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Inhibitory Effects of Butylated Hydroxyanisole and Butylated Hydroxytoluene on the Genotoxicity of Some Anticancer Drugs

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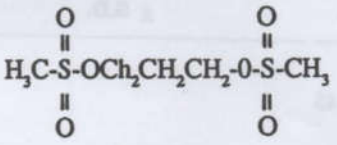
Busulfan, chlorambucil and cyclophosphamide, the three anticancer agents, did not exhibit direct DNA damaging capacity in *in vitro* test using the Rec assay. However, in *vivo* studies using the micronucleus test, showed that these drugs are mutagenic and clastogenic.

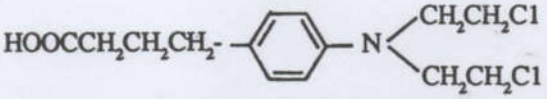
Two antioxidants, butylated hydroxyanisole and butylated hydrozytoluene reduced the genotoxic effects of the three enticancer agents as shown by the significant reduction of micronucleated polychromatic erythrocytes in bone marrow cells of mice.

Keywords: butylated hydroxyanisole,butylated hydroxytoluene, genotoxicity antioxidants.

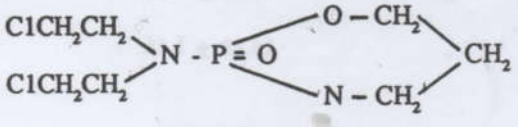
Three anticancer drugs were used in this study, busulfan, chlorambucil and cyclophosphamide. Their structures are given as follows:



Busulfan



Chlorambucil



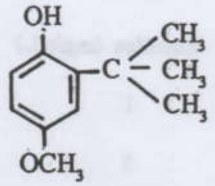
Cyclophosphamide

Busulfan is used for the treatment of chronic myelocytic leukemia [1]. It has been used to cause mutation in rice seeds [2]. Its genotoxic activity was shown when it induced chromosomal aberrations in human lymphocytes [3].

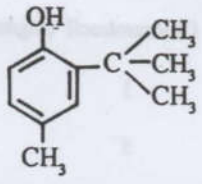
Chlorambucil is used in the treatment of chronic lymphocytic leukemia and malignant lymphomas [4]. It is also genotoxic because it has been shown to induce chromosomal damage [5].

Cyclophosphamide induces chromosome breaking effects [6]. Its anticancer property is associated with its ability to alkylate DNA [7]. Its teratogenicity is also associated with its alkylating ability [8]. Genotoxicity to germ cells was indicated when it induced sterility and testicular atrophy [9]. This drug has been used for all kinds of solid tumors [10].

This report confirms the genotoxicity of these three anticancer drugs. It also shows the inhibitory effects of two antioxidants on the genotoxicity of these drugs [11]. These antioxidants are butylated hydroxyanisole and butylated hydroxytoluene, whose structures are shown as follows:



Butylated hydroxyanisole



Butylated hydroxytoluence

MATERIALS AND METHODS

Busulfan, chlorambucil and cyclophosphamide were obtained from local drugstores. Butylated hydroxyanisole and butylated hydroxytoluene (analytical grade) were purchased from Sigma Chemical Company, (USA).

The Rec assay [13] was used to study the direct DNA damaging capacity of the anticancer agents. Mutant strains of *Bacillus subtilis*, the Rec⁻ and the Rec⁺ were used. These strains were streaked separately across the surface of top agar. A sterile paper disc, containing 0.02 mL of the anticancer drug solutions was placed at the starting point of each streak. The places were incubated at 37°C for 20 h, after which the length of zones of inhibition was measured.

The micronucleus test [14] was used to investigate the mutagenicity and clastogenicity potential of the anticancer agents and the antioxidants. Mice weighing 20-25 g were used. For every 20 g weight, 0.25 mL of the test system was administered orally by gavage. Administration was done twice, 30 h and 6 h prior to the preparation of the bone marrow. Bone marrow of the femur was flushed into a test tube containing fetal calf serum and centrifuged. The air-dried smear was stained and examined for micronucleated polychromatic erythrocytes.

The micronucleus test was also used to study the inhibitory effects of butylated hydroxyanisole and butylated hydroxytoluene.

RESULTS AND DISCUSSION

Effects of Anticancer Agents

The three anticancer drugs did not exhibit direct DNA damaging effects (Table 1). No zones of inhibition were observed indicating that they did not induce direct damage to the DNA of the test organisms. Although these drugs are known alkylating agents of DNA, it is possible that they have to undergo metabolic activation before they can alkylate DNA. If the drugs directly alkylated the DNA of the test organisms, zones of inhibition could have been observed especially with the Rec⁻ strain which does not possess the recombination repair system. This was not observed.

