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KF19418, a new compound for hair growth promotion in vitro and in vivo mouse models

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Abstract

KF19418, a newly synthesized compound, stimulated proliferation of cultured hair bulb cells from new born mice in concentration-dependent manner in the range under 10 μM. In the culture system of whole skin pieces from 4-week-old mice which we earlier established, KF19418 promoted hair follicle elongation as in the case of minoxidil. After topical application for 2 weeks of KF19418 or minoxidil to dorsal skin of hair-clipped mouse alopecia model, KF19418 at 1% suspension accelerated hair regrowth at a rate comparable to 1% minoxidil solution. Thus, it was shown that KF19418 directly stimulated hair follicle in vitro and had hair growth promoting activities in vivo.

Keywords: Hair bulb cell; Hair follicle; Organ culture; Mouse alopecia model

1. Introduction

Many substances have been investigated in attempts to cure male pattern baldness. One of the well-known effective substance is minoxidil (MXD). MXD is used as a hypotensive drug for its vasodilative effect with hypertrichosis as one of its side effects. It was suggested that the hair growth promotion by MXD was due to increase of cutaneous blood flow [1]. Topically applied, MXD was shown to improve blood flow in human balding scalps [2]. Recent in vitro studies demonstrated MXD or its sulfated metabolite act directly on hair follicles of mouse vibrissae [3,4] and human scalp hair follicles [5]. MXD was also shown to stimulate proliferation of cultured hair bulb cells (HBCs) from newborn mice [6]. In the culture system of whole skin pieces from 4-week-old mice which we earlier established, MXD promoted hair follicle elongation [7]. Potassium channel openers, including MXD, stimulates DNA synthesis [8] or protein synthesis [9] in whole-organ cultures of mouse vibrissa hair follicles. However, MXD or MXD sulfate failed to activate potassium channel current in hair follicles [10,11].

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For the purpose of discovering natural products which possess hair growing activity, we examined about 1000 kinds of plant extracts and found proanthocyanidins from grape seeds and procyanidin oligomers [12,13] promoted proliferation of mouse HBCs in vitro and activated hair follicle growth in vivo. We also screened more than 1000 of synthesized compounds using in vitro and in vivo models. In this report, we showed a novel compound, KF19418 had hair growth promotion in vitro and in vivo mouse models.

2. Materials and methods

2.1. Materials

The structure of KF19418, synthesized by Kyowa Hakko Kogyo Co. Ltd., Pharmaceutical Research Laboratories, is shown in Fig. 1. KF19418 has been known to possess various pharmacological activities, including immunosuppression shown by plaque forming cell assay, expansion of bronchia by passive Schultz-Dale reactions and anti-inflammation by inhibition effect on platelet activation factor-induced mortality (data not shown). MXD was obtained from Sigma (St. Louis, MO, USA). KF19418 was dissolved in dimethyl sulfoxide at 10mM and stored at –30°. Unless otherwise stated, plastic tissue culture dishes and plates were purchased from Iwaki (Chiba, Japan).

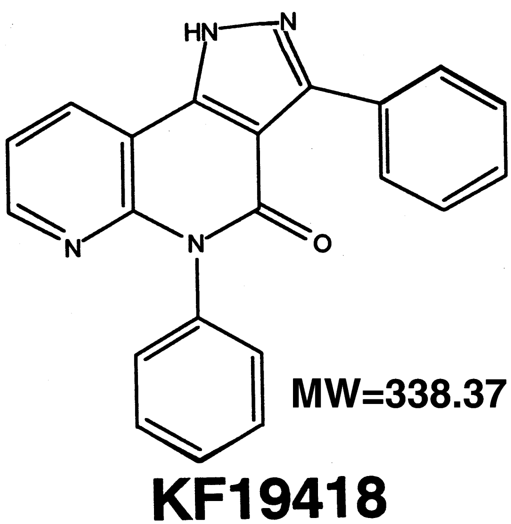

Fig. 1. The chemical structure of KF19418.

