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INHIBITION BY BUTYLATED HYDROXYTOLUENE OF EXCISION REPAIR SYNTHESIS AND SEMICONSERVATIVE DNA SYNTHESIS

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SUMMARY

Butylated hydroxyutoluene (BHT), a commonly used food antioxidant, inhibited excision repair synthesis in normal human peripheral lymphocytes damaged by uv light. Inhibition increased with increasing drug concentration to give 50% inhibition at approximately 20 μM. The acid, aldehyde, and alcohol derivatives at the 4-methyl position did not inhibit significantly DNA repair synthesis while semiconservative DNA synthesis was inhibited by both BHT and the metabolites. The significance of these findings to the reported biological effects of the antioxidant is unknown.

INTRODUCTION

BHT* is used widely in human and animal foods as an antioxidant and preservative (1,2). The maximum concentration of BHT allowed in foods is 0.02% based on the fat and oil content (3). Since its introduction, numerous studies have examined the possible effects of BHT consumption (for reviews see 2,4,5), and it is generally considered harmless.

Studies on the relationship of BHT consumption to tumor incidence in animals indicate both harmful and beneficial effects. The carcinogenicity of several chemicals was inhibited by dietary BHT (6-10), and the acute toxicity of several potent chemicals was decreased (4). The acute lethality of ionizing radiation was potentiated by BHT in one strain of mice (4),

*Abbreviations: BHT, butylated hydroxytoluene (3,5-di-tert-butyl-4-hydroxytoluene); S-MEM, minimal essential medium with Earle's salts (14); BHT-CH_2OH, 3,5-di-tert-butyl-4-hydroxybenzyl alcohol; BHT-CHO, 3,5-di-tert-butyl-4-hydroxybenzaldehyde; BHT-COOH, 3,5-di-tert-butyl-4-hydroxybenzoic acid; PBS, 0.025M $NaPO_4$ buffer in 0.85% NaCl, pH 7.2.

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but decreased in another strain (11); and Drosophila sperm were sensitized to gamma rays (12). In addition, BHT has been reported to behave as a promoter of squamous cell carcinomas of the forestomach induced by diethylnitrosamine (8), lung tumors induced by urethan (13), and liver tumors induced by 2-acetylaminofluorene (14). Furthermore, BHT was a complete carcinogen in inducing lung tumors (8).

The biological effects of BHT deserve further investigation, especially in view of the extensive human consumption of the compound. In this paper we report the effect of BHT and three of its metabolites on thymidine incorporation into resting and UV-damaged human lymphocytes as measures of semiconservative and DNA repair synthesis.

MATERIALS AND METHODS

Minimum essential medium with Earle's salts (S-MEM) (15) for suspension (Spinner) culture, without L-glutamine, was purchased from ISI Biologicals, Inc. BHT was purchased from Eastman Kodak Chemical Company and ICN Pharmaceuticals, Inc. The following BHT metabolites were purchased from Aldrich Chemical Company: BHT-CH_2OH; BHT-CHO; and BHT-COOH. Aquasol and [^{3}H] thymidine were purchased from New England Nuclear, and all other chemicals from Sigma Chemical Company.

Blood samples were collected from normal human donors (4 females, 9 males; ages 18-46) in 30 ml heparinized vacutainer tubes, and filtered through a column of nylon fibers (16). Lymphocytes were separated from the filtered blood using 5% dextran in 0.9% sodium chloride (17). The lymphocytes were centrifuged and resuspended in PBS, pH 7.2, at 9.6 x 10^6 cells/ml. For the repair studies cells were irradiated for 12 seconds with stirring at a maximum suspension depth of 4mm in open 100 ml beakers under a Gates Raymaster germicidal lamp emitting primarily at 254 nm (approximately 6 ergs/mm^2/sec). Lymphocytes were centrifuged and resuspended at a density of 2.4 x 10^6 cells/ml in S-MEM containing 1.0 mM hydroxyurea to inhibit semiconservative DNA synthesis (18). Following the addition of BHT or one of its metabolites and [^{3}H] thymidine, 5 μCi/ml (20-40 Ci/mmol), the cells were incubated for 2 hours at 37°C. An aliquot of cells was then lysed as described previously (19), nuclei collected on nucleopore filters, 8 μm pore diameter, and washed successively with PBS, 5% trichloroacetic acid containing 1% sodium pyrophosphate, and 95% ethanol. The filters were air dried and the radioactivity determined in 10 ml of Aquasol with a Beckman Model DPM-100 Liquid Scintillation Counter. Cell numbers were determined using a Coulter Counter (Model ZBI) and the data expressed as CPM/10^6 cells. Semiconservative DNA synthesis was determined as described for repair synthesis with the exception that the cells were not irradiated and were not incubated in the presence of hydroxyurea.