The three anticancer drugs induced the formation of micronucleated polychromatic erythrocytes in bone marrow cells of mice (Table 1). This suggests that these drugs fragmented the chromatin material of the bone marrow cells. After telophase when the nucleus is expelled, some fragments are left behind to form the micronuclei in the cytoplasm of the bone marrow cells.

Table 1. Genotoxic effects of busulfan, chlorambucil and cyclophosphamide.

	Using Rec assay		Using micronucleus test
	Zone of inhibition (mm)		No. micronucleated polychromatic erythrocytes per 1000 ± S.D.
	Rec+	Rec-	
Positive control, 4-NQO	29.61±2.14	38.20±1.45	-
Distilled water control)	0	0	1.12 ± 0.03
Busulfan (mg/mL)			
1	0	0	-
5	0	0	9.30 ± 0.14
10	-	-	16.89 ± 1.16
Chlorambucil (mg/mL)			
1	0	0	-
5	0	0	4.35 ± 0.02
10	-	-	6.50 ± 0.15
Cyclophosphamide (mg/mL)			
1	0	0	-
5	0	0	5.56 ± 0.61
10	-	-	9.44 ± 0.45

Two concentrations of the anticancer drugs were used: the lower concentration which is below the therapeutic dose and the higher concentration which is above the therapeutic dose. For both concentrations, there was an appreciable formation of micronucleated polychromatic erythrocytes as a consequence of the fragmentation of the chromatic after metabolic activation.

The results showed that busulfan had the strongest effect on DNA, chlorambucil had the weakest while cyclophosphamide had an intermediate effect. This confirms the genotoxicity of these drugs as reported by others. Since the micronucleus test is indicative of mutagenic and clastogenic activities, the results show that the three anticancer drugs are mutagenic and clastogenic.

Effects of BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene)

The two antioxidants, BHA and BHT were shown to be clastogenic (Table 2). They did not induce the formation of micronucleated polychromatic erythrocytes in bone marrow cells of the experimental mice, indicating that they did not fragment the chromatic material of the bone marrow cells.

Table 2. Effect of BHA (Butylated Hydroxyanisole) and BHT (Butylated Hydroxytoluene) on genotoxicity of some anticancer agents.

	No. of micronucleated polychromatic erythrocytes per 1000 ± S.D.
Control	2.43 ± 1.16
BHA, 10 mg/kg	2.67 ± 0.11
BHT, 10 mg/kg	2.34 ± 0.56
Busulfan alone, 10 mg/kg	16.89 ± 1.16
Busulfan + BHA, 0.2 mg/kg	2.33 ± 0.14
Busulfan + BHT, 0.2 mg/kg	6.50 ± 0.98
Chlorambucil alone, 0.4 mg/kg	6.50 ± 0.04
Chlorambucil + BHA, 0.2 mg/kg	2.55 ± 0.11
Chlorambucil + BHT, 0.2 mg/kg	2.56 ± 0.07
Cyclophosphamide alone, 10 mg/kg	9.44 ± 0.54
Cyclophosphamide + BHA, 0.2 mg/kg	2.78 ± 0.24
Cyclophosphamide + BHT, 0.2 mg/kg	3.11 ± 0.56

When these antioxidants were administered with the anticancer drugs, the chromosome breaking potential of the anticancer drugs was greatly reduced as indicated by the reduction of the formation of micronucleated polychromatic erythrocytes (Table 2). This indicates that the two antioxidants had inhibitory effects on the genotoxicity of the anticancer drugs. Since the anticancer drugs are alkylating agents, these possess electrophilic sites. The antioxidants are phenolic compounds and can easily ionize the hydrogen from the phenolic hydroxyl groups to form nucleophiles that can trap the anticancer drugs by interacting with the electrophilic sites. The interaction of the electrophilic sites can reduce or prevent the alkylating ability of the anticancer drugs.

Butylated hydroxyanisole had a greater inhibitory effect on the genotoxicity of busulfan than butylated hydroxytoluene. The inhibitory effects of both antioxidants on the genotoxicity of chlorambucil and cyclophosphamide are of similar magnitude. The greater inhibitory effect of butylated hydroxyanisole on the genotoxicity of busulfan can be a consequence of the greater nucleophilicity of the phenolate from butylated hydroxyanisole. While it is possible for nucleophilic strength to account for the greater inhibitory effect of butylated hydroxyanisole against busulfan, this may not be critical in the case of cholorambucil and cyclophosphamide.

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