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Butylated hydroxytoluene does not protect Chinese hamster ovary cells from chromosomal damage induced by high-dose rate ^{192}Ir irradiation

C.A.Grillo and F.N.Dulout*

Centro de Investigaciones en Genética Básica y Aplicada (CIGEBA), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina

Previous reports showed the protective effect of the synthetic antioxidant butylated hydroxytoluene (BHT) against the chromosomal damage induced by bleomycin (BLM), cadmium chloride and potassium dichromate. To test the hypothesis that this effect was exerted by inhibition and/or scavenging of reactive oxygen species (ROS), the effect of BHT on the chromosomal damage induced by a high dose-rate gamma rays (HDR ^{192}Ir). Experiments were carried out by irradiating G_1 CHO cells with nominal doses of 1, 2 or 3 Gy. BHT (doses of 1.0, 2.5 or 5.0 μg/ml) was added to the culture immediately before or immediately after irradiation. Cells were then incubated in the presence of BHT for 13 h until harvesting and fixation. Results obtained showed that BHT did not decrease the chromosomal damage induced by radiation in any consistent fashion. On the contrary, in cells post-treated with 5.0 μg/ml of BHT the yield of chromosomal aberrations increased in several experimental points. These results with ionizing radiation suggest that the previous observed protective effects of BHT on the chromosomal damage induced by chemical genotoxicants may not be mediated solely through the scavenging or inactivating reactive oxidative species. The decrease of the yield of chromosomal damage induced by BLM could be due to the union of BHT with a metallic ion, in this case Fe (II), required for the activation of BLM. In the same way, the protective effect of BHT on the chromosomal damage induced by cadmium chloride and potassium dichromate could be due to the decrease of the effective dose of both salts in the cell through the chelation of the cations by BHT.

Introduction

Butylated hydroxytoluene (BHT; 3,4-di-tert-butyl-4-hydroxytoluene; CAS number 128-37-0) is an effective synthetic antioxidant widely used in processed foods especially in oil and oil-based foods, where it has had a great economic impact. The biological effects of BHT have been evaluated in different *in vivo* and *in vitro* assays. Although most published data indicate that BHT and the related compound butylated hydroxyanisole (BHA) have beneficial effects there are some indications that their effects can be detrimental. For instance, BHT produces a marked delay in the development of the advanced stages of cataract formation (1). The compound decreases the chromosome aberrations induced in mammalian cells by bleomycin (BLM) (2), cadmium chloride and pottasium dichromate (3,4). BHT can either enhance or inhibit mutagenic potency, depending on the tested substance (5). In the Ames test, BHT is antimutagenic towards benzo(*a*)pyrene but it increases the number of *Salmonella* revertants induced by aflatoxin B1 (6,7). In *Drosophila melanogaster*, BHT exerts a radioprotective effect in sex-linked recessive lethal mutations induced by 2 krad of X-rays (8). Antioxidants also are able to modify neoplasic development, thereby enhancing or inhibiting the development of lesions, depending on the target organ and the animal model (9–15).

Several studies indicate that BHT inhibits photocarcinogenesis in animal models (16–18). Moreover, it inhibits aflatoxin B1 hepatocarcinogenesis in male Fisher 344 rats (19), In addition, BHT exerts protective effects against BLM-induced lung fibrosis and the development of associated hyperplastic lesions in hamsters (20).

On the other hand, BHT has been utilized in experimental studies to induce pulmonary inflamation (21), renal and hepatic damage (22). A metabolic derivative of BHT, BHT-quinone methide, interferes with glutathione-*S*-transferase P1-1 regulation of stress kinases, decresing cellular protection from electrophiles and oxidants (23). Faine and co-workers (24) reported that BHT induced toxic effects on serum lipids in Wistar rats through increased triacylglycerols, VLDL and LDL-cholesterol concentrations, but interestingly, the antioxidant enzymes were elevated, indicating that BHT constituted a long-term stressor agent.

Several possible mechanisms may account for the decrease of chromosomal aberrations produced by BHT in genotoxicant-treated cells. The first is that BHT acts as a true antioxidant, either preventing the formation of reactive oxigen species (ROS) or scavenging them. The second is that, BHT interferes competitively with BLM chelating Fe (II) and thus inhibiting the formation of 'activated BLM', a chelated complex formed after addition of oxigen to Fe (II) BLM (25). In the same way, chelation of cadmium and chromium cations by BHT could be the cause of the decrease of chromosome aberrations observed after combined treatments with cadmium and chromium salts and BHT (4).

