GENETICS

In Vivo Cytogenetic Effects of Acrylamide, Acrylonitryl, and their Combination with Verapamil

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Acrylamide significantly increased the number of cells with chromosome aberrations in BALB/c and C57Bl/6, but not in CBA mice. No difference was found between the BALB/c and C57Bl/6 strains in the clastogenic effect of acrylamide. Within the studied concentration range acrylonitrile exerted no genotoxic effects. Verapamil significantly potentiated the clastogenic effect of acrylamide in BALB/c mice, while in C57Bl/6 mice potentiation was observed only after the repeated intragastric administration of verapamil in a dose of 2.5 mg/kg. Acrylonitrile in combination with verapamil also produced a slight clastogenic effect after single and repeated administrations. The *in vivo* comutagenic activity of verapamil depended on the dose, administration route and schedule, and genotype of experimental animals.

Key Words: *acrylamide; acrylonitrile; verapamil; cytogenetic effect; mice*

Environmental mutagenic pressure is sufficiently counterbalanced by the protective systems of the organism. It is confirmed by epidemiological studies showing that the incidence of spontaneous mutations in blood cells of healthy individuals rarely exceeds 2% [10].

Large-scale genetic monitoring provides reasons to hope that this balance will not be destroyed by the appearance of new mutagens in our environment. However, the danger can come from comutagens, compounds that possess no intrinsic genotoxic properties, but can considerably potentiate minor effects of environmental mutagens.

It has been recently shown that calcium channel antagonists, in particular verapamil, can significantly potentiate the effects of various mutagens in cultures of human and Chinese hamster cells [12].

The aim of the present study was to investigate the clastogenic effects of acrylamide and acrylonitrile and their modulation by verapamil. Considering that qualitative and quantitative differences in xenobiotic mutagenity are usually explained by genotypic peculiarities of test-systems [3], this study was performed on inbred mice of different strains.

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MATERIALS AND METHODS

The study was carried out on 1.5-2-months-old BALB/c, C57Bl/6, and CBA male mice purchased from Stolbovaya Breeding Center, Russian Academy of Medical Sciences. The animals were divided into several groups (4-14 mice per group) and maintained under standard vivarium conditions at a 12h:12h light:dark cycle with free access to water and food.

Verapamil hydrochloride (Ferein) in therapeutic doses was administered by standard routes. Acrylamide and acrylonitrile were purchased from Sigma. These compounds are widely spread in both industrial and domestic environment and used for the production of various polymers and co-polymers [1,2].

Acrylamide was dissolved in saline and administered intraperitoneally in single doses of 50 and 100 mg/kg or in a daily dose of 50 mg/kg for 5 consecutive days (0.1 ml solution per 10 g body weight).

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Acrylonitrile was applied according to the same schedules in the following doses: 5 and 10 mg/kg for single, 20 mg/kg for repeated oral administrations (in oil), and 10 mg/kg for single and repeated intraperitoneal injections (water solution). Single doses of verapamil were administered intraperitoneally (0.1, 0.2, 0.4, 2.5, 5, and 10 mg/kg) or through a gastric tube (2.5, 5, and 10 mg/kg). Repeated administration was performed through a gastric tube for 5 days (2.5, 5, and 10 mg/kg) with 24-h intervals. Control animals received an equal volume of NaCl.

A clastogenic effect was measured by counting chromosome aberrations in bone marrow metaphase cells [6]. Cytogenetic preparations were prepared by a conventional dry air technique 24 h after single administration and 6 h after the last of repeated administrations. Two and a half hours before killing the mice were pretreated with intraperitoneal colchicine (2.5 mg/kg). In each cytogenetic preparation (one bone marrow preparation from each mice) 100 metaphases were analyzed.

A Standard-20 microscope (×1000) was used for the microscopic analysis. Cells with single and paired fragments and chromosome exchanges were counted. The presence of 5 and more abnormalities was considered as multiple chromosome aberrations.

The data were analyzed by comparing the proportion of cells with chromosome aberrations in the control and experimental groups (φ-test).

RESULTS

The count of bone marrow cells with chromosome aberrations (Table 1) was the same in intact BALB/c, C57Bl/6, and CBA mice, hence there is no interstrain difference in the level spontaneous mutations in somatic cell.

After single and repeated administrations of acrylamide the count of cells with chromosome aberrations in BALB/c and C57Bl/6 mice significantly exceeded that in intact animals. Hence, the clastogenic effect of the xenobiotic was the same in BALB/c and C57Bl/6 mice and did not depend on administration schedule.

Acrylamide exerted no clastogenic effect in CBA mice (Table 1).

