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Tumor-promoting Activity of Staurosporine, a Protein Kinase Inhibitor on Mouse Skin

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ABSTRACT

Staurosporine, which is a potent inhibitor of protein kinases, such as protein kinase C, inhibited both inductions of adhesion of mouse promyelocytic leukemia cells (50% effective dose = 9.0 nM) and 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ornithine decarboxylase activity in mouse epidermis. The two-stage carcinogenesis experiments of staurosporine were carried out at two different doses. Experiment 1 revealed that the group treatment with a single application of 100 μg of 7,12-dimethylbenz(a)anthracene alone gave 6.7% and 85.7% of tumor-bearing mice treated with teleocidin, but staurosporine has some potency to inhibit tumor promotion by teleocidin.

INTRODUCTION

TPA-type tumor promoters, such as TPA, telocidin, and aphisloxatin, activate calcium-activated, phospholipid-dependent protein kinase (protein kinase C) and, thus, induce various biological activities and tumor promotion (1, 2). If inhibitors of protein kinase C block this phosphorylation, they should also inhibit the tumor promotion induced by TPA-type tumor promoters. In fact, naltrokoxycarnitine, a protein kinase C inhibitor, has this effect (3, 4).

A potent protein kinase C inhibitor, staurosporine (Fig. 1), was isolated from Streptomyces sp by Tamaoki et al. (5). Staurosporine inhibited protein kinase C activity within a nm range of concentration, as well as the activity of cyclic AMP-dependent protein kinase, cyclic GMP-dependent protein kinase, and the phosphotyrosine kinase (5, 6). Although staurosporine did not selectively inhibit protein kinase C, the effects of staurosporine on various biological activities induced by phorbol esters had been reported. Staurosporine enhanced differentiation of HL-60 cells (7) and inhibited induction of EB virus EA in Raji cells (8) and NADPH-oxidase activation in human neutrophils (9). The study with staurosporine suggested the presence of dual effects on tumor promotion.

Here, we report that staurosporine induced irritation on mouse skin and HDC activity in mouse skin and had a weak tumor-promoting activity on mouse skin initiated with DMBA, although staurosporine showed inhibition of biological activity of teleocidin in HL-60 cells and Raji cells. Staurosporine is a unique tumor promoter possessing dual effects: tumor-promoting activity and anti-promoting activity on mouse skin.

MATERIALS AND METHODS

Materials

Staurosporine was kindly provided by Dr. H. Nakano, Tokyo Research Laboratories, Kyowa Hakko Kogyo, Tokyo, Japan. Telocidin was isolated from Streptomyces mediocutis (12). DMBA was purchased from Sigma Chemical Co., St. Louis, MO, and TPA was from LC Services Corporation, Waurus, MA. Sodium L-carnitine was obtained from Wako Pure Chemical Industries, Ltd, Osaka, Japan. 48 tPA was from American, United Kingdom, and 20 tTPA was from Sigma Chemical Co., St. Louis, MO. L-

Animals

Female CD-1 mice were purchased from the Japanese Charles River Co., Ltd, Kanagawa, Japan, and kept as reported previously (13).

Assay of Inhibition of Activation of Protein Kinase C in Filter

The effect of staurosporine on protein kinase C activated by 2.5 μM telocidin was examined as described previously (14). Protein kinase C was purified from mouse brain by DEAE-cellulose column chromatography (15).

Assay of Inhibition of Induction of HL-60 Cell Adhesion

Adhesion of HL-60 cells cultured in RPMI-1640 medium with 10% fetal calf serum was induced by incubation with 4.5 nmol of teleocidin for 48 h (16). The inhibitory effect of various concentrations of staurosporine on cell adhesion was examined in the presence of teleocidin.
staurosporine dose dependently at concentrations of 6 to 30 nM, determined by trypan blue staining. Incubation of Raji cells with 2.3 nM teleocidin for 48 h induced expression of EB virus EA in 55% of the cells, whereas in the absence of staurosporine were applied, twice a wk. In addition, this experiment included a group treated with DMBA and staurosporine plus 2.5 μM sodium n-butyrate. These two experiments showed that staurosporine strongly inhibited two of the biological activities induced by teleocidin. However, a single application of teleocidin in the presence of 2.3 nM teleocidin and 4 mM sodium n-butyrate resulted in a dose-dependent decrease in the percentage of EA-positive cells, the ED50 value of staurosporine being 3.4 nM. The viability of Raji cells was more than 80% at concentrations of up to 1 μM staurosporine in the presence of 2.3 nM teleocidin and 4 mM sodium n-butyrate. These two experiments showed that staurosporine strongly inhibited two of the biological activities induced by teleocidin. However, a single application of 10 nmol of staurosporine clearly enhanced the effects of teleocidin on ODC activity (Table 1).

