Induction of Anagen in Telogen Mouse Skin by Topical Application of FK506, a Potent Immunosuppressant

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The effect of topical application of FK506 on the normal hair cycle of C57BL/6J mice was investigated. When telogen mice (7 weeks of age) were treated topically with 1 μmol FK506 on days 0 and 3, 50% of the tested mice entered anagen by day 9 and 100% by day 16. With 0.1 μmol of FK506, 50% of the tested mice entered anagen by day 13 and 80% by day 19, indicating that the effect of FK506 is dose dependent. In control mice, a spontaneous shift from telogen to anagen started on day 14, and 30% of the control animals were in anagen at day 19. Histologic studies revealed that FK506 markedly stimulated the skin and thickened it. The depth and size of hair follicles were also markedly increased in FK506-treated skin compared to control skin. The data on hair growth also support the contention that FK506 induces early onset of anagen and stimulates hair growth. The hair growth stimulated by FK506 looked normal and the hairs were of normal length. The hair growth was restricted to the site of application. These results clearly demonstrate that topical application of FK506 induces anagen hair growth in telogen mouse skin and indicate that the hair-growth–stimulating effect of FK506 is due at least in part to its promoting effect on the hair cycle. ***Key words: hair growth/hair follicle/ C57BL/6J mouse. J Invest Dermatol 104:523–525, 1995***

FK506, a macrolide antibiotic, is a T-cell–specific immunosuppressant [1,2]. Topical application of FK506 stimulates hair growth in several species of animals, including mice [3]. In contrast, oral administration of FK506 at a dose that induces marked immunosuppression did not stimulate significant hair growth [3], consistent with the fact that FK506 does not induce hypertrichosis in clinical trials [4–7]. The hair-growth–stimulating effect of FK506 is due at least in part to its direct stimulation of hair follicles and may be essentially unrelated to its immunosuppressive effect [3]. Although at present the precise mechanism of this hair-growth–stimulating effect is not clear, it seems important to examine the effect of FK506 on the normal hair cycle. Therefore, we investigated whether FK506 induces anagen hair growth in telogen mouse skin.

MATERIALS AND METHODS

FK506 was supplied by Fujisawa Pharmaceutical Co. (Osaka, Japan). Female C57BL/6J mice were purchased from Japan Clea Co. (Tokyo, Japan). Mice were housed in an air-conditioned room (22–23°C) with a light period from 6 AM to 6 PM. Food and water were available *ad libitum*. The dorsal hair of each mouse was shaved with clippers at least 2 d before use. All of the experiments were started at 7 weeks of age, when all the mice used were in telogen phase. FK506 (0.1 or 1 μmol) was applied topically once each on days 0 and 3. FK506 was dissolved in acetone and applied to the shaved area in a volume of 0.2 ml using a micropipet. The control animals were treated topically with 0.2 ml of acetone alone. The area of dorsal skin treated with FK506 was approximately 3 cm^2.

The anagen induction assay was performed as described by Paus *et al* [8]. In brief, the back skin of each mouse was observed once daily during the experiment. The number of animals in telogen or anagen, as judged by their skin color, i.e., bright pink in telogen and gray to black in anagen, was counted and expressed as the percentage of animals in anagen. Each group consisted of 10 animals. Histologic examination also was conducted to confirm anagen induction. The skin was stretched and fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin. Thickness of the skin and depth of the hair follicles were measured microscopically. The length of dorsal hairs also was measured. All of the experiments were repeated twice on different occasions using animals from different litters. The mice used were chosen randomly from 50–100 animals in each experiment.

All experimental protocols were approved by The Experimental Animals Committee of Keio University School of Medicine.

Statistical analysis was done by Student t test.

RESULTS AND DISCUSSION

At the age of 7 weeks, C57BL/6J mice had pink skin, indicating telogen (resting) hair follicles. When the mice entered anagen, hair regrowth began just as described previously [8], as detected by increasing skin pigmentation, i.e., gray to black. After the mice were treated topically with 1 μmol FK506 on days 0 and 3, 50% of the tested animals entered anagen by day 9 and 100% by day 16 **(Fig 1)**. With 0.1 μmol of FK506, 50% of the tested mice entered anagen by day 13 and 80% by day 19 **(Fig 1)**, indicating that the effect of FK506 was dose dependent. In the control animals, a spontaneous shift from telogen to anagen started on day 14, and 30% were in anagen phase at day 19 **(Fig 1)**. These results indicate the induction of anagen by FK506.