In order to evaluate these possible mechanisms the effects of BHT on the yield of chromosome aberrations induced by a high-dose-rate (HDR; ^{192}Ir) of irradiation in Chinese hamster ovary (CHO) cells, was studied. Gamma radiation causes DNA damage and chromosome aberrations both directly and indirectly. The indirect mechanism involves the generation of DNA-damaging radicals by water radiolysis without the intermediation of any metallic cations. If the protective effect of BHT is exerted mainly through its ability to prevent the formation or the scavenging of free radicals, a decrease of the yield of chromosomal aberrations induced by radiation should be expected.

*To whom correspondence should be addressed at: CIGEBA, Facultad de Ciencias Veterinarias, UNLP, 60 y 118, CC 296, 1900 La Plata, Argentina; Email: dulout@fcv.unlp.edu.ar

Materials and methods

CHO cell line was originally obtained from the American Type Culture Collection (ATCC, Rockville, MD). Mycoplasma-free cells were grown in Ham's F10 medium (Gibco, Invitrogen, Argentina) supplemented with 10% heat-inactivated fetal calf serum (FCS), 50 IU/ml of penicillin and 50 µg/ml of streptomycin sulfate. The cells were cultured in T-25 plastic cell culture flasks, with 10 ml of culture medium at 37°C in a humidified atmosphere with 5% CO_2 in air. The duration of the cell cycle is checked periodically using the BrdU-Giemsa method (26) and varies between 12 and 15 h.

BHT (Sigma, St Louis, MO) was diluted in dimethylsulfoxide (DMSO; Sigma) before treatment and used at final concentration of 1.0, 2.5 and 5.0 µg/ml. The dilutions were made in order to add 0.1 ml of the solution to each culture to obtain the desired BHT concentration. DMSO final concentration was 1%.

The experiments were performed in order to analyse the effects of BHT on radiation-induced chromosomal aberrations were determined in the first metaphase after treatment. Chromosomal aberrations were classified following the criteria recommended by Archer and co-workers (27) and World Health Organization (28).

In all the experiments, quiescent CHO cells were dislodged with trypsin, subcultured into flasks containing fresh medium and irradiated after 2 h (G_1 phase of the cell cycle). The cells were irradiated with nominal doses of 1, 2 and 3 Gy of gamma rays at a HDR using Microselectron Nucleotron® equipped with a small source of ^{192}Ir. The device was controlled by Indy® software run on a Silicon Graphics® computer.

For each radiation treatment T-25 culture flasks were placed on an acrylic plate containing six parallel needles separated by 10 mm. To obtain the programmed isodose curve the cell monolayers were placed at 7 mm over the needles. In the first experiment, the cells were irradiated and subsequently treated with 1.0, 2.5 and 5.0 µg/ml of BHT until the cells were harvested and fixed 13 h later. In the second experiment, the cells were irradiated in the presence of the different doses of BHT incubated for 13 h in medium containing BHT, and then harvested and fixed. Colchicine (0.1 µg/ml final concentration) was added to all the cultures for the last 2 h of the incubation period. The cells were harvested and fixed and slides prepared and air-dried according to routine protocols (29,30).

The frequencies of chromosomal aberrations were determined in the first metaphase after treatment. Each experiment was repeated three times. A total at 300 metaphases per treatment (100 per repetition) were scored. Statistical analysis was performed using the χ^2 homogenicity test comparing each treatment with its corresponding control.

Results

Tables I, II and III summarize the results of the first experiment in which CHO cells were irradiated and subsequently treated with BHT. As expected (26), treatment with BHT alone induced a significant increase in the frequency of chromatid and isochromatid breaks ($P < 0.001$) in relation to the DMSO control. Similarly, treatment with 1, 2 and 3 Gy of radiation significantly increased the frequency of abnormal metaphases ($P < 0.001$) in relation to control. In combined treatments with radiation and 1.0 and 2.5 µg/ml BHT, the frequencies of abnormal metaphases were similar than those induced by radiation alone. The 5.0 µg/ml BHT treatment, however, increased the frequency of abnormal metaphases compared with irradiation alone ($P < 0.01$). With regard to the different types of aberrations, post-treatment with 1.0 µg/ml BHT increased the frequency of isochromatid breaks induced by

