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Effects of Polybrominated Diphenol Ethers from a Marine Sponge *Phyllospongia dendyi* on IL-8 Production in a PMA-stimulated Promyelocytic Leukemia Cell Line

Taiko Oda [1,*], Hongwei Liu [2] and Michio Namikoshi [2]

[1] Department of Basic Biological Sciences, Kyoritsu College of Pharmacy, Shibakoen 1-chome, 5-30, Minato-ku, Tokyo, 105-8512, Japan

[2] Department of Ocean Sciences, Tokyo University of Marine Science and Technology, Minato-ku, Tokyo, 108-8477, Japan

*Author to whom correspondence should be addressed; Tel. +81-3-5400-2497, Fax +81-3-5400-2497, E-mail: oda-ti@kyoritsu-ph.ac.jp

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Abstract: The effects of five polybrominated diphenol ethers from a Marine Sponge, *Phyllospongia dendyi*, on the relative plating efficiencies in V79 cells and the cell proliferation and IL-8 production in PMA-stimulated HL-60 cells were examined. Three compounds, possessing two phenol units, were reported to inhibit the assembly of microtubule proteins and showed the inhibition of colony formation of V79 cells and increase in IL-8 production. Interestingly, a monomethoxy compound exhibited the stronger activity than the three active compounds in both bioassays. This compound did not inhibit the assembly of microtubule proteins. A dimethoxy compound was not active in either bioassays. In these experimental conditions, the biological activities were not high, but in future, these compounds will be expected to be made stronger by structural modifications.

Keywords: polybrominated diphenol ether, relative plating efficiency, V79 cell, IL-8, HL-60 cell.

Introduction

Marine sponges are prolific sources of biologically active agents [1,2]. In the course of our studies on biologically active natural products from marine organisms, we isolated eleven polybrominated diphenol ethers from the ethanol extract of a marine sponge, *Phyllospongia dendyi*, and reported the structures and the inhibitory activity on the assembly of microtubules in the purified porcine brain microtubule proteins of these compounds [3]. Three compounds (**1**-**3**) showed weak inhibition of the assembly of microtubules in the *in vitro* system.

In this study, we attempted to show the effects of these compounds on colony formation in Chinese hamster V79 cells, because the inhibition of microtubules correlates to cell proliferation. Moreover, the effects of these compounds on inflammatory cytokine, interleukin-8 (IL-8), production were determined. IL-8 is a member of the superfamily of C-X-C chemokines and a chemotactic factor for T cells, neutrophils and basophils [4]. The expression of IL-8 has been detected in a variety of human cancers and is suggested to be a factor in tumor progression and metastasis [5–14]. Therefore, the regulation of IL-8 production is an important medical problem.

Compounds **1**–**3** and **5** showed weak inhibitory activity on colony formation in V79 cells. The IL-8 production was stimulated by compounds **1**–**3** and **5** at higher concentrations, but **4** was not active at 50 μM. Interestingly, compound **5**, a monomethoxy derivative of **2**, exhibited the stronger activity than **2** and the other compounds.

