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# Effects of protein kinase C activators on mouse skin in vivo

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Tumor-promoting phorbol ester, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) is known to have many biological effects on epidermal cells [14]. TPA activates protein kinase C directly both in vivo and in vitro [3], and protein kinase C is the receptor of TPA [5, 8]. Therefore, it is possible that biological effects of TPA on epidermis are mediated by protein kinase C, which is also known to be activated by diacylglycerol generated from phosphoinositides of cell membrane [9]. It has been reported that synthetic diacylglycerol, 1-oleoyl-2-acetylglycerol (OAG), as well as TPA, is able to penetrate cell membrane and activate protein kinase C in intact cells [1, 4, 9]. OAG has some biological effects on epidermal cells as does TPA [7, 12]. We investigated marco- and microscopic changes that could be induced by topical application of OAG, as compared with application of TPA.

Dorsal skin (6 $cm^2$) of Balb/c and Swiss-Webster mice aged 10 weeks were shaved with an electric shaver so as to avoid injury. TPA (20 μg) and OAG (40 mg) were each dissolved in 100 μl acetone. Each 100-μl acetone solution was applied with a microsyringe onto the shaved back skin of the mice. In consideration of previous reports [7, 11] these concentrations of TPA and OAG could be thought to be effective biologically on epidermal cells; 100 μl of acetone only was used as control. Five mice were used per group. The mice were treated with each solution once weekly for 3 weeks. They were killed 1 week after the last treatment, and the skins were taken and stained with HE.

The hair regrowth patterns in each group of Balb/c mice are shown in Fig. 1a–c. Hair regrowth was much accelerated by both OAG and TPA (Fig. 1b, c), compared with acetone control (Fig. 1a). Histological findings of these samples are shown in Fig. 1d–f. Downward elongation of hair follicles was observed in both OAG- and TPA-treated mice (Fig. 1e, f). However, the epidermis of both OAG- and TPA-treated mice showed no change either macroscopically or microscopically. With Swiss-Webster mice, the hair regrowth patterns were almost the same as those with Balb/c mice (data not shown). Epidermal changes, such as epidermal thickening, hyperkeratosis, and hypergranulosis, were induced by the TPA treatment (Fig. 2c), but not by OAG (Fig. 2b).

Previously Ogawa et al. [11] had reported that among various biologically active substances hair growth of mouse was remarkably accelerated by TPA. In our present study, it was found that OAG has a similar effect on hair growth as TPA. As both TPA and OAG may activate protein kinase C, it is possible that their effects on hair growth shown in this report were mediated by protein kinase C.

With regard to epidermis of Swiss-Webster mice, OAG did not induce any morphological change (Fig. 2b), whereas TPA induced significant changes on epidermis, such as epidermal thickening, hyperkeratosis, and hypergranulosis (Fig. 2c). The result was different in the case of Balb/c mice: Neither OAG nor TPA induced any morphological change on epidermis of Balb/c mice. It is well known that all strains of mice do not have the same sensitivity to TPA. This could explain why TPA has an effect on Swiss-Webster mice and not on Balb/c mice.

It has been generally believed that OAG induces the same biological effects as TPA in many cell systems [1, 9]. However, recently it has been reported [12, 13] that in some cell systems OAG does not induce the same biological effects at TPA. The reasons for the differences between effects of TPA and OAG on epi-

