Effects of Butylated Hydroxyanisole on Ornithine Decarboxylase Activity and Its Gene Expression Induced by Phorbol Ester Tumor Promoter

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Butylated hydroxyanisole (BHA) is a phenolic antioxidant that has been found to suppress the activity of skin tumor promoters. In this study, we investigated the effect of BHA on the activity of ornithine decarboxylase (ODC, an indicator of tumor promotion) and its gene expression induced by 12-O-tetradecanoyl-phorbol-13-acetate (TPA) in mouse skin. TPA-induced ODC activity was markedly inhibited by the topical application of 55 µmol of BHA (the inhibition rate at 6 h was about 80%). In Northern and dot-blot analysis, the TPA-induced increase in ODC mRNA was shown to be markedly reduced by the same dose of BHA (the inhibition rate at 4 h was about 60%). These results suggest the involvement of a decrease in ODC gene expression in the mechanism of the inhibition of ODC activity by BHA. J Invest Dermatol 96:289–291, 1991

Some antioxidants, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and nordihydroguaiaretic acid (NDGA), have been shown to suppress the skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate (TPA), benzoyl peroxide, or ultraviolet light [1–3]. Kozumbo et al [1] have reported that BHA, a synthetic phenolic antioxidant, strongly suppressed the TPA-induced activity of ornithine decarboxylase (ODC), an indicator of skin tumor promotion [4] and hyperproliferation [5]. However, the mechanism of this effect remains unexplained.

Recently, an increase in ODC gene expression has been demonstrated to be induced by tumor promoters [6–8]. In the present study, we investigated the effects of BHA on ODC activity and the level of its gene expression induced by TPA in mouse skin.

MATERIALS AND METHODS

Treatment of Mice Female CD-1 mice aged 7–9 weeks were obtained from Charles River Breeding Laboratories (Wilmington, MA) for use in all experiments. The dorsal skin of the mice was shaved 3–4 d before the experiment, and only those mice not exhibiting hair regrowth over this period were used. TPA (17 nmol in 0.2 ml of acetone) was applied to the shaved skin. BHA (55 µmol in 0.2 ml of acetone) was topically applied 30 min before TPA treatment. Control mice were treated with the same volume of acetone. TPA and BHA were purchased from Sigma Chemical Co. (St. Louis, MO).

Assay of ODC Activity Mice were sacrificed at the indicated times and the dorsal epidermis was separated by brief heat treatment [9] and homogenized [1,4]. ODC activity was assayed in the 30,000 × g supernatant of the homogenate, as described previously [10].

Assay of ODC mRNA Level Extraction and assay of ODC mRNA was performed by a modification of the method of Gilmour et al [6] and Verma [7], as described previously [8]. In brief, after the treated skin was excised and the dermis was removed, the specimen was immediately frozen and homogenized in guanidinium thiocyanate. After total RNA was extracted by Chomczynski’s phenol-chloroform method [11], the mRNA was purified using poly(A)+ RNA affinity paper (Message Activated Paper, Orgenics, Yavne, Israel) [12]. To assay the ODC mRNA level, isolated poly(A)+ RNA was analyzed by Northern [13] and dot-blotting [14] procedures, using an ODC cDNA probe (kindly donated by Dr. C. Kahana, The Weizmann Institute of Science, Rehovot, Israel) [15] labeled with 32P by the multiprime method [16]. As a control house-keeping gene, actin cDNA (Oncor Inc., Gaithersburg, MD) was used. The resulting autoradiogram was scanned using a soft-laser densitometer and the relative amount of hybridizable ODC mRNA was quantitated [6,7].

RESULTS

Figure 1 shows the time course of the changes in ODC activity induced by TPA. The ODC activity was markedly enhanced by TPA and reached a peak at 6 h. It then gradually decreased to the control level by 12 h. Topical application of BHA (55 µmol) 30 min
before TPA treatment suppressed the peak activity to about 20% of that without BHA. These results confirm those in previous reports [1,4].

Figure 2 demonstrates the results of Northern blot analysis. TPA induced a marked increase of ODC mRNA (lane 2, single band of 2.1 kb), and this increase was reduced by BHA treatment (lane 3). In contrast, the expression of the actin gene, which is an important housekeeping gene, was not affected by TPA or BHA treatment (Fig 3).

Figure 4 shows the dot-blot analysis of ODC mRNA and Fig 5 illustrates the relative amount of hybridizable ODC mRNA. Induction of ODC mRNA by TPA was suppressed to about 40% of the control level by BHA pretreatment (p < 0.001).

DISCUSSION

The important role of reactive oxygen and free radical species in skin tumorigenesis has been much discussed [17,18], and from this standpoint the effects of some antioxidants in suppressing tumor
promotion have been studied [1–3]. In particular, the food additives BHA and BHT have been studied in this regard [1,3].

Kozumbo et al have demonstrated that BHA strongly suppresses TPA-induced OCD activity in mouse skin [1], and in the present study we confirmed their results (Fig 1). Furthermore, we showed that the TPA-induced increase in ODC mRNA was suppressed by pretreatment with BHA (Figs 2, 4, and 5). As BHA or TPA treatment did not activate the change in actin gene expression (Fig 3), the effect of BHA is considered to be specific.

Although there was about 80% inhibition of OCD activity (Fig 1), the mRNA expression was only reduced about 60% (Fig 5). This difference has been observed by several investigators using other systems, and has been ascribed to the involvement of post-transcriptional regulation [19–21].

The importance of the lipoxygenase system in OCD induction has been demonstrated previously [22], and the inhibitory effect of BHA on lipoxygenase may contribute to its inhibition of OCD induction. Kozumbo et al [1] and Black et al [3] have noted that the antioxidant effect alone does not fully explain OCD inhibition. Recently, the relationship of protein kinase C to OCD induction has been elucidated [20], and the indirect effects of antioxidants, such as BHA and NDGA, on protein kinase C have been suggested in a T-cell system [23]. Although further studies are needed, our results suggest the involvement of decreased OCD gene expression in the mechanism of inhibition of OCD activity by BHA.

REFERENCES


3. Black HS, Tiggs J: Evaluation of structurally-related phenols for anti-

Figure 5. Densitometric relative quantification of the autoradiogram shown in Fig 4. Results are expressed as the amount of induction (fold-induction) over the ODC mRNA levels found after treatment with acetone alone. Each value is the mean of four experiments. Bar, SE.