Table I. Frequencies of structural chromosome aberrations in CHO cells treated with 1, 2 and 3 Gy gamma radiation during G_1 phase and then treated with 1.0 µg/ml BHT

Treatment	Abnormal metaphases (%)[1]	Chromosomal aberrations per 100 cells							Mitotic index %
		AL	B″	B′	RB′	Frag	DIC	RING	
—	0.3	0.3 (0.05)	0.3 (0.05)	—	—	—	—	—	6.1
DMSO	1.6	1.3 (0.11)	1.3 (0.11)	0.3 (0.05)	—	—	—	—	6.0
BHT	9.0	0.6 (0.08)	3.6 (0.18)	5.0 (0.22)	—	1.7 (0.12)	0.3 (0.05)	0.3 (0.05)	6.0
1	19.0	—	7.6 (0.26)	7.0 (0.25)	1.3 (0.11)	—	5.3 (0.22)	1.3 (0.11)	5.9
2	23.3	0.3 (0.05)	6.0 (0.24)	3.3 (0.17)	1.3 (0.11)	4.7 (0.20)	12.3 (0.33)	2.3 (0.14)	5.6
3	27.8	—	4.9 (0.22)	4.9 (0.22)	1.5 (0.12)	9.2 (0.30)	16.0 (0.36)	3.0 (0.17)	5.8
1 + BHT	21.0	—	7.6 (0.26)	6.3 (0.24)	—	—	7.6 (0.26)	2.0 (0.14)	5.8
2 + BHT	27.3	0.3 (0.05)	7.3 (0.26)	8.0 (0.27)	2.0 (0.14)	—	14.0 (0.35)	4.0 (0.19)	6.0
3 + BHT	29.0	—	5.0 (0.22)	4.5 (0.21)	—	5.0 (0.22)	18.5 (0.39)	5.5 (0.22)	5.9

For each experimental point 300 cells were scored. Standard error of mean is shown in the parentheses. AL, achromatic lesions; B″, chromatid breaks; B′, isochromatid breaks; RB′, chromatid exchanges; Frag, chromosome fragments; DIC, dicentric chromosomes; RING, chromosome rings.
[a]Abnormal metaphases are metaphases with at least one chromosomal aberration. Metaphases exhibiting only achromatic lesions were not scored as abnormal.

Table II. Frequencies of structural chromosome aberrations in CHO cells treated with 1, 2 and 3 Gy gamma radiation during G_1 phase and then treated with 2.5 µg/ml BHT

Treatment	Abnormal metaphases (%)[a]	Chromosomal aberrations per 100 cells							Mitotic index (%)
		AL	B″	B′	RB′	Frag	DIC	RING	
—	0.3	0.6 (0.08)	0.3 (0.05)	—	—	—	—	—	6.0
DMSO	1.6	2.0 (0.14)	1.0 (0.09)	0.6 (0.08)	—	0.3 (0.05)	—	—	6.2
BHT	8.3	0.3 (0.05)	5.6 (0.23)	5.0 (0.22)	—	0.3 (0.05)	—	—	5.0
1	20.0	—	3.0 (0.17)	5.3 (0.22)	0.6 (0.08)	—	9.3 (0.29)	2.6 (0.16)	6.0
2	26.3	0.6 (0.08)	4.3 (0.19)	4.3 (0.19)	2.6 (0.16)	5.8 (0.23)	17.6 (0.38)	2.6 (0.16)	6.2
3	37.0	—	7.6 (0.26)	2.7 (0.16)	1.5 (0.12)	19.0 (0.40)	24.4 (0.43)	5.7 (0.23)	5.8
1 + BHT	20.3	—	4.0 (0.19)	7.6 (0.26)	1.0 (0.09)	1.7 (0.12)	8.6 (0.28)	1.0 (0.09)	5.5
2 + BHT	29.3	0.3 (0.05)	3.0 (0.17)	5.0 (0.22)	2.3 (0.15)	4.0 (0.19)	14.0 (0.35)	5.6 (0.23)	5.0
3 + BHT	39.0	1.8 (0.13)	5.3 (0.22)	6.0 (0.24)	1.3 (0.11)	9.4 (0.30)	20.6 (0.40)	10.0 (0.30)	5.4

For each experimental point 300 cells were scored. Standard error of mean is shown in the parentheses. AL, achromatic lesions; B″, chromatid breaks; B′, isochromatid breaks; RB′, chromatid exchanges; Frag, chromosome fragments; DIC, dicentric chromosomes; RING, chromosome rings.
[a]Abnormal metaphases are metaphases with at least one chromosomal aberration. Metaphases exhibiting only achromatic lesions were not scored as abnormal.

