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

吉澤, 滋

https://doi.org/10.11501/3062580
Tumor-promoting Activity of Staurosporine, a Protein Kinase Inhibitor on Mouse Skin

Shigeru Yoshizawa, Hirotaka Fujiki, Hiroko Suguri, Masanori Suganuma, Michie Nakayasu, Rie Matsushima, and Takashi Sugimura

National Cancer Center Research Institute, Tsukuba, Japan; and National Cancer Center, 7-5, Chao-ka, Tokyo 104, Japan.

ABSTRACT

Staurosporine, which is a potent inhibitor of protein kinase, inhibited both inductions of adhesion of human promyelocytic leukemia cells (90% effective dose = 0.01 nM) and histamine release from early asthmatic patients (50% effective dose = 3.4 nM) by teleocidin. However, staurosporine induced irritation on mouse ear and histidine decarboxylase activity in mouse skin. It did not induce ornithine decarboxylase activity in mouse epididymis. The in vivo experiments of staurosporine were carried out in two different doses. Experiment 1 revealed that the group treatment with a single application of 150 µg of 7,12-dimethylbenz(a)anthracene, followed by repeated applications of 50 µg of staurosporine, resulted in 85.7% of tumor-bearing mice at Wk 15, whereas group treatment with staurosporine alone or 7,12-dimethylbenz(a)anthracene alone gave 6.1% and 6%, respectively. Experiment 2 showed that group treatment with 7,12-dimethylbenz(a)anthracene followed by applications of 18 µg of staurosporine resulted in 33% of tumor-bearing mice at Wk 30. In addition, staurosporine treatment reduced the percentages of tumor-bearing mice treated with teleocidin from 100% to 67% in Wk 15. These results demonstrated that staurosporine is a weak tumor promoter of mouse skin compared with teleocidin, but staurosporine has some potency to inhibit tumor promotion by teleocidin.

INTRODUCTION

TPA, a tumor promoter, such as TPA, teleocidin, and aplysiaxin, activate calcium-activated, phospholipid-dependent protein kinase (protein kinase C) and, thus, induce both biological activities and tumor promotion (1). If inhibitors of protein kinase C block this phosphorylation, they should also inhibit the tumor promotion induced by TPA-type tumor promoters. In fact, naltrexone, a protein kinase C inhibitor, has this effect (3). A potent protein kinase inhibitor, staurosporine (Fig. 1), was isolated from Streptomyces spp. 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 p34^tyrosine 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). Two research groups independently found that staurosporine inhibited tumor promotion of TPA in two-stage carcinogenesis experiments on mouse skin (10, 11). Staurosporine induced morphological changes of primary mouse epidermal cells similar to those of 12,13-dihydroxy (9). Thus, 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 mediterranei (12). DMBA was purchased from Sigma Chemical, St. Louis, MO, and TPA was from LC Services Corporation, Woburn, MA. Sodium n-butyrate was obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan. [3H]TPA was purchased from American, United Kingdom, and [20H]TPA, [1-14C]ornithine monohydrochloride, and 9-(4-chloroethyl)ethyl(dimethyl)amine, were from New England Nuclear, Boston, MA. An immunoserum against EB virus EA (1:140) was a generous gift from Dr. N. Yamamoto of Yamaguchi University, Japan. Anti-human IgG conjugated with fluorescein isothiocyanate was obtained from Dakopatts A/S, Glostrup, Denmark.

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 Fibroblasts

The effect of staurosporine on protein kinase C activity was assayed 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 nM teleocidin for 48 h (16). The inhibitory effect of various concentrations of staurosporine on cell adhesion was examined in the presence of teleocidin.

Assay of Inhibition of Induction of EB Virus EA

Raji cells (3 x 10^6 per ml) were incubated in 1 ml of RPMI-1640 medium containing 10% fetal calf serum with 2.3 nM teleocidin, 4 nM n-butyrate, and 0.21 nM staurosporine. After 48 h incubation, EA-positive cells stained by the indirect immunofluorescence method were counted (17). Cell numbers were counted after staining with trypan blue.
Irritant Test on Mouse Ear

Various amounts of staurosporine in 10 μl of acetone were applied to the ears of 8-wk-old CD-1 mice. The extent of irritation was expressed as the minimum dose of the compound causing irritation, as described previously (18).

Induction of HDC in Mouse Skin

Staurosporine in 0.1 ml of acetone was applied to the skin of the backs of CD-1 mice, and a crude enzyme extract was obtained from the skin 18 h later as described previously (19). HDC activity was expressed as pmol of CO2 released per mg of protein per 1 h of incubation.

