GENETICS

Effects of β-Carotene and Aspartame on Clustogenic Activity of Cyclophosphamide and Dioxidine in Mice

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Antimutagenic effects of combination of aspartame (0.4 and 4 mg/kg) and β-carotene (0.15-15 mg/kg) were studied by estimation of chromosome aberrations in bone marrow cells of C57Bl/6 mice. Single and 5-day treatment with this combination decreased the clastogenic effects of dioxidine and cyclophosphamide and produced a more potent and universal antimutagenic effect than its constituents.

Key Words: *β-carotene; aspartame; antimutagens; mice; chromosome aberrations*

Antimutagens attract much attention as possible protectors of human heredity [4,6,7].

Antimutagenic effects of β-carotene (BC) and aspartame have been previously demonstrated; these effects depend on the dose and administration schedule [3-5]. Both compounds are widely used in medicine and food industry; they are often used in combination [4,5].

We found no reports on combined use of antimutagens, and therefore the effects of their combinations on induced mutagenesis are interesting from both theoretical and practical viewpoints.

We studied the effect of combinations of aspartame and BC on clastogenic effects of dioxidine (DN) and cyclophosphamide (CP) in mice.

MATERIALS AND METHODS

Male C57Bl/6 mice aged 8-12 weeks (18-22 g) from Stolbovaya Breeding Center (Russian Academy of Medical Sciences) were used. Mice were kept under standard vivarium conditions at 12-h day/night regimen with free access to water and food.

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Mutagenesis was induced by DN (Farmakon, St. Petersburg) in a dose of 300 mg/kg and CP (Biokhimik, Saransk) in a dose of 20 mg/kg. Antimutagens were injected in the following doses: 1) 0.4 mg/kg aspartame (NutraSweet) with 30% oil suspension of BC (alimentary carotene stain E160a, Hoffman La Roche) in doses 0.5, 5, and 50 mg/kg (0.15, 1.5, and 15 mg/kg pure BC, respectively) and 2) aspartame (4 mg/kg) with BC in the three above doses. The doses of BC were chosen basing on previous clinical experience (up to 180 mg/day) [1]. The maximum daily dose of aspartame is 40 mg/kg [4].

In series I the animals received mutagen and combination of the test compounds simultaneously 24 h before sacrifice (acute experiment). In series II mutagen was injected once after 5-day pretreatment with aspartame and BC. Cytogenetic preparations in this series were made 24 h after mutagen treatment. In series III mutagen and combination of antimutagens were given in parallel for 5 days and the animals were sacrificed 6 h after the last injection. DN and CP were injected intraperitoneally, aspartame and BC were given orally.

Cytogenetic studies were carried out by counting chromosome aberrations in mouse bone marrow cells

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[7]. Solitary and paired chromosome fragments, exchanges, achromatic gaps, and cells with multiple chromosome aberrations (more than 5) were counted under a microscope. The percentage of abnormal cells in the control and experimental groups was compared using φ test. Each experimental group consisted of 4-6 mice, 100 metaphases were obtained and analyzed from each animal.

RESULTS

Acute experiments demonstrated a statistically significant decrease of clastogenic activity of CP under the effect of 0.4 mg/kg aspartame in combination with BC in all doses (Table 1).

Similar results were obtained with aspartame (4 mg/kg). The combination aspartam+BC suppressed the clastogenic effect of CP (Table 1). The number of cells with DN-induced damage decreased significantly in animals treated with 0.4 mg/kg aspartame in combination with the maximum dose of BC (Table 1). Increasing the dose of aspartame to 4 mg/kg extended the protective potential of the combination, and the clastogenic effect of DN essentially decreased after treatment with BC in both maximum and intermediate doses.

It was previously shown that BC alone exerted no antimutagenic effect after treatment with DN or CP in a 24-h acute experiment [3]. Under the same conditions aspartame in doses of 0.4 and 4 mg/kg showed a pronounced antimutagenic activity towards CP, but not DN [4].

Hence, acute experiment demonstrated more potent and universal antimutagenic activity of aspartame-BC combination in comparison with both agents alone.

In series II, the antimutagenic combination significantly decreased the clastogenic effect of CP in all doses (Table 2). The clastogenic effect of DN decreased only after 5-day treatment with 4 mg/kg aspartame and 1.5 or 15 mg/kg BC. Decreasing the dose of aspartame to 0.4 mg/kg did not reduce the effect of the combination on the clastogenic activity of DN. After 5-day pretreatment with aspartame (0.4 mg/kg) in combination with BC (1.5 or 15 mg/kg) the clastogenic effect of this mutagen markedly decreased (Table 2).

It was shown previously that pretreatment with BC in doses of 1.5 and 15 mg/kg decreased the clastogenic effect of DN, but not of CP [3]. In contrast to BC, aspartame in the above doses considerably reduced the effect of CP [4].

Hence, pretreatment with test antimutagens significantly reduces the mutagenic effects of CP and DN, which confirms its more potent antimutagenic effect in comparison with the effects of its constituents.

After 5-day parallel treatment with CP and aspartam+BC a notable antimutagenic effect was observed after BC in doses of 1.5 and 15 mg/kg with aspartame in both doses (Table 3).

