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A POSSIBLE NATURALLY OCCURRING TUMOR PROMOTER, TELEOCIDIN B FROM *STREPTOMYCES*

Hirota Fujiki, Masami Mori, Michie Nakayasu, Masaaki Terada and Takashi Sugimura

National Cancer Center Research Institute
5-1-1, Tsukiji, Chuo-ku, Tokyo, Japan

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SUMMARY: Dihydroteleocidin B, a derivative of teleocidin B, when painted on mouse skin, caused marked induction of ornithine decarboxylase within 4 hrs. This induction of ornithine decarboxylase was inhibited by painting the skin with 13-cis-retinoic acid one hour before dihydroteleocidin B. Dihydroteleocidin B induced cell adhesion of human promyelocytic leukemia cells (HL-60) to the surface of culture flasks, and inhibited terminal differentiation of Friend erythroleukemia cells induced by dimethyl sulfoxide. Its effective dose for these actions was comparable to that of the potent tumor promoter, 12-O-tetradecanoyl-phorbol-13-acetate. Teleocidin B seems to be a new type of promoter of carcinogenesis.

INTRODUCTION

12-O-Tetradecanoyl-phorbol-13-acetate (TPA) isolated from oil of the seeds of *Croton tiglium L.* is a very strong tumor promoter (1,2). We are looking for other naturally occurring promoters.

When painted on mouse skin, TPA induces ornithine decarboxylase (ODC) activity (3). It also induces dilatation of the blood vessels of mouse ear (4), and cell adhesion of human promyelocytic leukemia cells (HL-60) (5,6), and it inhibits terminal differentiation of Friend erythroleukemia cells (7).

Using these biological properties as parameters, we found a compound that may be a strong promoter, dihydroteleocidin B. This compound is a derivative of teleocidin B, which was isolated from the mycellia of *Streptomyces* 2A 1563 (8). Dihydroteleocidin B and teleocidin B are strong

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ABBREVIATIONS: ODC, ornithine decarboxylase; TPA, 12-O-tetradecanoyl-phorbol-13-acetate.

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irritants of the skin of humans, rabbits and mice (9). The structurally related compound lyngbyatoxin A from a blue-green alga is also reported to produce dermatitis (10).

This paper reports that dihydroteleocidin B induced ODC when painted on mouse skin, and also caused dilatation of the blood vessels of mouse ear, cell adhesion of human promyelocytic leukemia cells, and inhibition of terminal differentiation of Friend erythroleukemia cells. Induction of ODC by dihydroteleocidin B was inhibited by painting the skin with 13-cis-retinoic acid one hour before dihydroteleocidin B. The amount of dihydroteleocidin B required for these biological actions was comparable to that of TPA. Dihydroteleocidin B is a new type of chemical which may be as strong a tumor promoter as TPA *in vivo*.

MATERIALS AND METHODS

Animals: Female CD-1 mice were obtained from Japanese Charles River Co., Ltd, Kanagawa, Japan and were used when 8 weeks of age. They were given free access to commercial basal diet CE-2, CLEA Japan Inc., Tokyo and water. The hair of the back of each mouse was shaved with an electrical animal clipper, and a depilatory agent from Kanebo Co., Ltd. Tokyo, Japan was applied to the shaved area 1-2 days before experiments. The depilatory agent had no detectable effect on either ODC and the morphology. Only nude areas (about 2.5 x 4 cm) were used for experiments. Test compounds were applied as solutions in acetone (0.2 ml). Mice were routinely killed between 2 and 4 p.m. to avoid variations due to the circadian rhythm.

Preparation of epidermal extracts: Epidermal extracts were prepared essentially as described by Boutwell (3). The mice were killed by cervical dislocation without anesthesia, and the dorsal nude area was rapidly excised and placed in ice-water for 1 min. It was then transferred to water at 55° for 30 sec and then reimmersed in ice-water. The epidermis was scraped off with a razor blade and the tissue was sonicated in a Sonifier cell disruptor, Branson Sonic Power Co., L.I., N.Y., for 3 min at 0°, in 0.6 ml of 50 mM sodium phosphate buffer (pH 7.2), containing 0.1 mM pyridoxal phosphate and 0.1 mM EDTA. Insoluble material was removed by centrifugation at 30,000 x g for 20 min, and the protein content of the supernatant was determined by micro-assay as described by Heil and Zillig (11).