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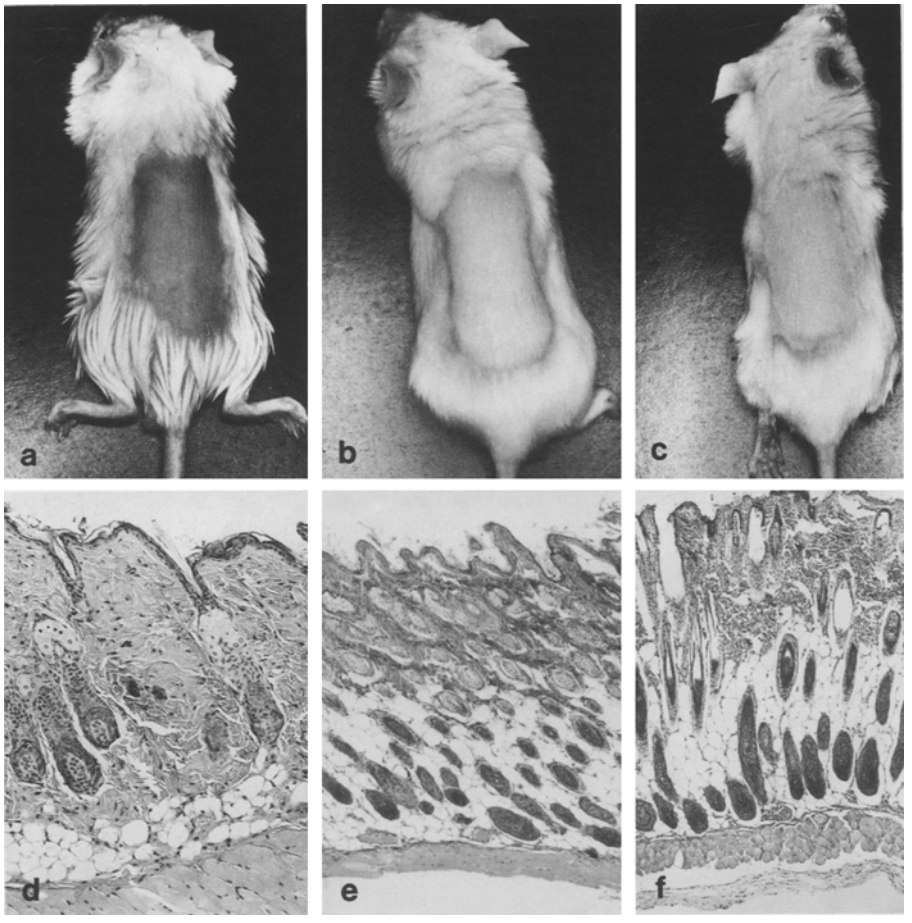


**Fig. 1a–f.** Effect of protein kinase C activators on hair regrowth. Macroscopic features induced by acetone control **(a)**, OAG **(b)**, TPA **(c)**. Microscopic features induced by acetone control **(d)**, OAG **(e)**, TPA **(f)**. (HE × 100

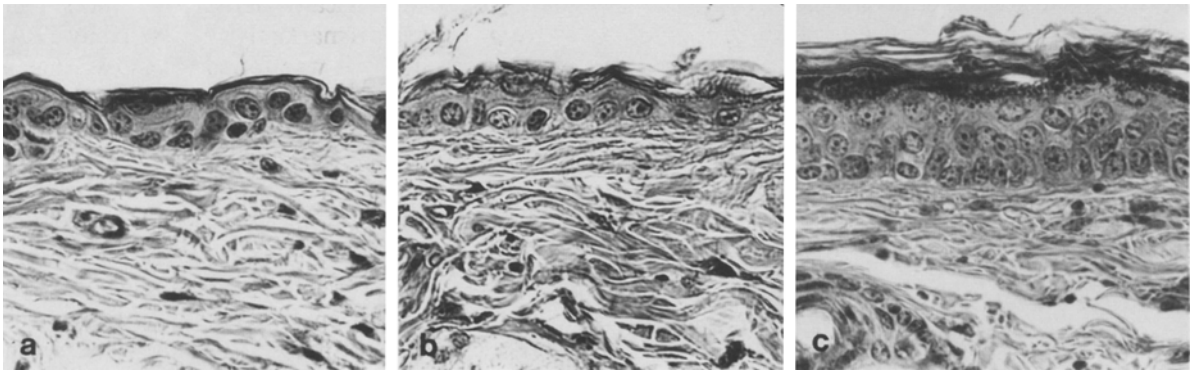


**Fig. 2a–c.** Effects of protein kinase C activators on epidermis. Microscopic features induced by acetone control **(a)**, OAG **(b)**, TPA **(c)**. (HE × 400)

dermis of Swiss-Webster mice may be speculated to be as follows: (a) OAG is more rapidly metabolized than TPA in vivo [9]. Therefore, it is possible that OAG is a weaker activator of protein kinase C than TPA. (b) An alternative possibility is that TPA has other activities than those mediated by protein kinase C. (c) Recently it has been reported that several forms of protein kinase C are expressed in cells [6]. The functions of these different forms are not yet understood. However, perhaps TPA and OAG modulate the activity of several isozymes of protein kinase C, which in turn may exert different biological effects. Further investigation is needed in order to determine which reason is correct in epidermis.

It is well known that penetration of a compound applied topically is greater via hair follicles than via

interfollicular epidermis. This could explain why OAG and TPA have an effect on hair growth and not on epidermis of Balb/c mice.

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