Table III. Frequencies of structural chromosome aberrations in CHO cells treated with 1, 2 and 3 Gy gamma radiation during G_1 phase and then treated with 5.0 μg/ml BHT

Treatment	Abnormal metaphases (%)[a]	Chromosomal aberrations per 100 cells							Mitotic index (%)
		AL	B″	B′	RB′	Frag	DIC	RING	
—	0.6	0.3 (0.05)	0.6 (0.08)	—	—	—	—	—	5.9
DMSO	2.0	2.0 (0.14)	1.3 (0.11)	0.6 (0.08)	—	—	—	—	5.8
BHT	7.0	0.3 (0.05)	5.6 (0.22)	2.0 (0.14)	—	—	—	—	4.1
1	22.6	—	7.3 (0.26)	3.3 (0.17)	0.3 (0.05)	1.7 (0.12)	7.6 (0.26)	1.0 (0.09)	6.0
2	28.0	0.3 (0.05)	8.6 (0.28)	2.3 (0.15)	1.3 (0.11)	7.4 (0.26)	8.3 (0.27)	3.3 (0.17)	6.0
3	32.6	—	5.3 (0.22)	3.0 (0.17)	1.6 (0.12)	14.7 (0.35)	14.3 (0.35)	4.3 (0.20)	4.2
1 + BHT	25.3	—	7.3 (0.26)	6.6 (0.25)	0.3 (0.05)	4.1 (0.19)	10.3 (0.30)	0.6 (0.08)	4.0
2 + BHT	34.6	—	10.3 (0.30)	3.3 (0.17)	0.6 (0.08)	1.0 (0.09)	16.0 (0.36)	4.0 (0.19)	5.0
3 + BHT	38.0	0.3 (0.05)	10.0 (0.30)	3.6 (0.18)	1.3 (0.11)	11.4 (0.32)	11.6 (0.32)	8.3 (0.27)	3.9

For each experimental point 300 cells were scored. Standard error of mean is shown in the parentheses. AL, achromatic lesions; B″, chromatid breaks; B′, isochromatid breaks; RB′, chromatid exchanges; Frag, chromosome fragments; DIC, dicentric chromosomes; RING, chromosome rings.
[a]Abnormal metaphases are metaphases with at least one chromosomal aberration. Metaphases exhibiting only achromatic lesions were not scored as abnormal.

Table IV. Frequencies of structural chromosome aberrations in CHO cells treated with 1, 2 and 3 Gy gamma radiation in the presence of 1.0 μg/ml during G_1 phase

Treatment	Abnormal metaphases (%)[a]	Chromosomal aberrations per 100 cells							Mitotic index (%)
		AL	B″	B′	RB′	Frag	DIC	RING	
—	1.0	0.3 (0.05)	1.0 (0.09)	—	—	—	—	—	6.0
DMSO	1.3	—	1.0 (0.09)	0.3 (0.05)	—	—	—	—	6.2
BHT	4.3	0.3 (0.05)	2.3 (0.15)	2.3 (0.15)	—	—	—	—	5.9
1	21.0	—	4.6 (0.21)	6.6 (0.25)	2.0 (0.14)	4.0 (0.19)	8.0 (0.27)	—	5.8
2	23.3	0.3 (0.05)	7.3 (0.26)	3.0 (0.17)	1.6 (0.12)	2.4 (0.15)	13.3 (0.34)	2.3 (0.15)	6.0
3	31.3	0.3 (0.05)	3.3 (0.18)	5.3 (0.22)	1.6 (0.12)	7.7 (0.26)	18.3 (0.38)	5.3 (0.22)	6.1
1 + BHT	24.0	1.0 (0.09)	6.6 (0.25)	4.6 (0.21)	0.6 (0.08)	4.3 (0.20)	9.0 (0.28)	2.0 (0.14)	5.9
2 + BHT	24.6	—	3.6 (0.19)	5.6 (0.23)	1.3 (0.11)	2.0 (0.14)	12.0 (0.32)	3.3 (0.18)	5.6
3 + BHT	34.3	—	4.0 (0.19)	6.0 (0.24)	2.0 (0.14)	12.7 (0.33)	20.6 (0.40)	5.3 (0.22)	5.8

For each experimental point 300 cells were scored. Standard error of mean is shown in the parentheses. AL, achromatic lesions; B″, chromatid breaks; B′, isochromatid breaks; RB′, chromatid exchanges; Frag, chromosome fragments; DIC, dicentric chromosomes; RING, chromosome rings.
[a]Abnormal metaphases are metaphases with at least one chromosomal aberration. Metaphases exhibiting only achromatic lesions were not scored as abnormal.