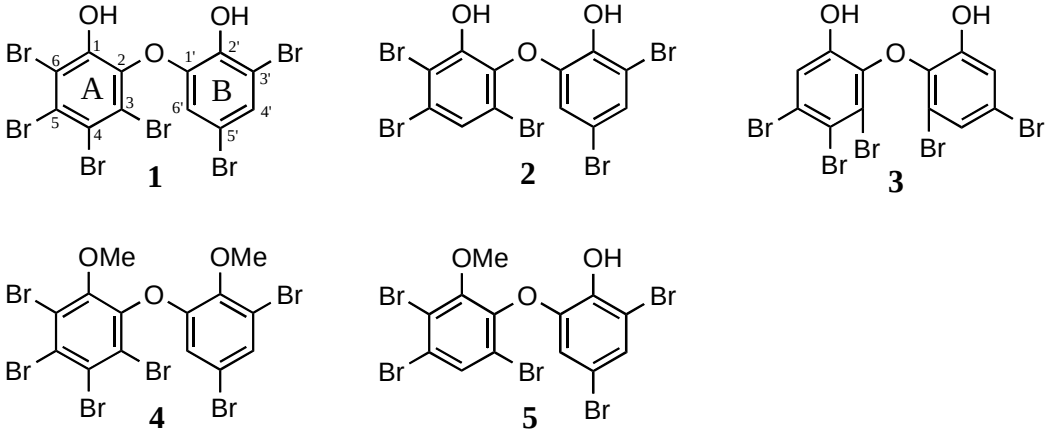

Figure 1. Structures of compounds **1-5.**

Materials and Methods

Materials

2-(3',5'-Dibromo-2'-hydroxyphenoxy)-3,4,5,6-tetrabromophenol (**1**) [15,16], 2-(3',5'-dibromo-2'-hydroxyphenoxy)-3,5,6-tribromophenol (**2**) [15,16], 2-(4',6'-dibromo-2'-hydroxyphenoxy)-3,4,5-tribromophenol (**3**) [16], 2-(3',5'-dibromo-2'-methoxyphenoxy)-3,4,5,6-tetrabromoanisole (**4**) [15] and

4,6-dibromo-2-(3',4',6'-tri-bromo-2'-meyhoxyphenoxy)phenol (**5**) [16] (Figure 1) were prepared as described previously [3]. The structures of five compounds are shown in Figure 1. Dimethylsulfoxide (DMSO) was purchased from Pierce Chemical Co. (Rockfield, IL) and fetal bovine serum (FBS) was obtained from GIBCO after checking the lot. All other reagents and chemicals used were of the highest grade available commercially.

Cell lines and culture conditions

Cell culture for Chinese hamster V79 cells were grown in monolayer culture in Eagle's minimum essential medium (MEM, Nissui Seiyaku Co., Ltd., Tokyo, Japan) with 10(v/v)% heat-inactivated FBS.

The human promyelocytic cell line, HL-60, was obtained from the Japanese Cancer Research Resources Bank (JCRB, Kamiyoga, Tokyo, Japan). This cell line was maintained in tissue culture dishes in RPMI 1640 medium (Nissui Seiyaku Co., Ltd., Tokyo, Japan), supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM glutamine, 100 U/ml of penicillin G and 100 μg/ml of streptomycin.

Relative Plating Efficiency

The method was essentially the same as that described in our previous paper [17]. The relative plating efficiencies in the presence of different concentrations of drugs were determined as the ratio of the number of colonies at a given drug concentration to that obtained in the control culture in the absence of any drug. Two hundred cells were seeded onto 60/15-mm Petri dishes in 4 ml MEM with 10(v/v)% FBS and incubated overnight at 37˚C, after which the samples dissolved in DMSO (4 μl) were added. The relative plating efficiencies were determined as the ratio of the number of colonies in the presence of different sample concentrations to that obtained in the control culture.

Detection of human IL-8 by ELISA

The IL-8 concentrations of the culture supernatants under control and various test conditions were measured by ELISA using a combination of monoclonal and polyclonal antibodies [18]. All samples were assayed at least in duplicate. Data are presented as the mean ± SE of three independent experiments.

Determination of cell proliferation

The cell proliferation was evaluated by enumerating the viable cells using the MTT formazan production method [19]. HL-60 cells (1×10^6 cells/ml) were treated with PMA (with or without test compounds) and then transferred to 96-well microtiter plates. After a 24 h incubation, 20 μl of MTT reagent (5 mg/ml in PBS) was then added to each well. After incubation for 3 h, formazan production was assessed by measuring the optical density (OD_{570} nm).