### RESULTS

**Inhibitory Effects of Staurosporine on Biological Activities Induced by Teleocidin.** Based on our evidence that staurosporine inhibited the activation of protein kinase C by 2.3 μM teleocidin, and that the effective dose for 50% inhibition was 2.6 nM, comparable to the results of Yamashita et al. (4), we then studied whether it inhibited the other biological activities induced by teleocidin. Treatment with 4.5 μM teleocidin for 48 h induced adhesion of HL-60 cells, and this induction was inhibited by staurosporine dose dependently at concentrations of 6 to 30 nM (Fig. 2). The ED50 value was 9.0 nM. In this experiment, the treatment with teleocidin and staurosporine did not show any toxicity to HL-60 cells at concentrations of staurosporine up to 30 nM, determined by trypan blue staining. Incubation of Raji cells with 2.3 nM teleocidin for 48 h induced expression of EB virus EA in 55% of the cells, whereas in the absence of staurosporine, less than 1% of the cells expressed EB virus EA. As Fig. 3 shows, incubation of the cells with staurosporine at concentrations of 0.21 μM to 2.1 μM together with 2.3 μM teleocidin and 4 mM sodium n-butyrate resulted in a dose-dependent decrease in the percentage of EA-positive cells, the ED50 value of staurosporine being 3.4 nM. The viability of Raji cells was more than 80% at concentrations of up to 1 μM staurosporine in the presence of 2.3 μM teleocidin and 4 mM sodium n-butyrate. These two experiments showed that staurosporine strongly inhibited two of the biological activities induced by teleocidin. However, a single application of 10 nmol of staurosporine clearly enhanced the effects of teleocidin on ODC activity (Table 1).

**Biological Activities of Staurosporine.** Staurosporine induced irritation of mouse ear, the dose causing redness of the ear 24 h after its application being 200 ng (0.43 nmol). Since that of teleocidin was 0.008 nmol (12), the potency of staurosporine for irritation on mouse ear was about 50 times weaker than that of teleocidin. In addition, applications of 170 and 340 nmol of staurosporine induced ODC activity at levels of 196 and 173 pmol of CO2/mg of protein per 60 min, respectively, which was about 10 times weaker than that of teleocidin (Table 2).

Staurosporine, at concentrations of up to 500 nmol, did not induce ODC activity in mouse epidermis 4 h after application, whereas teleocidin induced it as usual (Table 1). Furthermore, staurosporine did not induce HL-60 cell adhesion at a concentration of 50 nM. In Raji cells, staurosporine itself, at concentrations of up to 2.1 μM, did not induce EB virus EA in the presence of 4 mM sodium n-butyrate. Table 3 summarizes the effects of staurosporine.

**Tumor-Promoting Activity of Staurosporine.** Since staurosporin-
in showed positive responses in the irritation of mouse ear, induction of HOC on mouse skin, and stimulation of prostaglandin E₂ production in rat macrophages. A tumor-promoting activity of staurosporine was examined in two-stage carcinogenesis experiments on mouse skin. In Experiment 1, the repeated applications of 50 μg of staurosporine to the skin of the backs of mice initiated with DMBA, twice a week, produced the first tumor at Wk 11 and gradually increased the percentages of tumor-bearing mice (Fig. 4). At Wk 30, the percentages of tumor-bearing mice in the groups treated with DMBA and staurosporine were 85.7% and 67.0%, respectively. The average tumors per mouse in these two groups were 1.9 and 1.0, respectively. The group treated with DMBA alone did not produce any tumors throughout the experiment. A weak tumor-promoting activity of staurosporine was statistically significant in both tumor-bearing mice (y% test and papilloma per mouse test). Fig. 5 shows the results of Experiment 2. The repeated applications of 10 μg of staurosporine to the DMBA-initiated mouse skin induced tumors in 33% of the mice with an average of 0.5 papillomas per mouse in Wk 30. The amount of staurosporine (10 μg) was not sufficient to induce a significant tumor-promoting activity, but the results with 50 μg of staurosporine were statistically significant, as shown in Fig. 4.