ODC assay: ODC activity was determined by measuring release of CO_2 as described by Russell and Snyder (12). The incubation mixtures (final volume 2 ml) in Warburg flasks consisted of 0.4 μmole pyridoxal phosphate, 1.0 μmole dithiothreitol, 100 μmoles sodium phosphate (pH 7.2) and 0.1 to 0.4 ml epidermal extract containing about 1.0 mg of protein. After preincubation at 37° for 10 min, 0.2 ml of solution containing 0.5 μCi DL-ornithine monohydrochloride [1-^{14}C] (52.80 mCi/mmol) and 0.2 μmole L-ornithine were added. Incubations were carried out for 30 min at 37° and then the reaction was

stopped by adding 0.8 ml of 2 M citric acid, and released CO_2 was trapped in 0.2 ml Protosol (New England Nuclear). Results represent the mean of assays from two or three separate mice per point.

Cell adhesion assay: HL-60 cells were grown in tissue culture flasks at an initial density of 4 x 10^5 cells/ml of RPMI-1640 medium (13), with and without dihydroteleocidin B or TPA in a total volume of 1 ml. After 43-hr culture, the cells were examined under a reverse-phase microscope, and the flasks were shaken for 1 min with a rotary shaker at a rate of 160 rpm. An aliquot of the medium was taken to measure the number of cells that were not firmly attached to the surface of the flask. The cells were then scraped off and the cell number was counted to calculate the number of cells attached to the flask (5,6).

Inhibition of terminal differentiation of Friend erythroleukemia cells: Friend erythroleukemia cells, clone DS 19, were cultured in the presence of 2% dimethyl sulfoxide with and without dihydroteleocidin B or TPA (7,14). After 5 days, a sample of the cell suspension was removed for determination of the number of cells containing hemoglobin by the benzidine method as described previously (15).

Chemicals: TPA was purchased from Consolidated Midland Corp. Brewster, N.Y. Croton oil was obtained from Wako Pure Chemical Industries Ltd, Osaka, Japan. 13-Cis-retinoic acid was a gift from Dr. K. Takano, Japan-Roche, Tokyo, Japan. DL-Ornithine monohydrochloride [1-^{14}C] was purchased from New England Nuclear, Boston, Mass. Dihydroteleocidin B, a derivative of the toxic substance teleocidin B isolated from a methanol extract of mycellia of *Streptomyces* 2A 1563 (which resembles *Streptomyces mediocidicus*) was a gift from Dr. M. Takashima, Fujisawa Pharmaceutical Industries, Ltd, Osaka, Japan. The structures of teleocidin B and dihydroteleocidin B are shown in Fig. 4.

RESULTS

ODC induction by dihydroteleocidin B: Fig. 1 a shows the induction of ODC in mouse skin by 5 μg dihydroteleocidin B. Activity was maximal 4-5 hrs after application of dihydroteleocidin B. Fig. 1 b shows the induction of ODC in mouse skin by 11 μg of TPA. As with dihydroteleocidin B, activity was maximal after 4 hrs.

Application of 5 μg of 13-cis-retinoic acid to the skin one hour before dihydroteleocidin B or TPA inhibited the induction of ODC, as shown in Fig. 1 a and b. The effect of 13-cis-retinoic acid in inhibiting ODC-induction by TPA has been reported (16).

