Inhibition of Teleocidin-Caused Epidermal Ornithine Decarboxylase Induction by Phospholipase A2-, Cyclooxygenase- and Lipoxygenase-Inhibitors

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Abstract—Teleocidin (5 μg/mouse), a potent tumor promoting indole alkaloid from Streptomyces, induced epidermal ornithine decarboxylase (ODC) in CD-1 mice. Teleocidin-caused ODC induction was inhibited by the treatment of indomethacin (2 μmol/mouse), a selective cyclooxygenase inhibitor, and p-bromo-phenacyl bromide (BPB) (30 μmol/mouse), a phospholipase A2 inhibitor. Teleocidin-caused ODC induction inhibited by indomethacin was completely restored by concurrent application of prostaglandin E2 (PGE2) (140 nmol/mouse). On the other hand, teleocidin-caused ODC induction inhibited by BPB was not restored by the treatment of mice with PGE2, but partially restored by the treatment with arachidonic acid (1 μmol/mouse). Treatment of mice with lipoxygenase inhibitors such as BW755C (30 μmol/mouse), nordihydroguaiaretic acid (NDGA) (30 μmol/mouse), quercetin (10 μmol/mouse), and 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA861) (10 μmol/mouse) clearly suppressed ODC induction by teleocidin. Moreover, both NDGA (30 μmol/mouse) and quercetin (10 μmol/mouse) inhibited the restoring effect of PGE2. Therefore, our present results suggest that arachidonate metabolites, i.e., not only cyclooxygenase product(s) but also lipoxygenase product(s), are involved in the mechanism of ODC induction by teleocidin.

Teleocidin isolated from Streptomyces is a potent tumor promoter in two-stage carcinogenesis of mouse skin (1-3). Teleocidin, an indole alkaloid, is structurally different from 12-O-tetradecanoylphorbol-13-acetate (TPA), which is the most potent tumor promoter of the series of phorbol esters. Teleocidin, like TPA, shows many biological and biochemical effects on mouse skin and in various types of cells (1-4). The induction of ornithine decarboxylase (ODC) is a typical and prominent biochemical alteration elicited by tumor promoters (3-5) and has been thought to be one of the representative biochemical parameters of strong tumor activity. However, the mechanism and the meaning of induction of ODC by tumor promoters have not yet been fully understood.

The role of the arachidonate cascade in TPA actions in mouse skin has been investigated. In fact, Verma et al. (6) reported that cyclooxygenase products may play a crucial role in TPA-caused ODC induction and tumor promotion. Recently, we showed that not only cyclooxygenase products but also lipoxygenase products are involved in the mechanism of ODC induction (7, 8, 10, 11) and tumor promotion (9-11) by TPA. Fischer et al. (12) also suggested that lipoxygenase products are essential for tumor promotion. Therefore, we studied the effects of inhibitors of arachidonate metabolism on the teleocidin-caused mouse epidermal ODC induction by teleocidin.
induction in order to further evaluate our previous findings.

Materials and Methods

Female CD-1 mice (Charles River, Atsugi, Japan), 7 to 8 weeks old, were used. The dorsal hair of each mouse was shaved with clippers at least 2 days before using, and only those mice showing no regrowth of hair were used. All chemicals were dissolved in reagent grade acetone and applied to the shaved area in a volume of 0.2 ml using a micropipet. The inhibitors were applied to the mouse skin concurrently with teleocidin.

Five hours after the treatment of teleocidin, the mice were sacrificed by cervical dislocation, and the skins were excised. The epidermis was separated by a brief heat treatment and then homogenized in 10 vol. of 20 mM Tris-HCl buffer (pH 7.2) with a Polytron PT-10 homogenizer for 10 to 15 sec at 4°C. The homogenate was centrifuged at 30,000×g for 30 min at 4°C. ODC activity of the 30,000×g supernatant was determined by measuring the release of 14CO2 from DL-[1-14C]ornithine as described previously (5, 7). In short, incubation was carried out for 60 min at 37°C in the following medium: 0.4 μmol pyridoxal phosphate, 1.0 μmol dithiothreitol, 0.2 μmol L-ornithine, 1.0 μmol Tris-base (pH 7.2), 0.5 ml epidermal extract, and 0.5 μCi DL-[1-14C]ornithine (57.3 mCi/mmol) in a final volume of 2.0 ml. The generated CO2 and 14CO2 were absorbed into hyamine 10X placed in a central well of the incubation flask. The reaction was stopped by 2 M citric acid, and incubation was continued for an additional 30 min to ensure complete absorption of 14CO2. The hyamine solution was transferred into a scintillation vial containing 10 ml of toluene-based scintillator, and radioactivity was measured.

