Differentiation induction of murine erythroleukemia cells by butylated hydroxytoluene

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Butylated hydroxytoluene (BHT) which is widely used as an anti-oxidant in food has been found to induce the differentiation of murine erythroleukemia cells. BHT also amplifies the differentiation inducing activity of DMSO.

Butylated hydroxytoluene *Differentiation* *Erythroleukemia cell* *DMSO*

1. INTRODUCTION

During the studies on the differentiation-inducing substances of animal cells, we found that butylated hydroxytoluene (BHT) induces the differentiation of murine erythroleukemia cells.

BHT is widely used as an antioxidant or a stabilizer in food, feed, cosmetics and pharmaceutical products. Recently it has been reported that BHT shows a variety of biological activities; e.g., inactivation of viruses [2,3], antimutagenicity [4], anticarcinogenesis [5–7], and stimulation of DNA synthesis [8].

We report here that BHT also induces the differentiation of murine erythroleukemia cells with high efficiency, and amplifies the differentiation-inducing activity of DMSO.

2. MATERIALS AND METHODS

2.1. *Materials*

Butylated hydroxytoluene (BHT) was purchased from Wako Chemical Co. (Tokyo). Ham's F12 medium and fetal bovine serum were obtained from GIBCO and Flow Laboratory, respectively. DMSO was a product of Junsei Chemical Co. (Tokyo).

2.2. *Cell line and cell culture*

Mouse erythroleukemia cells (clone B8) [9] were kindly provided by Dr K. Nose. Cells were grown in Ham's F12 medium supplemented with 15% fetal bovine serum and kanamycin (60 μg/ml) at 37°C in a humidified incubator with 8% CO_2 atmosphere.

2.3. *Differentiation assay*

The cells (6×10^4 cells/ml) were cultured for 3–7 days at 37°C with the indicated concentrations of BHT (see text) and/or 1.5% DMSO. The differentiated cells were determined by the modified benzidine-staining method [10].

3. RESULTS AND DISCUSSION

3.1. *Effect of BHT on growth and differentiation of mouse erythroleukemia cells*

After the erythroleukemia cells were cultured with the various concentrations of BHT for 3 days, the differentiated cells were determined by the benzidine-staining method. Fig.1 shows that the

Studies on the differentiation inducing substances of animal cells II. See [1]

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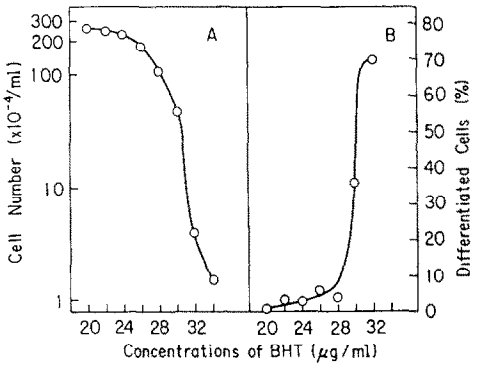

Fig.1. Effect of BHT on growth (A) and differentiation (B) of mouse erythroleukemia (B8) cells. The cells (6×10^4 cells/ml) were cultured for 3 days at 37°C with the indicated concentrations of BHT in 5 ml of Ham's F12 medium supplemented with 15% fetal bovine serum in humidified air containing 8% CO_2. The differentiated cells were examined by the benzidine-staining method [9].

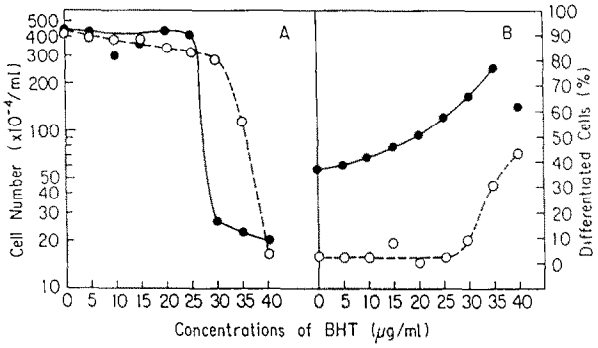

Fig. 3. Cooperative effect of 1.5% DMSO and the different concentrations of BHT on growth (A) and differentiation (B) of mouse erythroleukemia (B8) cells. The cells were cultured for 6 days with 1.5% DMSO and the indicated concentrations of BHT (●), and with BHT only (○). The experimental details are described in the legend of fig. 1, except the days in culture (this experiment was repeated 3 times, and the results were confirmed).

differentiation occurs at about 30 μg/ml of BHT maximally.

3.2. *Time-course analysis of the differentiation induced by BHT and DMSO*

Time-course of differentiation was examined (fig.2). Maximum cell differentiation by BHT was observed after 3 days culture, whereas maximum differentiation induction by DMSO was observed after 6 days culture.

3.3. *Cooperative effect of BHT and DMSO on the differentiation of erythroleukemia cells*

Fig.3 shows the cooperative effect of 1.5% DMSO and the indicated concentrations of BHT on the growth (A) and the differentiation (B) of mouse erythroleukemia (B8) cells. The cells were cultured for 6 days with 1.5% DMSO and the various concentrations of BHT (●), and with BHT only (○). This figure shows inducibility of about 0% with BHT at the concentration range of 5–25 μg/ml, and of about 40% with DMSO, respectively. Inducibilities, however, by the cooperation of DMSO and BHT (5–25 μg/ml) were higher than the sums of inducibilities caused by the individual inducers. Somewhat weak synergism was observed.

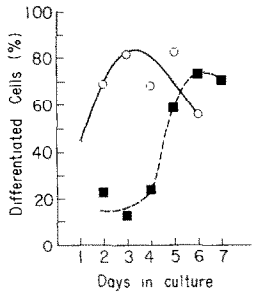

Fig. 2. Time-course analysis of differentiation by 30 μg/ml of BHT (○) and 1.5% DMSO (■). The culture conditions and examination method of the differentiated cells were the same as described in the legend of fig. 1, except the days in culture.

BHT is a strong antioxidant. Other antioxidants such as retinoic acid [11–13], tocopherol [7,14] and ascorbic acid [7,15] are also known to inhibit carcinogenesis or to influence cell differentiation. The redox system might take part in the mechanisms of differentiation and carcinogenesis.

BHT has been reported to possess a variety of biological activities; e.g., inactivation of viruses [2,3], anti-mutagenic activity [4], inhibition of physical [5] and chemical [6,7] carcinogenesis and the stimulation of DNA synthesis [8]. It was recently reported that BHT inhibits cell differentiation of mouse myeloid leukemia (M1) cells [16] induced by dexamethasone at the concentration of 10 μg/ml [17].

We have described here that BHT is a highly ef-

ficient inducer of differentiation. The fact that BHT, a widely used reagent, has differentiation controlling activity might present a serious problem to embryogenesis or development of animals.

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