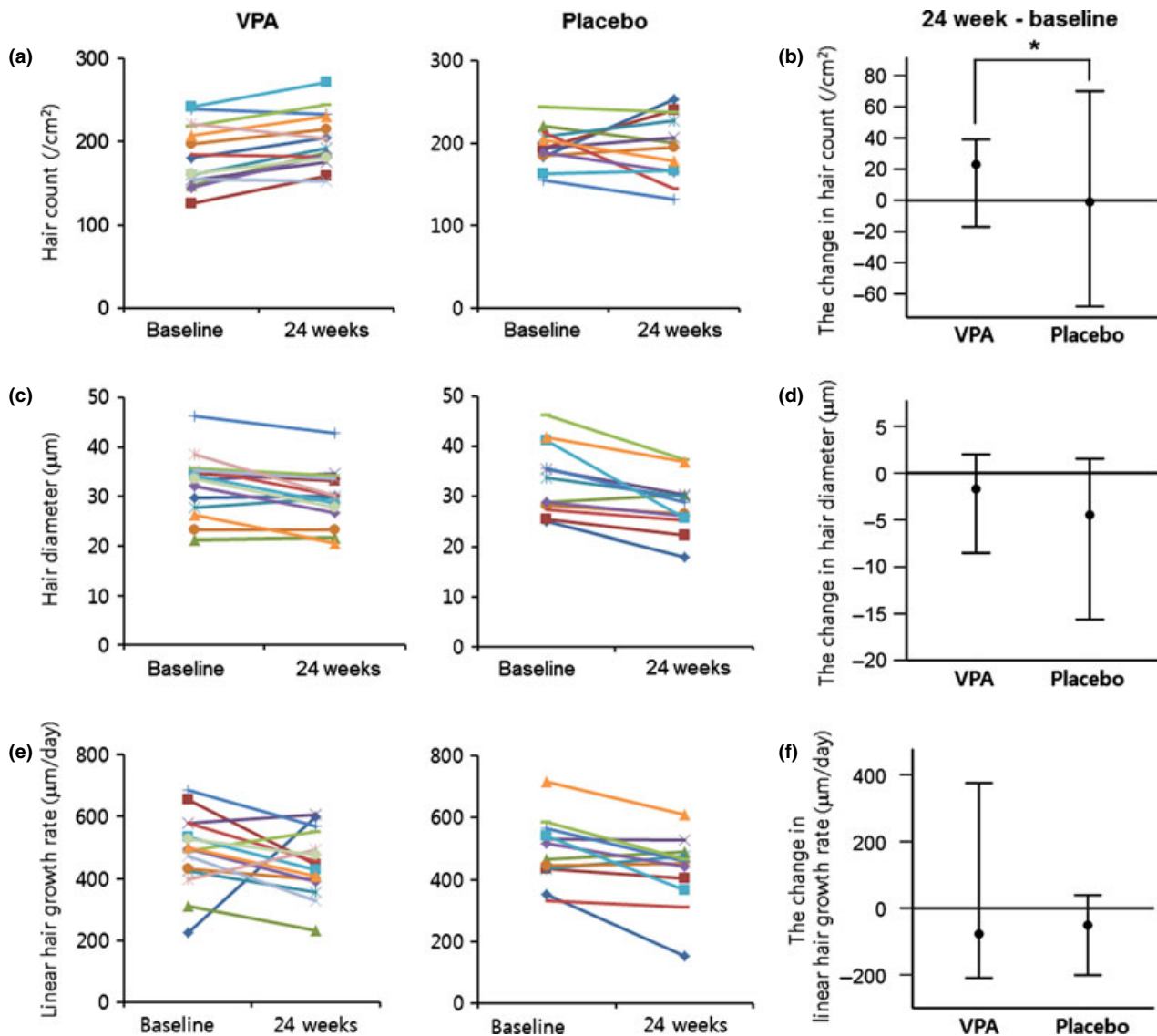


Figure 3. Phototrichogram data. The macrophotographs of the 1-cm² circular scalp area, 2-cm apart from the vertex, taken at baseline and after 24 weeks of treatment. The hair counts (a), hair diameters (c) and linear hair growth rates (e) were determined by the phototrichogram analysis. The changes in median hair count (b), hair diameter (d) and hair growth rate (f) in the VPA and placebo groups were compared. * $P < 0.05$, Mann-Whitney U -test. Error bar: range. VPA, valproic acid.

The present study had some limitations. First, the number of subjects was small. A large-scale study may be helpful to statistically confirm the efficacy and safety of topical VPA. Second, we did not run a minoxidil group. In previous studies, the increase in hair count ranged approximately 15–35/cm² in male or female patients with AGA after treatment with 1–5% topical minoxidil,^{37–41} while the median increase was 23/cm² after treatment with VPA in this study. However, the optimal dose and concentration of the VPA spray were not determined and higher concentration and dose of the VPA spray may be more effective for the treatment of AGA. Even if VPA is not as effective as minoxidil, it may be an alternative to minoxidil for the

patients with heart problems considering that long-term use of topical minoxidil can cause changes in cardiac function.⁴²

To the best of our knowledge, this study is the first clinical trial to analyze the feasibility of topical agent of VPA. The results of this study demonstrated that topical VPA increased the hair count in the patients with AGA although the dose of topical VPA was not optimized yet, and that the adverse events encountered by the VPA and placebo groups were not significantly different. A further study with a larger sample size and involving a comparative analysis of various VPA concentrations is necessary to evaluate the effects of topical VPA as a treatment option for AGA.

Table 2. Summary of the adverse reactions observed in each group after randomization

	VPA (n = 20)	Placebo (n = 20)
Adverse event profiles		
Itching		2
Dandruff	1	
Eczema	1	
Urticaria	1	
Dizziness		2
Insomnia	1	
Fever		1
Sexual dysfunction		1
Ventricular tachycardia	1	
Severity of adverse events		
Mild	4	6
Moderate	0	0
Severe	1	0
Life-threatening	0	0

VPA, valproic acid.

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CONFLICT OF INTEREST: The authors declare no competing financial interests.

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