Enzyme activity was expressed as nmol CO2 in 60 min per mg protein. The protein concentration of the epidermal extract was measured by the method of Lowry et al. (13), with bovine serum albumin as the standard. None of the drugs which we used directly interfered with the ODC assay system.

Teleocidin was isolated from Streptomyces as reported previously (2). It is a mixture of teleocidin A and teleocidin B. Indomethacin, nordihydroguaiaretic acid (NDGA) and arachidonic acid were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.; quercetin was from Tokyo Chemical Industry Co., Tokyo, Japan; and p-bromophenacyl bromide (BPB) was from Wako Pure Chemical Industries Ltd., Osaka, Japan. Prostaglandin E2 (PGE2), 3-amino-1-[m-(trifluoromethyl)-phenyl]-2-pyrazoline (BW-755C) and 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA-861) were supplied from Ono Pharmaceutical Co. Ltd., Osaka, Japan; Wellcome Research Laboratories, Beckenham, Kent, UK; and Takeda Chemical Industries, Ltd., Osaka, Japan, respectively.

Results

A single topical application of teleocidin (5 μg/mouse) resulted in a rapid transient increase in mouse epidermal ODC activity, as reported previously (4). A peak of activity was observed at 5 hr after treatment. The teleocidin-caused ODC induction was inhibited by BPB (5 μmol/mouse) (Fig. 1) and indomethacin (2 μmol/mouse) (Table 1). Inhibition of the teleocidin-caused ODC induction was dose-dependent as in the case of TPA-caused ODC induction (6, 7). The inhibition of teleocidin-caused ODC induction by indomethacin was fully counteracted by application of PGE2 (140 nmol/mouse) to the mice (Table 1). However, the inhibition of teleocidin-caused ODC induction by BPB could not be counteracted by PGE2 (Fig. 1). On the contrary, arachidonic acid (1 μmol/mouse) partially restored the teleocidin-caused ODC induction inhibited by BPB (Fig. 2). As reported previously (6, 7), application of PGE2 or arachidonic acid alone does not induce epidermal ODC activity. Moreover, PGE2 applied with TPA shows no potentiating action on ODC induction by TPA (6).

Table 2 shows the effects of several lipoxygenase inhibitors on the induction of ODC by teleocidin. BW755C (30 μmol/mouse). NDGA (30 μmol/mouse). quercetin (10 μmol/mouse) and AA861 (10 μmol/mouse) clearly suppressed the teleocidin-caused ODC induction. In addition, treatment
Fig. 1. Inhibitory effect of BPB on teleocidin-caused epidermal ODC induction and effect of PGE₂ on the inhibition of teleocidin-caused ODC induction by BPB. Mice were treated with BPB (5 μmol) and PGE₂ (140 nmol) concurrently with teleocidin (5 μg). Mice were killed for the determination of ODC activity 5 hr after teleocidin treatment. Each value is the mean of individual determinations from 5 mice. Vertical bars indicate standard errors. *P<0.05; NS, not significantly different.

Table 1. Counteracting effect of PGE₂ on indomethacin-caused inhibition of ODC induction by teleocidin in the absence or presence of lipoygenase inhibitors

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Ornithine decarboxylase activity (nmol CO₂/60 min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>3</td>
<td>0.08±0.01</td>
</tr>
<tr>
<td>Teleocidin (5 μg)</td>
<td>5</td>
<td>1.92±0.30</td>
</tr>
<tr>
<td>Teleocidin+indomethacin (2 μmol)</td>
<td>5</td>
<td>0.95±0.28*</td>
</tr>
<tr>
<td>Teleocidin+indomethacin +PGE₂ (140 nmol)</td>
<td>5</td>
<td>2.21±0.29**</td>
</tr>
<tr>
<td>Teleocidin+indomethacin +PGE₂ +NDGA (30 μmol)</td>
<td>5</td>
<td>0.79±0.06***</td>
</tr>
<tr>
<td>Teleocidin+indomethacin +PGE₂ +quercetin (10 μmol)</td>
<td>5</td>
<td>0.46±0.06***</td>
</tr>
</tbody>
</table>

Values represent the mean±standard error. *P<0.05 versus teleocidin, **P<0.05 versus teleocidin+indomethacin, ***P<0.01 versus teleocidin+indomethacin+PGE₂.
of mice with NDGA (30 μmol/mouse) and quercetin (10 μmol/mouse) counteracted the restoring effect of PGE2 (Table 1).