Fig. 2 shows the dose-response curve of ODC induction with doses of dihydroteleocidin B of 1 μg to 1 mg; induction was maximal with 5 to 7.5 μg of dihydroteleocidin B, and decreased at higher doses of dihydroteleocidin B,

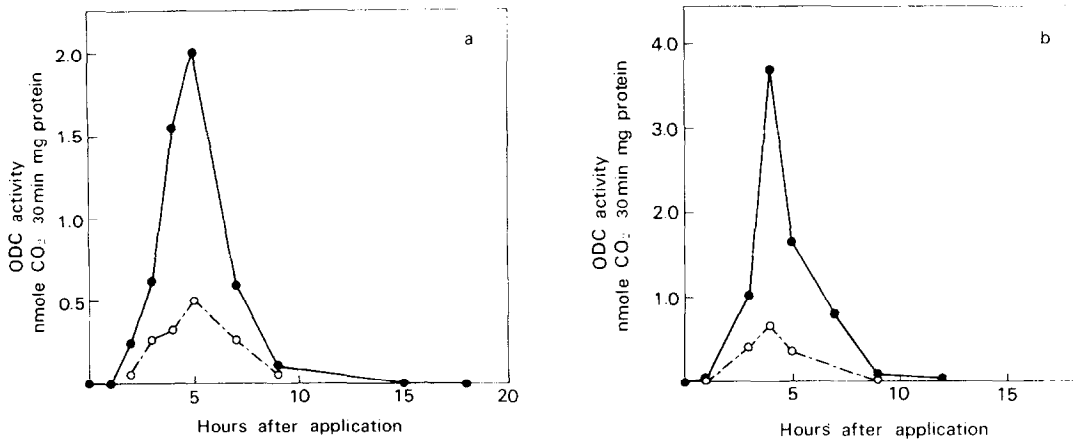

Fig. 1. ODC induction in mouse skin, (a) 5 μg dihydroteleocidin B dissolved in 0.2 ml acetone was applied (●—●). 0.2 ml acetone containing 5 μg 13-cis-retinoic acid was applied 1 hr before dihydroteleocidin B (o---o). (b) 11 μg TPA in 0.2 ml acetone was applied (●—●) and 13-cis-retinoic acid was applied 1 hr before TPA (o---o). ODC activity was assayed as described in the text.

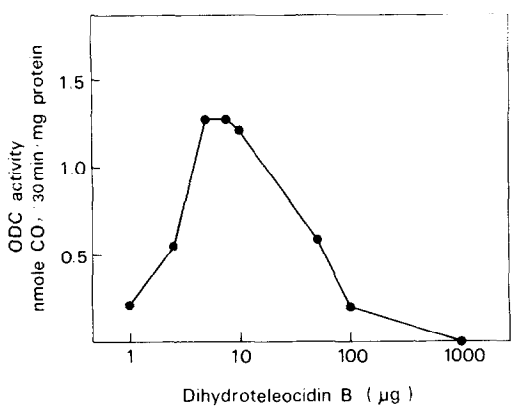

Fig. 2. Dose-response of ODC induction to dihydroteleocidin B. ODC was assayed 4 hrs after applying various amounts of dihydroteleocidin B as described in the text.

as in the case with TPA. Dilatation of the blood vessels of the ear of female mice was observed 3 hrs after painting the ear with 10 ng dihydroteleocidin B and after 24 hrs the ear was still red.

Induction of cell adhesion by dihydroteleocidin B: Table 1 shows the concentrations of dihydroteleocidin B and TPA required for around 50% adhesion of HL-60 cells to the flasks within 43 hrs. Both compounds induced cell

able 1. Concentrations of dihydroteleocidin B and TPA required for 50% adhesion of HL-60 cells

Compound	Concentration	Total cell number	Attached cells	
			Number *	%
None		1.45×10^6	0.06×10^6	4.1
Dihydroteleocidin B	0.3 ng/ml	0.97×10^5	0.50×10^6	51.5
TPA	1.5 ng/ml	0.92×10^6	0.53×10^6	57.6

* The numbers of cells attached to the surface of flasks after 43 hrs were determined as described in the text.

adhesion. The concentration of dihydroteleocidin B required for around 50% adhesion was one fifth that of TPA. The results on TPA confirm previous reports (5,6).

Inhibition of terminal differentiation of Friend erythroleukemia cells by dihydroteleocidin B: The effects of various concentrations of dihydroteleocidin B and TPA on terminal differentiation are shown in Fig. 3. The concentrations required for 50% reduction of benzidine reactive cells were 0.2 ng/ml and 1 ng/ml, respectively, and the concentrations required for 90% inhibition of induction of benzidine reactive cells were 1 ng/ml and 10 ng/ml, respectively. Concentrations up to 4 ng/ml of dihydroteleocidin B had no growth inhibitory effect on Friend erythroleukemia cells in the absence or presence of dimethyl sulfoxide.