Antipromoting Activity of Staurosporine. Although staurosporine is a tumor promoter, it has inhibitory activities for activation of protein kinase C and induction of HL-60 cell adhesion and of EB virus (EA). Therefore, we examined whether staurosporine inhibits the tumor promotion on mouse skin. As Fig. 5 shows, the group treated with DMBA and teleocidin plus staurosporine resulted in 67% tumor-bearing mice by Wk 15 and in 80% by Wk 30, whereas the group treated with DMBA and teleocidin induced tumors in 100% of the mice by Wk 15 and remained constant up to Wk 30. The staurosporine treatment reduced the average numbers of tumors per mouse from 6.1 to 3.9 in Wk 30. In the group treated with DMBA and staurosporine, mice did not show any significant body weight loss throughout the experiment, and the skins of the backs of these mice were not irritated, compared with those in the group treated with DMBA and teleocidin. The inhibitory effect of staurosporine was statistically significant at around Wk 10 of tumor promotion. Although statistically significant inhibition was not obtained with 10 μg of staurosporine in later weeks of the experiment, a decreasing trend in the percentage of tumor-bearing mice and in average numbers of tumors per mouse was apparent (Fig. 5). Thus, staurosporine slightly inhibited tumor promotion of teleocidin, even at the dose at which staurosporine itself induced tumors.

DISCUSSION
Staurosporine is a potent inhibitor of protein kinases, such as protein kinase C, cyclic AMP-dependent protein kinase, cyclic GMP-dependent protein kinase (8). Since staurosporine inhibited biological activities of teleocidin in cell lines such as HL-60 cells and Raji cells, it was expected that staurosporine could inhibit tumor-promoting activity of teleocidin. In fact, staurosporine slightly inhibited the tumor-promoting activity of teleocidin in a two-stage carcinogenesis experiment on mouse skin. During the preparation of this paper, two groups reported independently that staurosporine inhibited tumor promotion of TPA in a two-stage carcinogenesis experiment on mouse skin. Although our results with staurosporine were not strongly inhibitory, due to limited amounts of the compound, we obtained the results that staurosporine did not effectively inhibit the effect of phorbol esters on epidermal growth factor binding or induction of ODC and EMP-1, as well as tumor promotion in mouse primary epidermal cells, and that staurosporine itself induced morphological changes in keratinocytes, resembling those induced by the phorbol esters. Since staurosporine is a microbial alkaloid produced by Streptomyces spp. (5), staurosporine was structurally, from a new family of tumor promoters that those already reported, such as phorbol esters, teleocidin, aplysianin, and taxanes (17). These results suggest that K-252a might be a weaker tumor promoter than staurosporine. The antifungal monomeric, flavonoid acetonide, is reported to be a very strong inhibitor of tumor promotion induced by TPA in mouse skin (23). However, Fukui et al. (24) recently reported that flavonoid acetonide has a tumor-promoting activity in mouse skin, which is mediated by 3- methylcholanthrene. Retinoic acid strongly inhibits skin tumor promotion by phorbol esters (25). However, retinoic acid itself induced papillomas in mouse skin initiated with DMBA (26). Further study revealed that retinoic acid has the ability to act as a first stage tumor promoter as well as a weak complete tumor promoter (27). Bryostatin 1 also shows both effects on tumor promotion in a multistage carcinogenesis experiment on mouse skin. Bryostatin 1 inhibits the tumor-promoting activity of TPA in SENCAR mice (28), and further study revealed that bryostatin 1 inhibits the first stage of tumor promotion (29, 30). On the other hand, bryostatin 1 itself is a second stage tumor promoter (30). Thus, like staurosporine, flavonoid acetonide, retinoic acid, and bryostatin 1 have dual effects on tumor promotion and inhibition of tumor promotion. Staurosporine provides important new information for understanding the mechanisms of tumor promotion and inhibition of tumor promotion.

REFERENCES
TUMOR-PROMOTING ACTIVITY OF STAUROROSPORINE ON MOUSE SKIN


14. L. Fujiki, T. Yamanoto, S. Aki, E., and Kato, R. Inhibition of 1,2- 
tetradecanoylphorbol-13-acetate-induced tumorpromotion and epidermal 

15. L. Fujiki, T. Noma, H., Takahashi, T., Kato, Y., Nishida, M., and 
Tanaka, F. Staurorosporine, a potent inhibitor of phospholipid/Ca 

16. L. Fujiki, H., Mori, M., Akayasu, M., Terada, M., Yama 

17. L. Fujiki, T. Noma, H., Takahashi, T., Kato, Y., Nishida, M., and 
Tanaka, F. Staurorosporine, a potent inhibitor of phospholipid/Ca 