Induction of ODC in Mouse Skin

Staurosporine in 0.2 ml of acetone was applied to the skin of the backs of CD-1 mice. After 4 h, a crude enzyme extract was prepared from the epidermis, and its ODC activity was measured as described previously (16). Enzyme activity was expressed as nmol of CO2 per mg of protein per 30 min of incubation.

Two-Stage Carcinogenicity Experiments

Experiment 1. Initiation was achieved by a single application of 100 μg of DMBA dissolved in 100 μl of acetone to the skin of the backs of 8-wk-old female CD-1 mice (13). From 1 wk after initiation, 30 μg (107 nmoI) of staurosporine dissolved in 100 μl of acetone were applied to the initiated skin parts of the mice, twice a wk, until Wk 30. Control groups were treated with DMBA alone or staurosporine alone. The percentages of tumor-bearing mice and the average numbers of tumors per mouse were determined weekly as described previously (20).

Experiment 2. The experiment was carried out by the same procedure. From 1 wk after initiation with 100 μg of DMBA, 10 μg (21 nmoI) of staurosporine were applied, twice a wk. In addition, this experiment included a group treated with DMBA and staurosporine plus 2.5 μg (5.7 nmoI) of teleocidin, which was applied 15 min after each application of staurosporine. Control groups treated with DMBA and teleocidin or DMBA alone were also observed. In both Experiments 1 and 2, each group consisted of 15 mice, because of the limited availability of staurosporine.

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 Tamasaki 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 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 μM teleocidin for 48 h induced expression of EB virus EA in 55% of the cells, whereas in the absence of teleocidin, 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 nM to 2.1 μM together with 2.3 μM teleocidin and 4 mM sodium β-hexyrate 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 β-hexyrate. These two experiments showed that staurosporine strongly inhibited two of the biological activities induced by teleocidin. However, a single application of 10 nmoI 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 nM). Since that of teleocidin was 0.008 nmoI (12), the potency of staurosporine for irritation on mouse ear was about 5 times weaker than that of teleocidin. In addition, applications of 170 nmoI and 340 nmoI of staurosporine induced HDC 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 nmoI, 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 nmoI. 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 β-hexyrate. Table 3 summarizes the effects of staurosporine.

Tumor-Promoting Activity of Staurosporine

Since staurospor-
Staurosporine was a potent inhibitor of protein kinases, such as protein kinase C, cyclic AMP-dependent protein kinase, cyclic GMP-dependent protein kinase (6). Since staurosporine inhibited biological activities of telocidin in cell lines such as HL-60 and Raji cells, it was expected that staurosporine would inhibit tumor promotion activity of TPA-type tumor promoters. In fact, staurosporine significantly inhibited the tumor-promoting activity of telocidin in a two-stage carcinogenesis experiment on mouse skin. During the preparation of this paper, two groups reported independently that staurosporine inhibited tumor promotion activity of TPA-type tumor promoters. These results strongly suggested that staurosporine inhibited tumor promotion activity, 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 (6). Since staurosporine inhibited biological activities of telocidin in cell lines such as HL-60 and Raji cells, it was expected that staurosporine would inhibit tumor promotion activity of TPA-type tumor promoters. In fact, staurosporine significantly inhibited the tumor-promoting activity of telocidin in a two-stage carcinogenesis experiment on mouse skin. During the preparation of this paper, two groups reported independently that staurosporine inhibited tumor promotion activity of TPA-type tumor promoters. These results strongly suggested that staurosporine inhibited tumor promotion activity, even at the dose at which staurosporine itself induced tumors.

**REFERENCES**

Telomeres and functions on mouse skin

Tumor-promoting activity of staurosporine on mouse skin


14. Nakanishi, T., Yasumizu, S., Aino, K., Kato, S., Inhibition of 1,2-
tetradecanoylphorbol-13-acetate-induced tumor promotion and epidermal
ornithine decarboxylase activity in mouse skin by p-nitroanilinonine. Cancer

15. Tabata, T., Masui, H., Takahashi, L., Kani, Y., Nishimura, M.,
and Tanioka, F. Staurosporine: a potent inhibitor of protein kinase C.

16. Fujiki, H., Tanaka, Y., Miyake, K., Kikkawa, U., Takai, Y.,
E. Nakano, H., Kobayashi, E., Takahashi, T., Tamaoki, T.,
and Moto, H. Staurosporine: a novel protein kinase inhibitor, enhances HL-60
cell differentiation induced by various compounds. Exp. Hematol., 14: 42-48,
1986.