Figure 2 and **Table I** show the histology and the quantitative morphometric data, respectively, for the control and FK506-treated mouse skin. As is clearly shown, FK506 treatment markedly stimulated the skin and thickened it, without inducing an inflammatory reaction. The depth and size of hair follicles were also markedly

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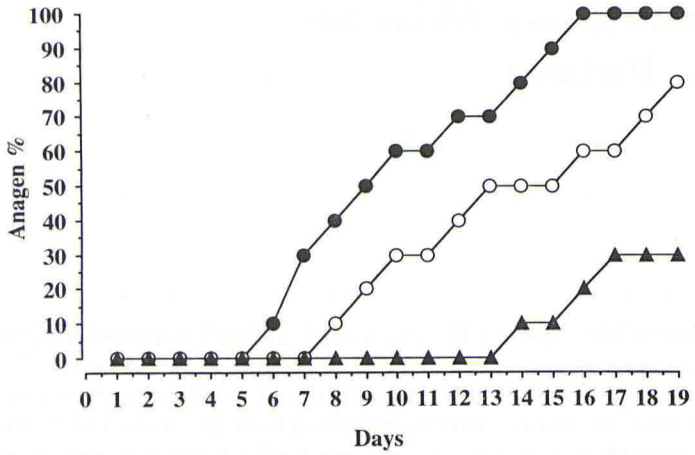

Figure 1. Induction of anagen in telogen mice by topical application of FK506. FK506, 0.1 μmol (*open circles*) or 1 μmol (*closed circles*), or vehicle (acetone; *triangles*) was applied topically to the dorsal skin of telogen C57BL/6J mice (7 weeks of age) once each on days 0 and 3. The number of animals in telogen or anagen, as judged by their skin color, i.e., bright pink in telogen and gray to black in anagen, was counted and expressed as percentage of animals in anagen. Each group consisted of 10 animals. All the experiments were repeated twice, and the results were reproducible.

increased in FK506-treated skin compared to control skin **(Fig 2; Table I)**. It has been reported that skin thickness increases from a thin telogen skin to a thickened anagen skin [8,9] and that the size and depth of follicles are markedly increased in anagen phase [9,10]. Changes in the hair length after FK506 treatment were also seen **(Fig 3)**. FK506 markedly stimulated hair growth, consistent with our

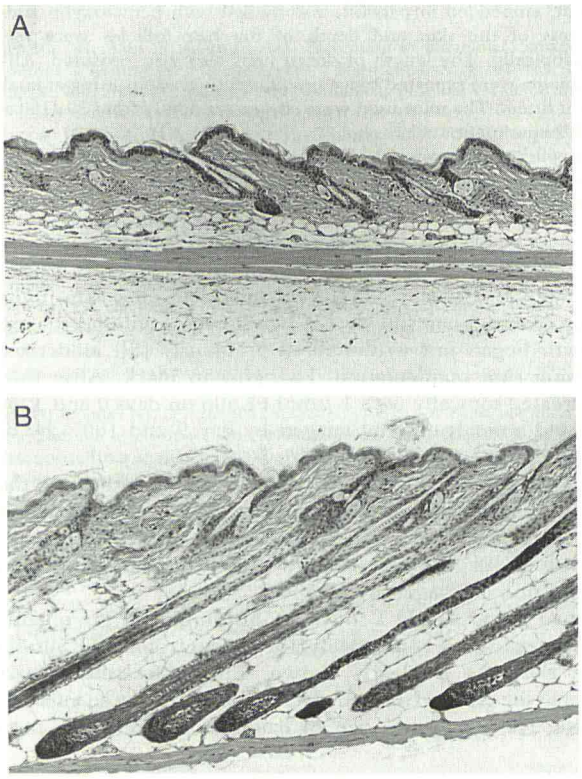

Figure 2. Histology of FK506-treated mouse skin. Vehicle (acetone) (*A*) or FK506 (1 μmol) (*B*) was applied topically to telogen C57BL/6J mice (7 weeks of age) once each on days 0 and 3. At day 15, skin was taken for histologic examination. Each section was stained with hematoxylin and eosin. Magnification × 25.