The doses of BPB, indomethacin, PGE2, arachidonic acid, BW755C, NDGA and quercetin are similar to the doses used in the studies concerning TPA-caused ODC induction (6–8, 10). Histological studies revealed that epidermal cells were not apparently damaged by the doses of the drugs used in the present study (8–10, 14).

Table 2. Effects of lipoxygenase inhibitors on teleocidin-caused epidermal ODC induction

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Ornithine decarboxylase activity (nmol CO₂/60 min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>3</td>
<td>0.13±0.03</td>
</tr>
<tr>
<td>Teleocidin (5 μg)</td>
<td>5</td>
<td>1.77±0.37</td>
</tr>
<tr>
<td>Teleocidin+BW755C (30 μmol)</td>
<td>5</td>
<td>0.63±0.08*</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>3</td>
<td>0.07±0.01</td>
</tr>
<tr>
<td>Teleocidin (5 μg)</td>
<td>4</td>
<td>1.52±0.30</td>
</tr>
<tr>
<td>Teleocidin+NDGA (30 μmol)</td>
<td>4</td>
<td>0.54±0.13*</td>
</tr>
<tr>
<td>Experiment 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>3</td>
<td>0.14±0.01</td>
</tr>
<tr>
<td>Teleocidin (5 μg)</td>
<td>5</td>
<td>2.39±0.33</td>
</tr>
<tr>
<td>Teleocidin+quercetin (10 μmol)</td>
<td>5</td>
<td>0.52±0.11*</td>
</tr>
<tr>
<td>Teleocidin+AA861 (10 μmol)</td>
<td>5</td>
<td>0.84±0.27*</td>
</tr>
</tbody>
</table>

Values represent the mean±standard error. *P<0.01 versus teleocidin

The main lipoxygenase product of arachidonic acid is 12-hydroxy-5, 8, 10, 14-eicosatetraenoic acid (12-HETE) in mouse epidermis (10, 23), as in human (23) and guinea pig epidermis (24). The main cyclooxygenase product of arachidonic acid is PGE2 in mouse epidermis as reported previously (14). NDGA (25), quercetin (10, 26) and AA861 (27) potently inhibit epidermal lipoxygenase activity (10, 14), but do not inhibit cyclooxygenase activity (14, 28). BW755C usually inhibits both lipoxygenase and cyclooxygenase (29), although we did not check it in mouse epidermis. Indomethacin does not inhibit epidermal lipoxygenase activity, but potently inhibits epidermal cyclooxygenase activity (28). The present results clearly show that teleocidin-caused ODC induction was inhibited by treatment of mice with BW755C, NDGA, quercetin and AA861. In addition, NDGA and quercetin counteracted the
restoring effect of PGE₂ on the teleocidin-caused ODC induction inhibited by indomethacin. These results further support our finding that lipoxygenase product(s) are involved in the mechanism of teleocidin-caused ODC induction.

Teleocidin inhibits the specific binding of phorbol ester to membrane receptors with a potency similar to that of TPA (15, 30). Thus, teleocidin and TPA appear to bind the same receptor, which is considered to be calcium-activated, phospholipid-dependent protein kinase (31), and produce similar biological effects. In agreement with this, the present results indicate that TPA and teleocidin induce ODC by acting through a similar mechanism which involves the lipoxygenase pathway of arachidonic acid (7–12). Interestingly, a similar contribution of lipoxygenase product(s) in the mechanism of teleocidin-induced insulin secretion from isolated rat pancreatic islets, which is also common to TPA- and glucose-induced insulin secretion (32, 33), has been suggested (18).

Further investigations are necessary to elucidate the mechanism of TPA and teleocidin actions on cellular function.

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References
14 Nakadate, T., Yamamoto, S., Aizu, E. and Kato, R.: Inhibition of mouse epidermal 12-lipoxygenase...
by 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodeca-
diylnyl)-1,4-benzoquinone (AA861). J. Pharm.