17. Laufman, J., Zempleni, C., Gritsko, S., Bara, G., Yamamoto, S.,
and Faggioni, A. TPA induction of Epstein-Barr virus early antigens in Raji
cells is blocked by selective protein kinase C inhibitors. J. Cancer Res., 40:

18. Sato, T., Tadano, A., Yagi, A., Yugi, T., and Blumberg, P. M.
Contracting action on arteries, a protein kinase C inhibitor, on human
prostatic carcinoma cells and primary mouse epidermal cells. Cancer Res., 47:

ester-induced induction of ornithine decarboxylase and tumor promotion in
mouse skin by epigallocatechin, a potent inhibitor of protein kinase C.

20. Yamada, S., Kiyono, T., Araki, E., Nakahara, T., Hondo, Y., and Kato,
K. Differential inhibition by staurosporine, a potent protein kinase C inhibitor,
of 12-O-tetradecanoyl-phorbol-13-acetate-caused skin tumors promotion, epider­
mal ornithine decarboxylase induction, hyperplasia, and inflammation.


22. Fujiki, H., Sugimura, T., and Takayama, S. Telocidin from Streptomyces
is a potent promoter of mouse skin carcinogenesis. Cancer Res., 37:

23. Fujiki, H., Tanaka, Y., Miyake, K., Kikkawa, U., Nishimura, Y., and Sagimura,
T. Activation of calcium-activated, phospholipid-dependent protein
kinase (protein kinase C) by new tumor promoters: telocidin and

24. Kikkawa, U., Iizuka, T., Inaba, S., and Nishibata, Y. Calcium-activated,
phospholipid-dependent protein kinase from rat brain. Subcellular distribution,

25. Fujiki, H., Mori, M., Nakanishi, M., Toda, M., Sugimura, T., and Moore,
R. E. Isolated alkaline phosphodiesterase B, natalisin, and lynphotrin A
as members of a new class of tumor promoters. Proc. Natl. Acad. Sci. USA,

26. Yasumizu, S., Kamata, T., Hirawas, Y., Hoshino, H., Wada, M., Fujiki,
H., and Sugimura, T. Induction of Epstein-Barr virus by a new tumor
promoter, telocidin, resolved by induction by TPA. Int. J. Cancer, 20:

27. Fujiki, H., Sugimura, T., Tada, T., Yusikawa, T., Nakayama, M., Endo,
Y., Shudo, R., Takahama, S., Mori, E., and Sugimura, T. New classes of tumor
promoter inhibitors: epipolacin, and palmatin. In: H. Fujiki, E.
Ishii, B. C. Moore, T. Sugimura, and J. M. Wrenn (eds.), Cellular
modulation of tumor promotion by tumor promoters and tumor promotion in

28. Kikkawa, U., Fujiki, H., Sugimura, M., Nakayama, M., Tada, T., Tamaoki,
T., Schuer, F. P., and Christensen, S. B. Thapsigargin, a bisubstrate acetyl­
carrier, is a non-12-O-tetradecanoylphorbol-13-acetate (TPA) 95% tumor

29. Fujiki, H., Sugimura, M., Nakanishi, M., Yuskipa, S., Yamashita, K.,
Thapar, J., Horikonyama, Y., Sakai, J., Shudo, R., and Sugimura, T. New
protein kinase C inhibitor, telocidin, resolved by induction by TPA in a two-stage
carcinogenesis experiment on the skin of CD-1 mice. Cancer Res., 48:

expression of epidermal growth factor receptors in mouse skin in vivo and in

31. Katz, J. H., Ishikawa, K., Nakazaki, S., Yamada, Y., Takahashi,
M., Minakuchi, C., Sato, A., and Kawase, M. K-252 (inovastat), a novel and
potent inhibitor of protein kinase C and cyclic nucleotide-stimulated protein

32. Fujiki, H., Sugimura, T., and Badger, G. H. Reversal of epidermal
ornithine decarboxylase induction by palmitoylcarnitine. Cancer Res., 46:

33. Fujiki, H., Sugimura, T., and Badger, G. H. Reversal of epidermal
ornithine decarboxylase induction by palmitoylcarnitine. Cancer Res., 46:

34. Thompson, C. B., and Kadon, T. Reversal of epidermal ornithine
decarboxylase induction by palmitoylcarnitine. Cancer Res., 46:

35. Fujiki, H., Sugimura, T., and Badger, G. H. Reversal of epidermal
ornithine decarboxylase induction by palmitoylcarnitine. Cancer Res., 46: