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

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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 induction of adhesion of mouse proteinase 2 + cyclic leukocytes (90% effective dose = 9.0 nM) and E-selectin, which was a late antigen on Raji cells (50% effective dose = 3.4 nM) to telecine. However, staurosporine induced irritation on mouse ear and inhibited dermolytic activity in mouse skin. It did not induce ornithine decarboxylase activity in mouse epidermis. The two-stage carcinogenesis experiments of staurosporine were carried out at two different doses. Experiment I revealed that the group treatment with a single application of 100 μg of 7,12-dimethylbenz(a)anthracene alone gave 6.7% and inhibition of induction of EB virus EA in Raji cells (8) and NADPH-oxidase activation in human neutrophils (9). Experiment 2 showed that group treatment with 7,12-dimethylbenz(a)anthracene alone gave 6.7% and 6.8%, respectively. Experiments II showed that treatment with staurosporine alone or 7,12-dimethylbenz(a)anthracene followed by applications of 10 μg of staurosporine resulted in 33% of tumor-bearing mice at Wk 10. In addition, stauros­porine treatment reduced the percentage of tumor-bearing mice treated with telecine from 100% to 67% in Wk 15. These results demonstrated that staurosporine is a weak tumor promoter of mouse skin compared with telecine, but staurosporine has some potency to inhibit tumor promotion by telecine.

INTRODUCTION

TPA-type tumor promoters, such as TPA, telecine, and aphiptycin, activate calcium-activated, phospholipase-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, nalmoxycarnitine, a protein kinase C inhibitor, has this effect (3, 4).

A potent protein kinase inhibitor, staurosporine (Fig. 1), was isolated from Streptomyces spp. by Tamao 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 p70/s60 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 phorbol 12,13-dibutyrate (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 HDCC 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 telecine in HL-60 cells and Raji cells. Staurosporine is a unique tumor promoter possessing dual effects: tumor-promoting activity and anti-inflammatory activity on mouse skin.

MATERIALS AND METHODS

Materials

Staurosporine was kindly provided by Dr. H. Nakano, Tokyo Research Laboratories, Kyowa Hakko Kogyo, Tokyo, Japan. Telecine was isolated from Streptomyces mediocutis (12). DMBA was purchased from Sigma Chemical Co., St. Louis, MO, and TPA was from LC Services, Wcura, MA. Sodium L-leucine was obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan, [3H]TPA was from Amersham, United Kingdom, and [32P]ATP, 32P-orthophosphate, and L-[methyl-3H]methionine were from Amersham, Buckinghamshire, UK. Staurosporine was kindly provided by Dr. H. Nakano, Tokyo Research Laboratories, Kyowa Hakko Kogyo, Tokyo, Japan. The abbreviations used are: TPA, 12-O-tetradecanoylphorbol-13-acetate; DMBA, 7,12-dimethylbenz(a)anthracene; HDCC, human promyelocytic leukemia cells; EB virus, Epstein-Barr virus; EA, early antigen; HDCC, histamine deacetylase; ODC, ornithine decarboxylase; EBV, 50% effective dose.

Methods

Assay of Inhibition of Activation of Protein Kinase C In Vitro

The effect of staurosporine on protein kinase C activated by 2,3-AMP-dependent protein kinase (13). Protein kinase C was purified from mouse brain by DEAE-cellulose column chromatography (15).

Assay of Inhibition of Induction of HDL Cell Adhesion

Adhesion of HDL-60 cells cultured in RPMI-1640 medium with 10% fetal calf serum was induced by incubation with 4.5 nM telecine for 48 h (16). The inhibitory effect of various concentrations of staurosporine on cell adhesion was examined in the presence of telecine.
staurosporine dose dependently at concentrations of 6 to 30 nM (Fig. 2). The ED50 value was 3.4 nM. The viability 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 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 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 mM 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 100 times weaker than that of teleocidin. However, applications of 50 mM 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 nM 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 nM 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 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 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 mM of staurosporine clearly enhanced the effects of teleocidin on ODC activity (Table 1).

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Table 1: Effect of staurosporine on induction of ODC in mouse epidermis

<table>
<thead>
<tr>
<th>Tumor-Promoting Activity of Staurosporine on Mouse Skin</th>
<th>ODC activity (nmol/mg of protein/30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>0.12 ± 0.07</td>
</tr>
<tr>
<td>Staurosporine</td>
<td>0.18 ± 0.06</td>
</tr>
<tr>
<td>Staurosporine + TPA</td>
<td>0.16 ± 0.04</td>
</tr>
<tr>
<td>Staurosporine + TPA + staurosporine</td>
<td>0.14 ± 0.03</td>
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</tbody>
</table>

Table 2: Biological activities of staurosporine

<table>
<thead>
<tr>
<th>Biological Activity</th>
<th>Activity in mouse skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staurosporine</td>
<td>231</td>
</tr>
<tr>
<td>Staurosporine + TPA</td>
<td>173</td>
</tr>
<tr>
<td>Staurosporine + TPA + staurosporine</td>
<td>170</td>
</tr>
<tr>
<td>Staurosporine + TPA + staurosporine + staurosporine</td>
<td>176</td>
</tr>
</tbody>
</table>

Stimulated with staurosporine, the percentage of tumor-bearing mice in the groups treated with DMBA and staurosporine was statistically significant as shown in Fig. 4.

Antipromoting Activity. Staurosporine is a tumor promoter, but it has the ability to inhibit the activation of protein kinase C and induction of HL-60 cell collagenase and of staurosporine was statistically significant as shown in Fig. 4. As reported previously, staurosporine inhibited the tumor-promoting activity of telocidin in a two-stage carcinogenesis experiment. This discrepancy between our results might be due to the doses of DMBA and of staurosporine that were used. The results of our experiment indicated that the doses of staurosporine which other groups used were not sufficient to induce any tumor-promoting activity in two-stage carcinogenesis experiments.

In addition to inhibition of tumor promotion, staurosporine itself did not show any tumor-promoting activity in mouse skin initiated with 3-methylcholanthrene. These results suggested that K-252a might be a weaker tumor promoter than staurosporine.

REFERENCES

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