Results and Discussion

*The effects of polybrominated diphenol ethers **1**–**5** on relative plating efficiencies of Chinese hamster V79 cells*

Compounds **1**–**3** showed inhibitory activities to the assembly of microtubule proteins (IC_{50}: 29.6, 33.5, and 20.9 μM, respectively) and to the meiotic maturation of starfish oocytes (IC_{50}: 3.6, 4.2, and 4.2 μM, respectively) [3]. On the other hand, we showed that the inhibition of microtubule polymerization correlated with the inhibition of colony formation in Chinese hamster V79 cells. However, it will be expected that the biological activities are generally different between *in vitro* and *in vivo* systems. Then, we examined the effects of these polybrominated diphenol ethers on colony formation in Chinese hamster V79 cells. Two doses (1 and 10 μM) of each compound were examined, and the results shown in Figure 2.

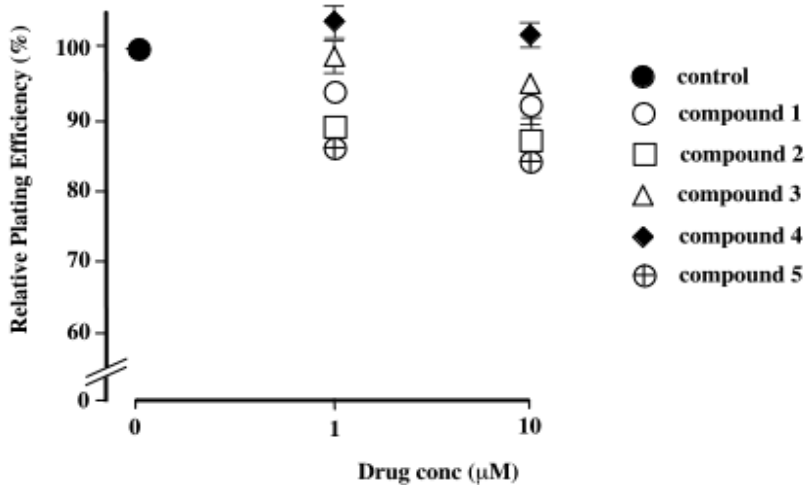

Figure 2. Relative plating efficiencies of Chinese hamster V79 cells treated with compounds **1–5** for 48 h with 1 and 10 μM concentrations of drugs. The experimental conditions were as described in the Materials and Methods. The data are the mean values of three independent experiments.

Compounds **2** and **5** showed stronger inhibition than those of **1** and **3** on colony formation. On the other hand, compound **4** had no effect at either concentration. These results indicated that the monomethoxy derivative (**5**) displayed the strongest inhibition compared with those of the two OH derivatives (**1**–**3**), and the dimethoxy derivative (**4**) did not inhibit its activity. This result shows that the inhibited activities of microtubule polymerization *in vitro* systems were not correlated with the inhibition of cell growth.

*The effects of polybrominated diphenol ethers **1**–**5** on IL-8 production by PMA-stimulated HL-60 cells*

To further examine the effects of compounds **1**–**5**, we used PMA-stimulated HL-60 cells because of their IL-8 production. Comparison of the results of relative plating efficiencies and IL-8 production were very interesting, because IL-8 production correlated to the stoppage of cell growth. We examined the effects of compounds **1**–**5** on IL-8 production by the PMA (2 or 20 nM)-stimulated HL-60 cells, and the results are shown in Figure 3. The high concentration of compounds **1**, **2**, **3** and **5** showed the increases in the IL-8 production under the additional two doses of PMA; on the other hand, compound **4** did not increase the IL-8 production under the same experimental conditions. Moreover, the cell proliferations of each IL-8 production condition were determined by MTT methods (data not shown). The results, these proliferations at 10 and 50 μM of compounds **1**, **2**, **3** and **5** were inhibited strongly (20-80%). Thus, IL-8 production correlated with cell proliferation in this case.