2 Gy ($P < 0.001$), the frequency of chromosome fragments induced by 1 Gy ($P < 0.05$) and the frequency of chromosome rings induced by 3 Gy ($P < 0.01$) (Table I). 2.5 μg/ml BHT increased the frequency of isochromatid breaks in cells irradiated with 3 Gy ($P < 0.001$) and the frequency chromosome rings in the cells irradiated with 2 and 3 Gy ($P < 0.01$) (Table II). Post-treatment with 5.0 μg/ml of BHT resulted in significant increases in the frequencies of chromatid breaks ($P < 0.001$), isochromatid breaks ($P < 0.01$), chromosome fragments ($P < 0.01$) and dicentric chromosomes ($P < 0.001$) in cells irradiated with 3, 1, 1 and 2 Gy, respectively (Table III).

Tables IV, V and VI show the results of the second experiment in which cells were irradiated in the presence of BHT and incubated with the compound until fixation. With this protocol, there were no differences in the frequency of abnormal metaphases between treatments conducted with radiation alone and treatments with HDR-^{192}Ir plus the three concentrations of BHT. Cells irradiated with 2 Gy in the presence of 1.0 μg/ml of BHT showed a decrease in the frequency of chromatid breaks ($P < 0.025$). On the other hand, irradiation with 2, 3 and 1 Gy increased the frequencies of isochromatid breaks ($P < 0.01$), chromosome fragments ($P < 0.025$) and chromosome rings ($P < 0.01$), respectively (Table IV). With the dose of 2.5 μg/ml BHT an increase of the frequency of chromatid breaks induced by 1 Gy irradiation ($P < 0.05$) and a decrease of the frequency of chromosome fragments in cells irradiated with 3 Gy ($P < 0.05$) was found. Finally, cells irradiated with 1 Gy in the presence of 5.0 μg/ml BHT exhibited a decrease in the frequency of chromatid breaks ($P < 0.025$) and an increase in the frequency of chromosome rings ($P < 0.01$). The dose of 5.0 μg/ml BHT also increased in the frequency of isochromatid breaks induced by 2 and 3 Gy ($P < 0.05$ and < 0.025, respectively).

The cytotoxicity of different treatment was established by scoring the mitotic index, which decreased with increasing doses of BHT.

Figure 1 shows that there are not differences between cells irradiated and post-treated with BHT (B) and in cells irradiated in the presence of the compound (C). In both cases, the bars corresponding to the combined treatments are similar to those corresponding to the cells treated only with radiation alone

Discussion

The results obtained, under the experimental conditions of this study, show that BHT does not protect cells from the chromosomal damage induced by ionizing radiation. A decrease in the frequency of some chromosome aberrations was detected in few experimental points (chromatid breaks

Table V. Frequencies of structural chromosome aberrations in CHO cells treated with 1, 2 and 3 Gy gamma radiation in the presence of 2.5 μg/ml during G_1 phase

Treatment	Abnormal metaphases (%)[a]	Chromosomal aberrations per 100 cells							Mitotic index (%)
		AL	B″	B′	RB′	Frag	DIC	RING	
—	1.3	0.3 (0.05)	1.3 (0.11)	—	—	—	—	—	5.8
DMSO	2.0	—	1.3 (0.11)	0.6 (0.08)	—	—	—	—	5.6
BHT	6.3	0.3 (0.05)	3.3 (0.18)	5.0 (0.22)	—	—	—	—	4.6
1	20.6	—	4.3 (0.20)	6.3 (0.24)	1.0 (0.09)	3.6 (0.18)	8.0 (0.27)	—	6.2
2	23.6	0.3 (0.05)	8.0 (0.27)	3.0 (0.17)	1.6 (0.12)	3.1 (0.17)	12.6 (0.33)	2.6 (0.16)	6.0
3	33.3	0.3 (0.05)	4.3 (0.20)	5.0 (0.22)	1.3 (0.11)	11.1 (0.32)	18.6 (0.39)	5.3 (0.22)	5.8
1 + BHT	18.6	—	7.0 (0.25)	4.0 (0.19)	1.6 (0.12)	4.7 (0.21)	8.0 (0.27)	1.3 (0.11)	4.7
2 + BHT	24.0	0.6 (0.08)	6.6 (0.25)	3.0 (0.17)	2.3 (0.15)	6.3 (0.25)	9.0 (0.28)	3.0 (0.17)	4.9
3 + BHT	34.3	—	5.3 (0.22)	4.0 (0.19)	3.3 (0.18)	5.0 (0.22)	16.3 (0.37)	6.0 (0.24)	4.5

