Differentiation induction of murine erythroleukemia cells by butylated hydroxytoluene

Yoko Ohno, Tomoko Takuma, Ken-ichi Asahi* and Kiyoshi Isono

The Institute of Physical and Chemical Research, Wako-shi, Saitama 351, Japan

Received 15 November 1983

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% CO₂ atmosphere.

2.3. Differentiation assay

The cells (6 x 10⁶ 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...
Fig. 1. Effect of BHT on growth (A) and differentiation (B) of mouse erythroleukemia (B8) cells. The cells (6 x 10⁶ 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% CO₂. The differentiated cells were examined by the benzidine-staining method [9].

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 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.

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-
sufficient 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.

REFERENCES