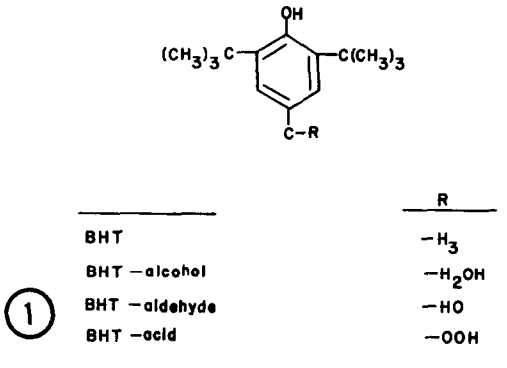
RESULTS AND DISCUSSION

The importance of DNA repair to the carcinogenic process has been

implicated from several lines of evidence (20-24), and inhibition of DNA repair was proposed as one possible mechanism for tumor promotion (24). Despite the contention that the lack of specificity of promoters as inhibitors of DNA repair precludes the importance of such effects in tumor promotion (25), DNA repair is a mechanism which needs additional exploration for its effects on the consequences of carcinogen exposure. Cells are still subject to DNA damage and repair even when not replicating. (Furthermore, many agents have more than one biological target). We were interested, therefore, in whether BHT, a "universal" food additive which exhibits carcinogenic, cocarcinogenic and anticarcinogenic activities, inhibited DNA repair replication and semiconservative DNA synthesis, although BHT was reported not to inhibit DNA repair in hamster embryo cells (26). Other studies with human cells seemed appropriate.

Butylated hydroxytoluene inhibited repair synthesis following UV irradiation of human lymphocytes (Fig. 2). The inhibition of [^{3}H] thymidine incorporation increased with increasing concentration of BHT, and the inhibition curve was linear between approximately 15 and 25 μM, with 50% inhibition at 20 μM. The 'cooperative' nature of the inhibition curve between 1 and 15 μM was unexplained, but could represent subsaturation of the system because of multi-site interactions of BHT, or a sequential increase in BHT affinity upon binding. BHT is hydrophobic in character and inhibits numerous cell membrane-associated functions (27, 28), and lipid-containing viruses (29). These findings are not in disagreement with the previous report which showed no inhibition of DNA repair in the hamster embryo cell line V79-4, at a maximum drug concentration of 10 μM (26).

Certain promoters, DNA-binding agents, and steroids were shown to inhibit semiconservative DNA synthesis at least as much as they inhibit DNA repair replication (25). Semiconservative DNA synthesis was also inhibited by BHT in the lymphocyte assay (Fig. 2). These findings are



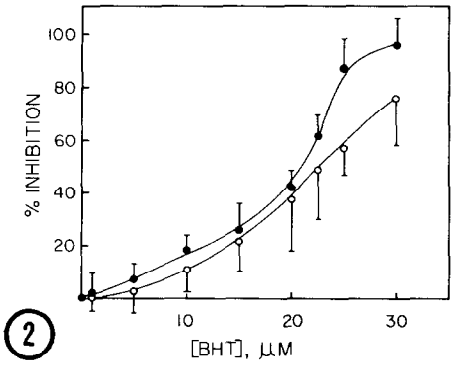


Fig. 1 Structure of BHT and BHT metabolites used.

Fig. 2 Inhibition of DNA repair synthesis and semiconservative DNA synthesis by BHT. Duplicate determinations were done for each experiment and each point represents the average of 3-9 experiments. ●, repair synthesis; O, semiconservative synthesis.

in agreement with the inhibition of DNA synthesis in monkey kidney cells grown in monolayer cultures (30). However, other studies have shown that BHT enhances the incorporation of [^{3}H] thymidine into pulmonary DNA in 7 strains of mice (31, 32), but not into DNA of liver, kidney, spleen or gastrointestinal tract (32).

The biological activity of any compound may be due to its native form or to its metabolites. Therefore, three metabolites of BHT (BHT-CH_2O, BHT-CHO, BHT-COOH; Fig. 1) were examined for their influence on DNA repair replication and semiconservative DNA synthesis (Table I). At a concentration of 25 μM, where BHT inhibited repair replication by 88%, the metabolites were without appreciable effect. In contrast, these metabolites did inhibit semiconservative DNA synthesis. Thus, small alterations in the methyl moiety of BHT resulted in drastic differences in the ability of the compound to influence differentially the semiconservative and repair replication processes.

Mammalian cells undergo unscheduled DNA synthesis following damage to their DNA by various chemical and physical agents (33-37), and the accumulation of damage is dependent, at least in part, on the enzymatic

Table I. Inhibition of semiconservative DNA synthesis and DNA repair synthesis. The concentration of each compound was 25 μM.

Compound	Inhibition	
	Semiconservative	Repair
BHT	57%	88%
BHT-CH_2OH	56%	11%
BHT-CHO	52%	15%
BHT-COOH	15%	9%

repair process. Defective repair has been well correlated with 'spontaneous' carcinogenesis in the human (21, 38, 39), ethylnitrosourea carcinogenesis in the rat (40), aging of human fibroblasts (41), and mammalian lifespans (42); and the relative effectiveness of DNA repair has provided the basis for a general theory of cellular aging (43). The potential importance of inhibition of DNA repair processes by BHT is unknown since such compounds have several effects and any combination of these, or a single one in particular, could be responsible for biological effects. It is yet to be determined whether a compound which inhibits both repair and replicative synthesis can lead to a net accumulation of DNA damage.

Although the possible effects of BHT on humans is unknown, the effects observed in these experiments occurred at concentration ranges close to those reached in human tissues at present levels of consumption. Concentrations of 1.30 $\pm$ 0.82 ppm have been reported in body fat (44). A level of 1.3 ppm is 0.6 x 10^{-5} M, which inhibited DNA repair slightly in the present experiments.

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