15 Umezawa, K., Weinstein, I.B., Horowitz, A.,
Fujiki, H., Matsushima, T. and Sugimura, T.: 
Similarity of teleocidin B and phorbol ester
tumor promoters in effects on membrane

16 Sakamoto, H., Terada, M., Fujiki, H., Mori, M.,
Nakayasu, M., Sugimura, T. and Weinstein, I.B.: 
Stimulation of prostaglandin production and
cholesterol turnover in HeLa cells by lyngbyatoxin A
and dihydroteleocidin B. Biochem. Biophys.
Res. Commun. 102, 100–107 (1981)

17 Horowitz, A.D., Fujiki, H., Weinstein, I.B., 
Jeffrey, A., Okin, E., Moore, R.E. and Sugimura,
T.: Comparative effects of aplysiatoxin, de
bromoaplysiatoxin, and teleocidin on receptor
binding and phospholipid metabolism. Cancer
Res. 43, 1529–1535 (1983)

18 Yamamoto, S., Nakadate, T., Fujiki, H. and 
Kato, R.: Insulinotropic effect of the tumor
promoter teleocidin in isolated pancreatic islets. 
(1983)

19 Snoek, G.T. and Levine, L.: Requirements for 
protein synthesis and calcium for stimulation of 
prostaglandin synthesis in cultured rat liver cells 

20 Mufson, R.A., Defeo, D. and Weinstein, I.B.: 
Effects of phorbol ester tumor promoters on 
arachidonic acid metabolism in chick embryo 
fibroblasts. Mol. Pharmacol. 16, 569–578
(1979)

21 Levine, L. and Ohuchi, K.: Stimulation by 
carcinogens and promoters of prostaglandin 
production by dog kidney (MDCK) cells in 

22 Vallee, E., Gougat, J., Navarro, J. and Delahayes,
J.F.: Anti-inflammatory and platelet anti-agગ
gregant activity of phospholipase-A₂ inhibitors. 
J. Pharm. Pharmacol. 31, 588–592 (1979)

23 Hammarstrom, S., Lindgren, J.A., Marcelo, C., 
Duell, E.A., Anderson, T.F. and Voorhees, J.J.: 
Arachidonic acid transformation in normal and 
psoriatic skin. J. Invest. Dermatol. 73, 180–183
(1979)

24 Ruzicka, T., Vitto, A. and Printz, M.P.: Epidermal
arachidonate lipoygenase. Biochim. Biophys.

25 Hamberg, M.: On the formation of thromboxane
B₂ and 12-L-hydroxy-5,8,10,14-eicosatetraenoic
acid (12 ho-20:4) in tissues from the guinea pig. 

26 Hope, W.C., Welton, A.F., Fiedler-Nagy, C., 
Batula-Bernardo, C. and Coffey, J.W.: In vitro 
inhibition of the biosynthesis of slow reacting 
substance of anaphylaxis (SRS-A) and lipoygenase

27 Yoshimoto, T., Yokoyama, C., Ochi, K., Yamamoto, 
S., Maki, Y., Ashida, Y., Terao, S. and 
Shiraishi, M.: 2,3,5-Trimethyl-6-(12-hydroxy-
5,10-dodecadiynyl)-1,4-benzoquinone (AA
861), a selective inhibitor of the 5-lipoxygenase 
reaction and the biosynthesis of slow-reacting 
substance of anaphylaxis. Biochim. Biophys. 

28 Kato, R., Nakadate, T. and Yamamoto, S.: 
Involvement of lipoygenase products of 
arachidonic acid in tumor-promoting activity of 
TPA. In Icosanoids and Cancer, Edited by 
Thaler-Dao, H., De Paulet, A.C. and Paoletti, R., 

29 Higgs, G.A., Flower, R.J. and Vane, J.R.: A new 
approach to anti-inflammatory drugs. Biochem. 

30 Solanki, V. and Slaga, T.J.: Specific binding of 
phorbol ester tumor promoters to intact primary 

31 Nishizuka, Y.: The role of protein kinase C in cell 
surface signal transduction and tumor promotion. 

32 Yamamoto, S., Nakadate, T., Nakaki, T., Ishii, 
K. and Kato, R.: Tumor promoter 12-0-tetra-
decanoylphorbol-13-acetate-induced insulin 
secretion: inhibition by phospholipase A₂- 

33 Yamamoto, S., Ishii, M., Nakadate, T., Nakaki, T. 
and Kato, R.: Modulation of insulin secretion by 
lipoygenase products of arachidonic acid, 
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