2.2. Preparation and culture of HBCs

We modified the method reported earlier [13]. Briefly, each of the dorsal skin from 4-day-old C3H/HeN mice (Charles River Japan, Kanagawa, Japan) was cut into small pieces and soaked in 500 U/ml dispase (Godo Syusei, Tokyo, Japan) in Eagle's minimum essential medium (Nissui, Tokyo, Japan) supplemented with 10% fetal calf serum (FCS) at 4°C for 24 h. The epidermis was peeled off and discarded. The dermal pieces containing hair follicles were kept in phosphate buffer saline – (PBS –), followed by treatment with 0.25% collagenase at 37°C for 1 h, and gently pipetted to obtain dermal suspension containing hair bulbs. The pellet was repeatedly rinsed with PBS (–) and the supernatant was discarded to remove dermal fibroblasts, leaving only hair bulbs as pellet. This pellet was treated with 0.1% EDTA–0.25% trypsin in PBS (–) at 37°C for 5 min and gently pipetted. The suspension of hair bulb cells in Dulbecco's modified Eagle medium (Nissui, Tokyo, Japan) supplemented with 10% FCS was plated onto Type I collagen-coated 24-well plates and incubated at 37°C in an atmosphere of 5% CO_2 and 95% air. After 24 h of plating, the media were changed to K-GM (Kurabo, Osaka, Japan) containing test compound after brief washing with PBS (–).

2.3. Preparation and culture of whole skin pieces

The detailed method was described earlier [7]. Briefly, 4-week-old C3H mice in anagen phase were trimmed by hair clippers and electric shavers and their dorsal skins were cut off by ophthalmic scissors. After removal of the connective tissue and the fatty tissue by tweezers, the dorsal skin was cut into small pieces by razor blades and scalpels. The skin pieces were placed on Costar Nuclepore™ membrane (13 mm diameter, 12.0 μm pores) floating on minimum essential medium alpha medium (Life Technologies, Gaithersburg, MD, USA) supplemented with 20% FCS, 10 μg/ml insulin, 50 U/ml of penicillin and 50 μg/ml of streptomycin in GREINER 24-well plates. The skin pieces were cultured at 37°C in an atmosphere of 5% CO_2 and 95% air.

2.4. Measurement of hair follicle elongation

Areas of individual skin pieces were measured by TV image processor TVIP-4100II (Nippon Avionics Co., Ltd., Tokyo) before and after the culture. The rates of hair follicle elongation and the increase in tissue area correlates well up to 3 days of culture in the skin piece culture of newborn mice, and this parameter was shown to be applicable to 4-week-old mice [7].

2.5. Mouse alopecia model

We modified the method reported earlier [14]. Briefly, 8-week-old C3H mice in telogen phase were trimmed by electric clippers and electric shavers. The next day, mice without visible scratches were selected, randomized and separated in groups of five mice. Compounds were dissolved or suspended in standard solvent; 64% ethanol, 20% propylene glycol and 16% distilled water. After topical treatments of solutions or suspensions (0.15 ml per mouse per day) for 2 weeks, hair-regrowing activities of each compound were estimated. Dorsal skin was removed from each animal and was flattened on a wooden plate. Both hair-regrown area and shaved area were measured using digitizer.

3. Results

3.1. Stimulation of HBCs proliferation by KF19418

HBCs contains hair matrix cells rich in germinative epithelial cells that are to differentiate into hair shafts in vivo. We earlier demonstrated that proanthocyanidins from grape seeds and procyanidin oligomers stimulated proliferation of cultured HBCs isolated from newborn mice [12,13].