Table I. Comparison of Total Dermal Thickness and Hair Follicle Depth Between Control (Acetone-Treated) and FK506-Treated Mouse Skin[a]

Day	Treatment	Total Dermal Thickness[b] (μm)	Hair Follicle Depth[b] (μm)
0	None	388 ± 9	172 ± 8
8	Acetone	382 ± 4[c]	164 ± 7[c]
8	FK506, 0.1 μmol	490 ± 16[d]	311 ± 20[d]
15	Acetone	398 ± 15[c]	167 ± 8[c]
15	FK506, 0.1 μmol	719 ± 22[d]	548 ± 22[d]
15	FK506, 1 μmol	662 ± 19[d]	535 ± 17[d]

[a] FK506 was applied topically to the dorsal skin of C57BL/6J mice (7 weeks of age) on days 0 and 3.
[b] Values are expressed as mean ± SEM (n = 15–22) from three mice.
[c] Not significantly different versus none (day 0).
[d] $p < 0.01$ versus corresponding control (acetone).

previous observations [3]. The hair produced looked normal, and the hairs were of normal length **(Fig 3)**. Moreover, the hair growth was restricted to the site of application **(Fig 4)**. All of these results clearly demonstrate that topical application of FK506 induces anagen hair growth in telogen mouse skin.

As reported previously, topical application of FK506 markedly stimulates hair growth in mice, rats, and hamsters [3]. *In vitro* studies have revealed that FK506 stimulates [^{3}H]thymidine and [^{3}H]glycine uptake in the hair follicles [3]. Our present results indicate that the hair-growth–stimulating effect of FK506 is due at least in part to its promoting effect on the hair cycle.

Another T-cell–specific immunosuppressant, cyclosporin A (CsA), also stimulates hair growth both in laboratory animals [8,11–13] and in humans [14–18]. Hypertrichosis is a well-known side effect of CsA [14–18]. Moreover, CsA also induces anagen in telogen mouse skin [8]. Because of these findings, one can speculate that the immunosuppressive effect of FK506 is related to its stimulation of hair growth. However, the hair-growth–stimulating effect of FK506 does not occur after oral administration of an immunosuppressive dose of this drug [3]. Furthermore, FK506 stimulates hair growth even in SCID mice, which lack both B- and T-cell immunity [3]. Therefore, it seems unlikely that the hair growth-stimulating effect of FK506 is related to its immunosuppressive effect [3].

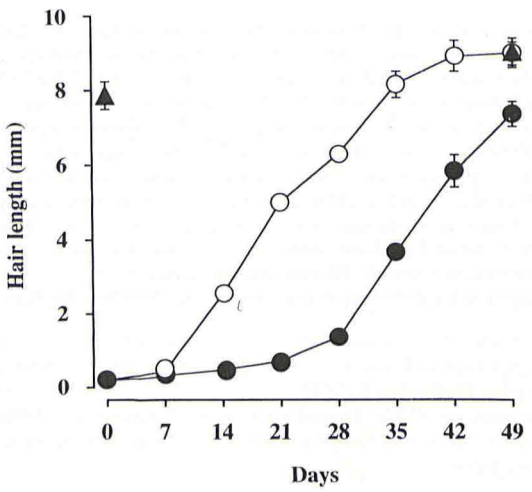

Figure 3. Changes in hair length after topical application of FK506 in telogen mice. The dorsal hair of C57BL/6J mice (7 weeks of age) was shaved with clippers 2 d before day 0. FK506 (1 μmol; *open circles*) or vehicle (acetone; *closed circles*) was applied topically on days 0 and 3. *Triangles* indicate non-shaved control mice (without treatment). Values are expressed as mean ± SEM of 35 hairs from four mice.