DISCUSSION

The present work shows that when painted on mouse skin dihydroteleocidin B induced ODC and dilatation of the blood vessels of the ear, and that it also induced cell adhesion of HL-60 cells and inhibition of terminal differentiation of Friend erythroleukemia cells. The doses of dihydroteleocidin B required for these effects were similar to, or lower than, those of the potent tumor promoter TPA.

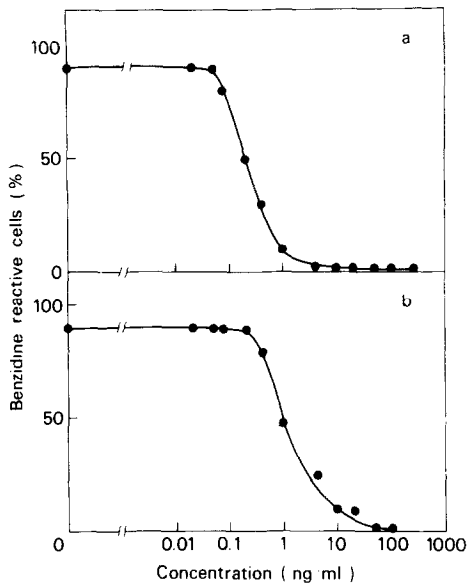

Fig. 3. Inhibitions by dihydroteleocidin B and TPA of terminal differentiation of Friend erythroleukemia cells. Friend cells were cultured with 2% dimethyl sulfoxide in the presence or absence of various concentrations of dihydroteleocidin B (a), or TPA (b) for 5 days. The percentage of benzidine reactive cells was determined as described in the text.

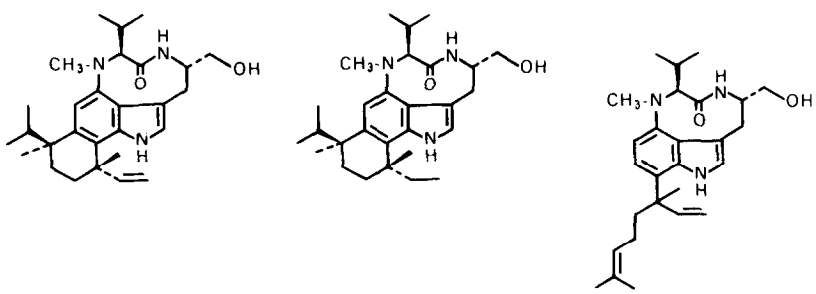

Fig. 4. Structures of teleocidin B, dihydroteleocidin B and lyngbyatoxin A.

Dihydroteleocidin B was obtained by catalytic hydrogenation of teleocidin B, which was isolated from *Streptomyces* (8). Dihydroteleocidin B and teleocidin B are reported to be very strong skin irritants (9), and as shown in Fig. 4 they are indole alkaloids (17). Recently lyngbyatoxin A was isolated from the blue-green alga, *Lyngbya majuscula* (10). This substance has the same skeleton as teleocidin B, and it is known to produce sea weed dermatitis (10). Similar sea weed dermatitis has been observed after

contact with a certain species of sea weed in Okinawa (18). Since dihydroteleocidin B has the same biological effects as TPA in short term tests, it is very likely that dihydroteleocidin B and teleocidin B may both possess tumor promoting activity *in vivo*. A long term experiment on the effect of painting mice with a limited amount of 7,12-dimethylbenz(a)anthracene and then dihydroteleocidin B is in progress in this laboratory. Experiments on the effects of teleocidin B and lyngbyatoxin A are also in progress. So far results have shown that a crude extract of the mycellia of *Streptomyces* containing teleocidin B induced ODC.

The possible presence of strong promoters in fungi and algae suggests that there may be other naturally occurring promoters besides phorbol ester type compounds. The final aim of this line of research is to determine the roles of these compounds in development of human cancer.

ACKNOWLEDGEMENTS

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