18. L. Fujiki, H., Sugimura, M., Tada, T., Yonamori, K., Akazawa, E., and 
Sugimura, T. New classes of tumor-promoting phorbol esters, aprotinin, and gramicidin. In: L. Fujiki, E. 
Inoue, R. C. Moore, T. Sugimura, and J. M. Wrenn (eds.), Cellular 

19. L. Fujiki, H., Sugimura, M., Tada, T., Yonamori, K., Akazawa, E., and 
Sugimura, T. New classes of tumor-promoting phorbol esters, aprotinin, and gramicidin. In: L. Fujiki, E. 
Inoue, R. C. Moore, T. Sugimura, and J. M. Wrenn (eds.), Cellular 

20. L. Fujiki, H., Sugimura, M., Tada, T., Yonamori, K., Akazawa, E., and 
Sugimura, T. New classes of tumor-promoting phorbol esters, aprotinin, and gramicidin. In: L. Fujiki, E. 
Inoue, R. C. Moore, T. Sugimura, and J. M. Wrenn (eds.), Cellular 

21. L. Fujiki, H., Sugimura, M., Tada, T., Yonamori, K., Akazawa, E., and 
Sugimura, T. New classes of tumor-promoting phorbol esters, aprotinin, and gramicidin. In: L. Fujiki, E. 
Inoue, R. C. Moore, T. Sugimura, and J. M. Wrenn (eds.), Cellular 

22. L. Fujiki, H., Sugimura, M., Tada, T., Yonamori, K., Akazawa, E., and 
Sugimura, T. New classes of tumor-promoting phorbol esters, aprotinin, and gramicidin. In: L. Fujiki, E. 
Inoue, R. C. Moore, T. Sugimura, and J. M. Wrenn (eds.), Cellular 

23. L. Fujiki, H., Sugimura, M., Tada, T., Yonamori, K., Akazawa, E., and 
Sugimura, T. New classes of tumor-promoting phorbol esters, aprotinin, and gramicidin. In: L. Fujiki, E. 
Inoue, R. C. Moore, T. Sugimura, and J. M. Wrenn (eds.), Cellular 

24. L. Fujiki, H., Sugimura, M., Tada, T., Yonamori, K., Akazawa, E., and 
Sugimura, T. New classes of tumor-promoting phorbol esters, aprotinin, and gramicidin. In: L. Fujiki, E. 
Inoue, R. C. Moore, T. Sugimura, and J. M. Wrenn (eds.), Cellular 

25. L. Fujiki, H., Sugimura, M., Tada, T., Yonamori, K., Akazawa, E., and 
Sugimura, T. New classes of tumor-promoting phorbol esters, aprotinin, and gramicidin. In: L. Fujiki, E. 
Inoue, R. C. Moore, T. Sugimura, and J. M. Wrenn (eds.), Cellular 

26. L. Fujiki, H., Sugimura, M., Tada, T., Yonamori, K., Akazawa, E., and 
Sugimura, T. New classes of tumor-promoting phorbol esters, aprotinin, and gramicidin. In: L. Fujiki, E. 
Inoue, R. C. Moore, T. Sugimura, and J. M. Wrenn (eds.), Cellular 

27. L. Fujiki, H., Sugimura, M., Tada, T., Yonamori, K., Akazawa, E., and 
Sugimura, T. New classes of tumor-promoting phorbol esters, aprotinin, and gramicidin. In: L. Fujiki, E. 
Inoue, R. C. Moore, T. Sugimura, and J. M. Wrenn (eds.), Cellular 

28. L. Fujiki, H., Sugimura, M., Tada, T., Yonamori, K., Akazawa, E., and 
Sugimura, T. New classes of tumor-promoting phorbol esters, aprotinin, and gramicidin. In: L. Fujiki, E. 
Inoue, R. C. Moore, T. Sugimura, and J. M. Wrenn (eds.), Cellular 

29. L. Fujiki, H., Sugimura, M., Tada, T., Yonamori, K., Akazawa, E., and 
Sugimura, T. New classes of tumor-promoting phorbol esters, aprotinin, and gramicidin. In: L. Fujiki, E. 
Inoue, R. C. Moore, T. Sugimura, and J. M. Wrenn (eds.), Cellular 

30. L. Fujiki, H., Sugimura, M., Tada, T., Yonamori, K., Akazawa, E., and 
Sugimura, T. New classes of tumor-promoting phorbol esters, aprotinin, and gramicidin. In: L. Fujiki, E. 
Inoue, R. C. Moore, T. Sugimura, and J. M. Wrenn (eds.), Cellular 