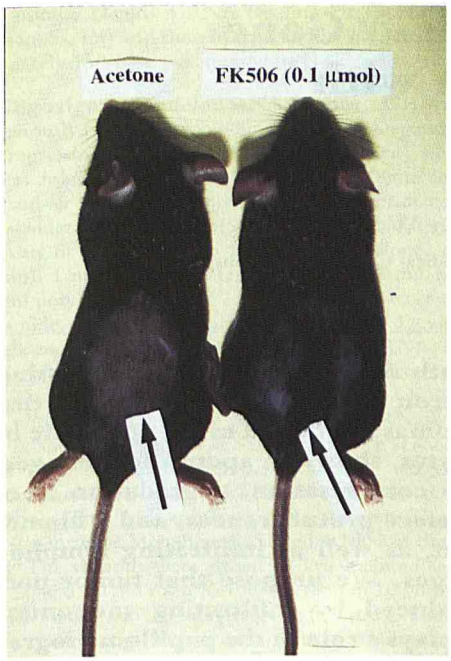

Figure 4. Hair growth is restricted to the site of topical application in FK506-treated C57BL/6J mice. FK506 (0.1 μmol) or vehicle (acetone) was applied topically only to the longitudinal center of the shaved area on days 0 and 3. The macrophotograph was taken 3 weeks after the application.

Both FK506 and CsA exert their immunosuppressive effects through inhibition of T-cell activation, interfering with the production of interleukin-2 by inhibiting interleukin-2 gene expression [1,2,19,20]. FK506 and CsA initially bind to intracellular binding proteins, i.e., FK506-binding protein and cyclophilin, respectively [21–24]. Both FK506-binding protein and cyclophilin, also called immunophilins, are identical to peptidyl-prolyl isomerase [21,22,25,26], i.e., rotamase, an enzyme that catalyzes the interconversion of the *cis*- and *trans*-rotamers of the peptidyl amide bond of peptides. Rotamase activity is inhibited by these immunosuppressants [21,25,26]; however, this inhibition is essentially unrelated to the immunosuppressive activities of these drugs [27,28]. Both FK506/FK506-binding protein and CsA/cyclophilin complexes bind to Ca^{++}- and calmodulin-dependent serine/threonine phosphatase calcineurin, resulting in inhibition of the phosphatase activity [29]. Inhibition of calcineurin by FK506 and CsA suppresses the assembly of the T-cell–specific transcription factor of the interleukin-2 gene, i.e., NF-AT, leading to the inhibition of interleukin-2 gene expression [30].

At present, the precise mechanism of the hair-growth–stimulating effect of FK506 is unknown. Moreover, it is also unclear whether FK506 and CsA stimulate hair growth through a common mechanism. Because cyclophilin, FK506-binding protein, and calcineurin are abundant and ubiquitous proteins, it should be considered that immunophilin-related or immunophilin/calcineurin-related mechanisms are involved in the hair-growth–stimulating effects of FK506 and CsA. Further studies are now under way in our laboratory to elucidate the precise mechanism.