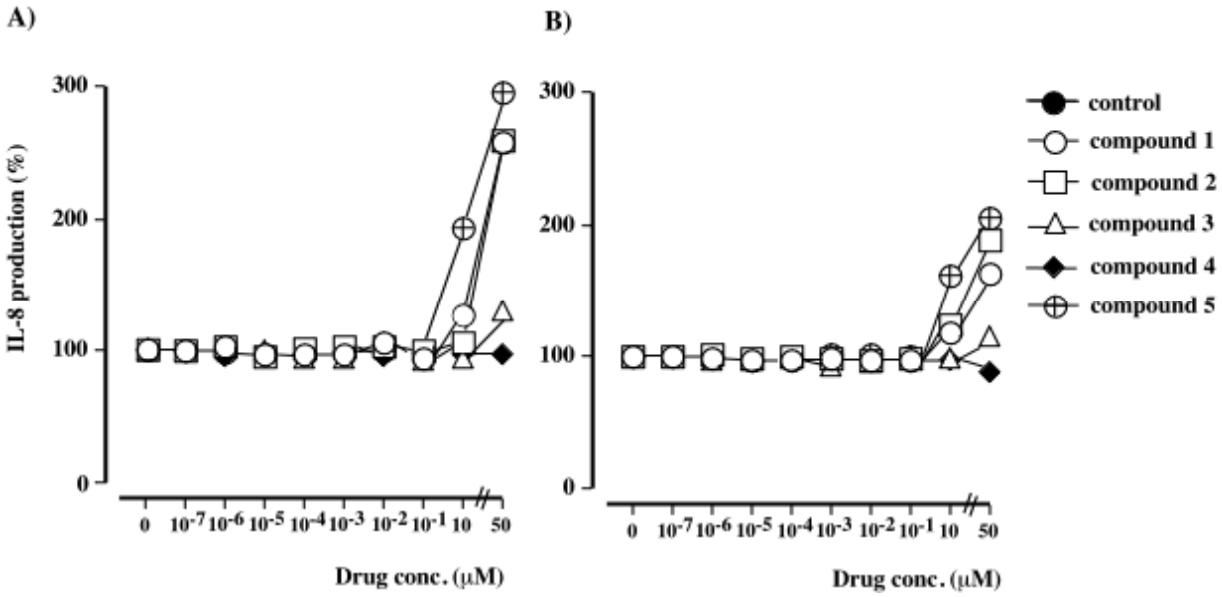

Figure 3. Effects of compounds **1–5** on IL-8 production and cell proliferation in PMA-stimulated HL-60 cells. A and B show the effects of compounds **1**–**5** on IL-8 production by 2 and 20 nM PMA added, respectively. HL-60 cells (1 x 10^6 cells/ml) were treated with PMA (2 or 20 nM) and the indicated concentration of each compound for 24 h. The IL-8 concentrations in the culture supernatant of the PMA-stimulated cells were determined to be ca. 14 and 40 ng/ml by ELISA, as described in the Materials and Methods. The data are shown as values relative (%) to the PMA-stimulated each IL-8 productions (14 and 40 ng/ml). The data are the mean values of three independent experiments.

On the other hand, the monomethoxy derivative (**5**) showed the strongest inhibition compared with those of the two OH derivatives (**1**–**3**), and the dimethoxy derivative (**4**) did not inhibit the activity. As compound **2** showed greater inhibition than **1**, it was suggested that the 4-Br in the ring A has a

negative effect on IL-8 production. Moreover, the relative inhibited activities of all compounds for IL-8 production agreed with these relative plating efficiencies, but did not agree with *in vitro* microtubule polymerization system, as shown in the previous report [3].

In this study, we elucidated the effects of polybrominated diphenol ethers from a Marine Sponge, *Phyllospongia dendyi*, on the relative plating efficiencies in V79 cells and the cell proliferation and IL-8 production in PMA-stimulated HL-60 cells. The monomethoxy compound (**5**) has greater activity than those of the compounds possessing two phenol units. One of the reasons suggested was that the monomethoxy derivative incorporates more easily into cells than the two OH derivatives. In this experimental data, the biological activities were so fine what weak, but after this, these compounds will be expect to have their activity enhanced by any structure modifications.

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