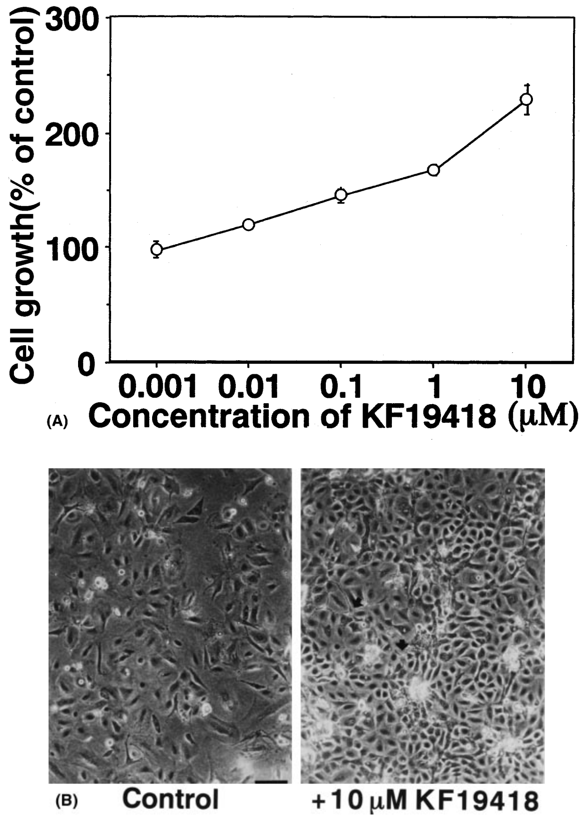

Fig. 2. (A) Stimulation of hair bulb cell proliferation. (B) Morphology of control cells (-KF19418) and KF19418 treated cells. Hair bulb cells prepared from 4-day-old C3H mice were plated at a density of 3×10^5 cells per well of 24-well plate. After 4 days of culture in the presence of various concentration of KF19418, cells were harvested and counted with a haemocytometer chamber. ($N = 3$, mean ± S.E.M.). Arrows: melanocytes. Bar = 0.1 mm. The experiment was repeated twice with similar results.

In the present study, we found that KF19418 stimulated proliferation of cultured HBCs in concentration-dependent manner in the range under 10 μM (Fig. 2A). Microscopic observation revealed that KF19418 not only kept HBCs small (immature morphology), but also maintained melanocyte population existed in cultured HBCs (Fig. 2B).

3.2. In vitro promotion of hair follicle elongation by KF19418

We earlier demonstrated that in the culture system of whole skin pieces from 4-week-old C3H mice in anagen phase, MXD promoted hair follicle elongation after 3 days of culture [7]. In this experiment, we found that KF19418 promoted hair follicle elongation as in the case of MXD (Fig. 3). KF19418 at 10 μM accelerated hair follicle elongation better than 0.5 mM MXD. By direct observation of cultured skin pieces, it was evident that KF19418 stimulated not only hair follicle elongation but also thickening of hair bulbs (data not shown).

3.3. Evaluation of KF19418 on C3H mouse alopecia model

MXD and KF19418 were examined in hair-clipped C3H mouse alopecia model. After topical application of 1% solution of MXD or suspension of KF19418 for 2 weeks, it became evident that both compounds facilitated hair regrowth (Fig. 4). In the mice that received either MXD or KF19418, hair regrowth began to be visible after 1 week of application. In contrast, the mice that received vehicle showed only a faint evidence of hair regrowth even after 2 weeks of application. Ratios of hair-regrown area to shaved area were summarized in Table 1. It was demonstrated that KF19418 promoted hair regrowth at a rate comparable to MXD. KF19418 did not cause any visible abnormal changes (e.g. inflammation) against skin.

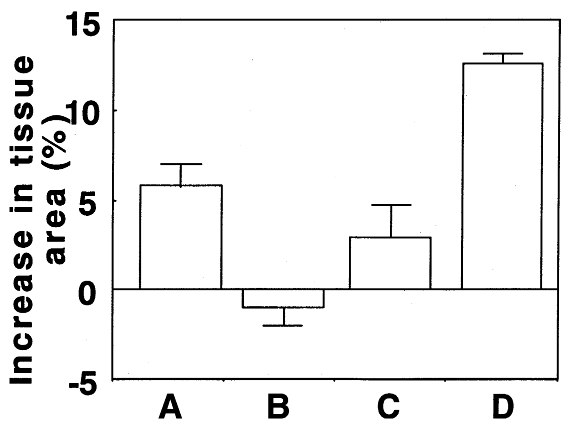

Fig. 3. In vitro promotion of hair follicle elongation by KF19418. Skin pieces from 4-week-old C3H mice were cultured in the presence or absence of each compound for 3 days. Areas of individual skin pieces were measured (as described in Section 2). (A) minoxidil (0.5 mM); (B) KF19418 (0 μM); (C) KF19418 (3 μM); (D) KF19418 (10 μM). ($N = 6$, mean ± S.E.M.). The experiment was repeated twice with similar results.