TABLE 1. Effects of Single Administration of BC and Aspartame (A) on Clastogenic Effects of DN and CP

Drug, dose, mg/kg		Number of cells	Per 100 cells					Abnormal metaphases
			gaps	fragments		exchanges	MA	
				single	paired			
CP,	20	500	4.2	11.0	1.8	0.2	0.6	17.8±1.7
+A, 0.4+BC	0.15	500	1.8	9.2	1.6	0.2	—	12.8±1.5*
	1.5	500	1.4	7.6	0.6	1.0	—	10.6±1.4*
	15	500	0.8	6.6	0.4	1.0	—	8.8±1.3*
CP, 20		400	1.5	8.3	1.5	4.5	—	14.8±1.6
+A, 4+BC	0.15	500	1.4	5	1.2	2.8	—	9.4±1.3*
	1.5	500	2.8	4.2	1.0	1.4	—	8.4±1.2*
	15	500	1.6	4.8	0.4	1.2	—	8.2±1.2*
DN, 300		500	2.2	5.2	2.2	2.2	8.8	20.6±1.8
+A, 0.4+BC	0.15	500	2.0	3.6	1.8	2.2	10.2	19.8±1.8
	1.5	500	1.6	4.0	1.8	2.0	7.6	17.0±1.7
	15	500	1.8	5.0	2.0	1.0	6.6	16.4±1.7*
DN, 300		500	0.2	4.2	—	0.6	12.2	17.2±1.6
+A, 4+BC	0.15	500	0.2	4.2	—	0.4	10.2	15.0±1.6
	1.5	500	—	4.4	0.2	0.2	7.8	12.6±1.6*
	15	500	0.2	4.0	—	0.2	7.6	12.0±1.6

Note. Here and in Tables 2 and 3: *$p<0.05$ *vs.* the control (effect of mutagen alone). MA: multiple aberrations.

TABLE 2. Effects of 5-Day Pretreatment with BC and Aspartame (A) on Clastogenic Effects of CP and DN

Drug, dose, mg/kg		Number of cells	Per 100 cells					Abnormal metaphases
			gaps	fragments		exchanges	MA	
				single	paired			
CP, 20		500	2.6	7.8	1.2	4.3	2.2	17.8±1.7
+A, 0.4+BC	0.15	500	2.8	6.8	0.8	4.6	—	14.4±1.6*
	1.5	500	1.9	6.8	0.6	2.8	—	11.6±1.4*
	15	500	1.6	6	0.2	1.6	—	10.2±1.4*
CP, 20		500	2.2	7.4	1.8	3	0.8	15.2±1.6
+A, 4+BC	0.15	400	2	6.8	1.8	2.3	—	12.8±1.5*
	1.5	500	2.4	6.2	1.2	1	—	10.8±1.4*
	15	500	0.8	4	1.2	1.4	—	7.4±1.2*
DN, 300		500	0.8	6.0	2.2	2.6	7.4	19.0±1.8
+A, 0.4+BC	0.15	500	1.0	3.2	2.0	2.8	6.8	15.8±1.6
	1.5	500	0.2	4.8	1.6	1.8	5.8	14.2±1.6*
	15	400	0.1	2.5	2.0	1.5	4.5	11.5±1.6*
DN, 300		500	0.4	4.4	—	0.4	12.0	17.2±1.6
+A, 4+BC	0.15	500	—	5.6	0.2	0.6	9.2	15.6±1.6
	1.5	500	—	4.2	—	—	5.8	10.0±1.4*
	15	500	0.2	3.8	—	—	3.8	7.8±1.2*

TABLE 3. Effects of BC and Aspartame (A) on Clastogenic Effects of CP and DN after 5-Day Parallel Treatment

Drug, dose, mg/kg		Number of cells	Per 100 cells					Abnormal metaphases
			gaps	fragments		exchanges	MA	
				single	paired			
CP, 20		500	0.2	15.8	0.8	1.6	4.2	19.2±1.8
+A, 0.4+BC	0.15	500	0.2	14.6	0.6	1.2	2	16.2±1.6
	1.5	500	0.1	12.8	0.6	1.2	1.8	13.6±1.5*
	15	500	0.2	10.6	0.5	1	0.8	12.4±1.5*
CP, 20		500	2.8	8.4	1.4	4	2.6	18.2±1.3
+A, 4+BC	0.15	500	3.4	6.6	0.8	3.4	0.6	14.8±1.6
	1.5	400	2	7	2	1.8	0.5	12.8±1.5*
	15	500	2	6.4	1.4	1	—	10.8±1.4*
DN, 300		400	1.75	10.0	1.75	2.5	14.5	30.5±2.3
+A, 0.4+BC	0.15	400	1.3	10.3	1.5	2.0	14.2	29.3±2.3
	1.5	500	0.8	7.6	1.2	2.0	14.2	25.8±1.9
	15	400	2.25	7.5	0.5	0.75	9.25	20.25±2.00*
DN, 300		400	—	8.6	0.2	0.4	25.6	34.8±2.1
+A, 4+BC	0.15	500	—	17.2	—	0.8	9.6	27.6±2.2*
	1.5	500	—	14.4	—	0.6	9.8	24.8±2.2*
	15	500	—	12.8	0.2	0.4	10.2	23.6±2.1*

In experiments with DN a statistically significant antimutagenic effect of the combination was observed with BC in a dose of 15 mg/kg and 0.4 mg/kg or 4 mg/kg aspartame with 0.15, 1.5, or 15 mg/kg BC (Table 3).

It was previously shown that 5-day treatment with BC in doses of 1.5 and 15 mg/kg in parallel with CP decreased its clastogenic effect and in doses of 0.15 and 15 mg/kg decreased the clastogenic effect of DN injected according to the same protocol [3]. Similar

treatment with aspartame did not decrease the clastogenic effects of DN and CP [4].

Comparison of various experimental series showed a pronounced protective effect of the combination in all variants and the absence of comutagenic activity. As was previously shown, pretreatment with both agents alone exerted a pronounced antimutagenic effect [3,4], which was not synergistic.

The inhibition of clastogenic effects of two chemical mutagens with different mechanisms of action by combined treatment with BC and aspartame suggests their use for prevention of induced mutagenesis and opens new vistas in the creation of genetically safe foodstuffs with antimutagenic effect on the basis of the studied combination.

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