For each experimental point 300 cells were scored. Standard error of mean is shown in the parentheses. AL, achromatic lesions; B″, chromatid breaks; B′, isochromatid breaks; RB′, chromatid exchanges; Frag, chromosome fragments; DIC, dicentric chromosomes; RING, chromosome rings.
[a]Abnormal metaphases are metaphases with at least one chromosomal aberration. Metaphases exhibiting only achromatic lesions were not scored as abnormal.

Table VI. Frequencies of structural chromosome aberrations in CHO cells treated with 1, 2 and 3 Gy gamma radiation in the presence of 5.0 μg/ml during G_1 phase

Treatment	Abnormal metaphases (%)[a]	Chromosomal aberrations per 100 cells							Mitotic index (%)
		AL	B″	B′	RB′	Frag	DIC	RING	
—	1.0	1.3 (0.11)	0.6 (0.08)	0.3 (0.05)	—	—	—	—	6.0
DMSO	1.6	1.3 (0.11)	1.6 (0.12)	—	—	—	—	—	6.2
BHT	7.3	0.6 (0.08)	5.6 (0.23)	4.0 (0.19)	0.3 (0.05)	0.3 (0.05)	—	—	3.9
1	18.0	—	6.3 (0.24)	3.6 (0.18)	2.0 (0.14)	—	8.6 (0.28)	0.3 (0.05)	6.4
2	23.6	0.3 (0.05)	10.0 (0.30)	2.6 (0.16)	0.6 (0.08)	4.3 (0.20)	11.3 (0.32)	2.0 (0.14)	5.6
3	29.3	0.3 (0.05)	5.6 (0.23)	3.0 (0.17)	1.6 (0.12)	3.3 (0.18)	18.0 (0.38)	3.3 (0.18)	5.7
1 + BHT	18.6	0.6 (0.08)	3.3 (0.18)	3.3 (0.18)	1.6 (0.12)	1.7 (0.12)	5.6 (0.23)	2.3 (0.15)	4.3
2 + BHT	25.0	—	7.6 (0.26)	4.6 (0.21)	1.3 (0.11)	5.0 (0.22)	11.3 (0.32)	2.3 (0.15)	4.0
3 + BHT	28.3	—	6.6 (0.25)	5.3 (0.22)	1.6 (0.12)	8.7 (0.28)	13.3 (0.34)	4.0 (0.19)	4.1

For each experimental point 300 cells were scored. Standard error of mean is shown in the parentheses. AL, achromatic lesions; B″, chromatid breaks; B′, isochromatid breaks; RB′, chromatid exchanges; Frag, chromosome fragments; DIC, dicentric chromosomes; RING, chromosome rings.
[a]Abnormal metaphases are metaphases with at least one chromosomal aberration. Metaphases exhibiting only achromatic lesions were not scored as abnormal.

with 2 Gy plus 1.0 μg/ml of BHT and 1 Gy plus 5.0 μg/ml of BHT; chromosome fragments with 3 Gy plus 2.5 μg/ml of BHT). However, most treatments involving combinations of BHT and irradiation resulted in chromosome aberrations frequencies that were not different from those produced by irradiation alone. In addition BHT co-treatment had less of an effect on the overall frequency of abnormal metaphases in irradiated cells. Except for the treatment with 5.0 μg/ml of BHT following irradiation, where a significant increase was observed. BHT had no significant effects on the overall radiation-induced aberration frequency. Previous studies indicated that BHT protected cells against the chromosomal damage induced by BLM, cadmium chloride and potassium dichromate (2,4).