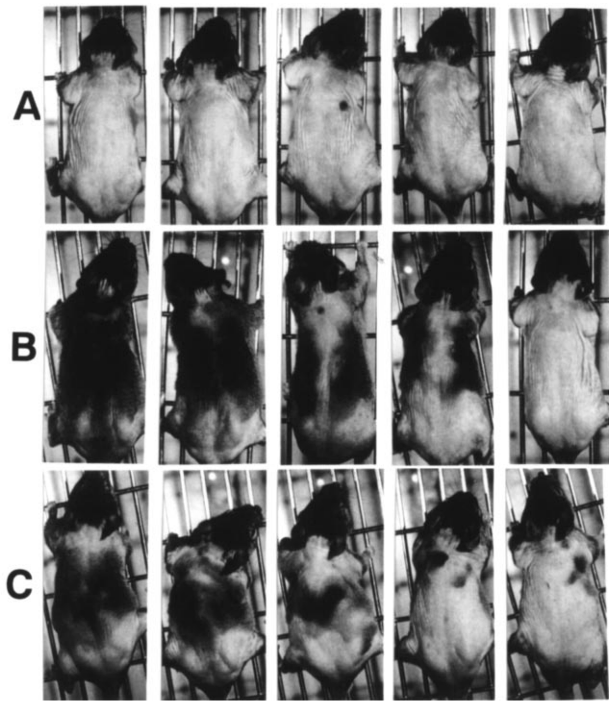

Fig. 4. Hair regrowth in C3H mouse alopecia model. After topical application of test compounds for 2 weeks, photographs of each animal were taken. (A) standard solvent; (B) 1% minoxidil (solution); (C) 1% KF19418 (suspension). The experiment was repeated twice with similar results.

Table 1
Qantification of hair-regrowth activities of minoxidil and KF19418 in C3H mouse alopecia model[a]

	Hair-regrown area/shaved area (%)	
Solvent	4.6	1.3
1% Minoxidil	53.8	14.5
1% KF19418	38.4	11.4
	(mean ± S.E.M.)	

[a] Both hair-regenerated area and shaved area were measured for the mice in Fig. 4 (as described in Section 2). The experiment was repeated twice with similar results.

4. Discussion

We demonstrated that KF19418 had hair growth promoting activities both in vitro and in vivo mouse models.

Morphological, histochemical and biochemical aspects of male pattern baldness have been revealed by a series of studies of stumptailed macaques which go bald as they become adult [15–20]. In male pattern baldness, telogen phase is prolonged and anagen phase is shortened. One of the histological characteristics of this disease is that anagen hair follicles cannot fully elongate. Thickness of produced hair is determined by the size of hair follicle. Thus, maintaining hair follicle elongation that occurs from early anagen to mid anagen is very important in normal hair cycle.

We used primary culture of mixed HBC population and found that KF19418 extensively potentiated the growth of HBCs. These HBCs are able to differentiate into inner root sheath cells, hair cortical cells and medulla cells [21]. We assume that KF19418 targeted hair matrix cells because the greater part of the population contained in cultured HBCs was considered to be hair matrix cells. The HBCs cultured in KF19418 containing medium maintained the rounded morphology, a characteristic of undifferentiated juvenile cells. This suggests that the mechanism of action of KF19418 may involve the prevention of cell differentiation and the retention of growing phase.

The in vitro organ culture system employed in this study used skin pieces obtained from mice that have elongating hair follicles in vivo. For some unknown reason, these hair follicles ceased to elongate in vitro and even retarded to catagen-like structure. Our earlier finding that MXD stimulates these hair follicles in this model [7] suggests that stimulation of in vitro hair follicle elongation by this compound is relevant to its effect on human alopecia. Now we demonstrated that KF19418 promoted hair follicle elongation as in the case of MXD.

KF19418 facilitated hair regrowth in C3H mouse alopecia model. It is possible that the hair follicles were stimulated to enter into anagen by this compound and the elongation of these immature hair follicles were accelerated, resulting in the shortening of time required for full hair regrowth. These results suggest that KF19418 has possibilities of promoting hair follicle elongation in humans, thus extending the anagen phase in male pattern baldness.

Specific activity of KF19418 for hair follicle elongation in vitro was much higher than that of MXD, whereas its in vivo activity was not superior to MXD. Both KF19418 and MXD at 0.1% concentration were soluble in the solvent used in in vivo experiment. Under these conditions, KF19418 failed to promote hair regrowth better than MXD (data not shown). This suggests at least the following three possibilities: (A) KF19418 was absorbed into skin much less than MXD; (B) After absorbed into skin, KF19418 were changed to inactive metabolites; and (C) Some unique mechanism other than revealed in our in vitro experiment was involved in the in vivo activity of MXD. In fact, it was suggested that MXD sulfate is the active metabolite in hair follicles [4]. In addition, it was demonstrated that MXD stimulates cutaneous blood flow in human scalps [2].

Although KF19418 and MXD showed similar hair growth promoting activities in vitro and in vivo in this study, KF19418 has quite different chemical structure from MXD. Moreover, KF19418 possesses various pharmacological activities not reported in MXD. Although it is not yet clear whether these activities of KF19418 are involved in its hair growth promoting activities, to clarify the mechanisms of action of KF19418 more precisely may lead to discover new candidates for treatment of human alopecia.

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