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REFERENCES

1. Schreiber SL, Crabtree GR: The mechanism of action of cyclosporine A and FK506. *Immunol Today* 13:136–142, 1992
2. Sigal NH, Dumont FJ: Cyclosporin A, FK-506, and rapamycin: pharmacologic probes of lymphocyte signal transduction. *Annu Rev Immunol* 10:519–560, 1992
3. Yamamoto S, Jiang H, Kato R: Stimulation of hair growth by topical application of FK506, a potent immunosuppressive agent. *J Invest Dermatol* 102:160–164, 1994
4. Armitage JM, Fricker FJ, del Nido P, Starzl TE, Hardesty RL, Griffith BP: A decade (1982 to 1992) of pediatric cardiac transplantation and the impact of FK506 immunosuppression. *J Thorac Cardiovasc Surg* 105:464–473, 1993
5. Starzl TE: FK506 versus cyclosporine. *Transplant Proc* 25:511–512, 1993
6. Starzl TE, Fung J, Jordan M, Shapiro R, Tzakis A, McCauley J, Johnston J, Iwaki Y, Jain A, Alessiani M, Todo S: Kidney transplantation under FK506. *JAMA* 264:63–67, 1990
7. Uemoto S, Tanaka K, Honda K, Tokunaga Y, Sano K, Katoh H, Yamamoto E, Takada Y, Ozawa K: Experience with FK506 in living-related liver transplantation. *Transplantation* 55:288–292, 1993
8. Paus R, Stenn KS, Link RE: The induction of anagen hair growth in telogen mouse skin by cyclosporine A administration. *Lab Invest* 60:365–369, 1989
9. Hansen LS, Coggle JE, Wells J, Charles MW: The influence of the hair cycle on the thickness of mouse skin. *Anat Rec* 210:569–573, 1984
10. Bogovski P: Tumors of the skin. In: Turusov VS (ed.). *Pathology of Tumours in Laboratory Animals, Vol. II. Tumours of the Mouse.* IARC Scientific Publications no. 23. International Agency for Research on Cancer, Lyon, 1979, pp 1–40
11. Pendry A, Alexander P: Stimulation of hair growth on nude mice by cyclosporin A. In: White DJG (ed.). *Proceedings of the International Conference on Cyclosporin A.* Elsevier Biomedical Press, New York, 1982, pp 77–81
12. Buhl AE, Waldon DJ, Miller BF, Brunden MN: Differences in activity of minoxidil and cyclosporin A on hair growth in nude and normal mice. Comparisons of *in vivo* and *in vitro* studies. *Lab Invest* 62:104–107, 1990
13. Sainsbury TSL, Duncan JI, Whiting PH, Hewick DS, Johnson BE, Thomson AW, Oliver RF: Differential effects of FK506 and cyclosporine on hair regrowth in the DEBR model of alopecia areata. *Transplant Proc* 23:3332–3334, 1991
14. Brown MD, Gupta AK, Ellis CN, Rocher LL, Voorhees JJ: Therapy of dermatologic disease with cyclosporine A. *Adv Dermatol* 4:1–28, 1989
15. Groisser DS, Griffiths CEM, Ellis CN, Voorhees JJ: A review and update of the clinical uses of cyclosporine in dermatology. *Dermatol Clin* 9:805–817, 1991
16. Kahan BD: Cyclosporine. *New Engl J Med* 321:1725–1738, 1989
17. Bencini PL, Montagnino G, Sala F, De Vecci A, Crosti C, Tarantino A: Cutaneous lesions in 67 cyclosporin-treated renal transplant recipients. *Dermatologica* 172:24–30, 1986
18. Wysocki GP, Daley TD: Hypertrichosis in patients receiving cyclosporine therapy. *Clin Exp Dermatol* 12:191–196, 1987
19. Siekierka JJ, Sigal NH: FK-506 and cyclosporin A: immunosuppressive mechanism of action and beyond. *Curr Opin Immunol* 4:548–552, 1992
20. Schreier MH, Baumann G, Zenke G: Inhibition of T-cell signaling pathways by immunophilin drug complexes: are side effects inherent to immunosuppressive properties? *Transplant Proc* 25:502–507, 1993
21. Siekierka JJ, Hung SHY, Poe M, Lin CS, Sigal NH: A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. *Nature* 341:755–757, 1989
22. Harding MW, Galat A, Uehling D, Schreiber SL: A receptor for the immunosuppressant FK506 is a cis-trans peptidyl-prolyl isomerase. *Nature* 341:758–760, 1989
23. Handschumacher RE, Harding MW, Rice J, Drugge RJ, Speicher DW: Cyclophilin: a specific cytosolic binding protein for cyclosporin A. *Science* 226:544–547, 1984
24. Harding MW, Handschumacher RE, Speicher DW: Isolation and amino acid sequence of cyclophilin. *J Biol Chem* 261:8547–8555, 1986
25. Takahashi N, Hayano T, Suzuki M: Peptidyl-prolyl cis-trans isomerase is the cyclosporin A-binding protein cyclophilin. *Nature* 337:473–475, 1989
26. Fischer G, Wittmann-Liebold B, Lang K, Kiefhaber T, Schmid FX: Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. *Nature* 337:476–478, 1989
27. Bierer BE, Somers PK, Wandless TJ, Burakoff SJ, Schreiber SL: Probing immunosuppressant action with a nonnatural immunophilin ligand. *Science* 250:556–559, 1990
28. Sigal NH, Dumont F, Durette P, Siekierka JJ, Peterson L, Rich DH, Dunlap BE, Staruch MJ, Melino MR, Koprak SL, Williams D, Witzel B, Pisano JM: Is cyclophilin involved in the immunosuppressive and nephrotoxic mechanism of action of cyclosporin A? *J Exp Med* 173:619–628, 1991
29. Liu J, Farmer JD Jr, Lane WS, Friedman J, Weissman I, Schreiber SL: Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell* 66:807–815, 1991
30. Flanagan WM, Corthesy B, Bram RJ, Crabtree GR: Nuclear association of a T-cell transcription factor blocked by FK-506 and cyclosporin A. *Nature* 352:803–806, 1991