The results obtained in this study support the proposal that BHT may decrease chromosome aberrations induced by these chemicals by a mechanism not involving the scavenging or inactivation of ROS. BHT could decrease the clastogenic effects of BLM by the union of BHT with a metallic ion, in this case Fe (II), which is required for the activation of BLM. In this way, the balance between the active and inactive forms of BLM could be altered, reducing the clastogenic properties of the drug (25). In the same way, the protective effect of BHT on the chromosomal damage induced by cadmium chloride and potassium dichromate could be due to the decreases in the effective doses of both salts in the cell through chelation of the cations by BHT (4). In addition, Gulcin and co-workers (31) demonstrated the metal chelating and hydrogen peroxide scavenging activity of BHT who exhibited a 72% inhibition of the formation of the Fe (II)-ferrozine complex and scavenged 23% of hydrogen peroxide.

The differences between the effect of BHT on the chromosomal damage induced by BLM and ionizing radiation are illustrated in Figure 1. The figure shows the frequency of cells with chromosome and chromatid-type aberrations as well as cells with both types of aberrations. All three frequencies were increased in cells treated with BLM or radiation during G_1. Figure 1A shows the effect of BHT on the chromosomal damage induced by BLM. BLM decreased the frequency of cells with chromosome-type aberrations, but it had no effect on cells with chromatid-type aberrations or with both types of aberrations (2). Figure 1B and C illustrates the effect of BHT in cells irradiated and post-treated with the antioxidant (B) and in cells irradiated in the presence of the compound (C). In all cases, the bars corresponding to the combined treatments are similar to those corresponding to the cells treated only with radiation alone. From the results obtained it can be inferred that BHT could interact with trace elements modifying cell homeostasis and metabolism. Thus, BHT might interact in cells, tissues and organisms exposed to genotoxic and non-genotoxic agents (other phenolic compounds) modifying their action. For these reasons we believe

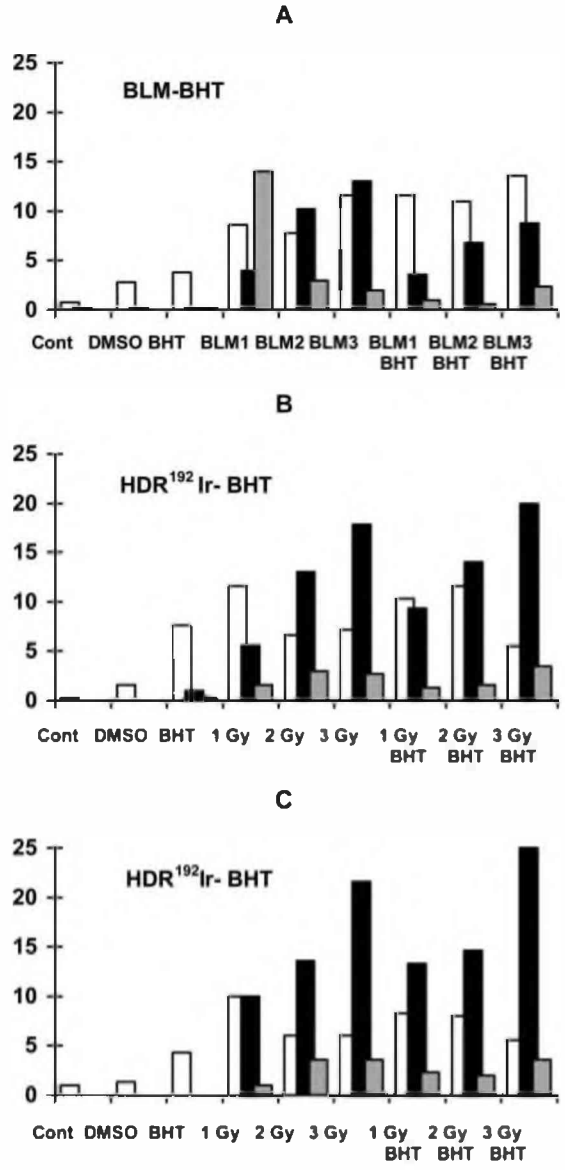

Fig. 1. Frequencies of cells with different types of aberrations treated (**A**) with BLM (doses of 0.1, 0.5 and 1.0 g/ml for BLM1, BLM2 and BLM3, respectively) plus 1.0 g/ml BHT (**B**) HDR ^{192}Ir and then treatment with 1.0 g/ml BHT (**C**) HDR ^{192}Ir plus 1.0 g/ml BHT. Black bars, frequency of chromosome-type aberrations. White bars, frequency of chromatid-type aberrations. Grey bars, frequency of cells with aberrations of both types.

that more extensive assessment of food additives in current use is warranted.

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