Our findings (in BALB/c and C57Bl/6 mice) confirm the data on weak mutagenic activity of acrylamide [11], while the absence of its mutagenic effect in CBA mice suggests that clastogenic activity depends on genotypic characteristics. It can be assumed that this factor determines the diversity of the results obtained in different studies of the genotoxic properties of acrylamide in mammalian somatic cells.

Acrylonitrile showed no clastogenic effect in BALB/c and C57Bl/6 mice, which is in line with published data [5].

Verapamil alone administered in all doses and administration schedules showed no genotoxic effect.

Intraperitoneal verapamil (0.1-10 mg/kg) did not modulate the clastogenic effect of single intraperitoneal injection of acrylamide in a dose of 100 mg/kg in BALB/c mice.

The clastogenic effect of intraperitoneal acrylamide was significantly (by 56%) potentiated by intragastric verapamil in a dose of 5 mg/kg (Table 2).

No significant potentiation of the clastogenic effect of acrylamide was found in acute experiments on C57Bl/6 mice.

TABLE 1. Clastogenic Effect of Acrylamide on Somatic Cells of BALB/c, C57Bl/6, and CBA Mice ($M \pm m$)

Strain	Acrylamide dose, mg/kg (administration schedule)	Cell count	Number of chromosome aberrations per 100 cells			Percent of abnormal cells
			single fragments	paired fragments	exchanges	
BALB/c	Control	500	1.6	—	—	1.6±0.6
	50 (1)	1100	5.0	0.2	—	5.1±0.7*
	100 (1)	1300	4.7	0.2	0.1	5.0±0.6*
	50 (5)	1400	4.4	0.1	0.1	4.6±0.6*
C57Bl/6	Control	600	2.0	—	—	2.0±0.6
	50 (1)	600	3.8	—	0.2	4.0±0.8**
	100 (1)	1200	4.1	0.3	—	4.4±0.6**
	50 (5)	1300	3.7	0.1	—	3.8±0.5**
CBA	Control	500	2.2	—	—	2.2±0.7
	50 (1)	500	1.8	0.2	—	2.0±0.6
	100 (1)	600	3.2	—	—	3.2±0.8
	50 (5)	600	3.5	0.2	—	3.6±0.8

Note. *$p<0.001$, **$p<0.05$ in comparison with the corresponding control.

TABLE 2. Effect of Verapamil (V) on Clastogenic Effect of Intraperitoneal Acrylamide and Acrylonitrile in BALB/c Mice in Acute Experiments (*M*±*m*)

Group	Cell count	Number of chromosome aberrations per 100 cells			Percent of abnormal cells
		single fragments	paired fragments	exchanges	
Acrylamide, 100 mg/kg+V, mg/kg					
0 (positive control)	1300	4.7	0.2	0.1	5.0±0.6
0.1	500	2.6	—	—	2.6±0.7
0.2	500	3.0	0.2	0.2	3.2±0.8
0.4	500	2.8	—	—	2.8±0.7
2.5	400	5.3	0.25	—	5.5±1.1
5	500	2.8	—	—	2.8±0.7
10	500	2.0	—	—	2.0±0.6
2.5 (i.g.)	400	4.6	0.3	—	6.0±1.2
5 (i.g.)	500	7.8	—	—	7.8±1.2*
10 (i.g.)	500	3.0	0.2	—	3.2±0.8
Negative control	500	1.6	—	—	1.6±0.6
Acrylonitrile, 10 mg/kg+V, mg/kg					
0 (positive control)	900	1.7	—	—	1.7±0.4
0.1	500	3.6	—	—	3.6±0.8*
0.2	500	3.2	—	—	3.2±0.8
0.4	500	2.6	—	—	2.6±0.7
2.5	500	3.8	—	0.2	4.0±0.9*
5	500	2.8	—	—	2.8±0.7
10	500	2.0	—		2.0±0.6
2.5 (i.g.)	500	3.6	0.2	—	3.8±0.9*
5 (i.g.)	500	2.0	—	—	2.0±0.6
10 (i.g.)	500	1.8	—	—	1.8±0.6

Note. Here and in Tables 3, 4: i.g., intragastric administration, *$p<0.05$ in comparison with the control.

In BALB/C mice treated with verapamil (2.5-10 mg/kg intragastrally) and acrylamide, the clastogenic effect of the latter was considerably (by 61%) potentiated by verapamil in doses of 2.5 and 5 mg/kg. In C57Bl/6 mice significant (84%) potentiation of the acrylamide effect was observed at a verapamil dose of 2.5 mg/kg (Table 3).

In BALB/c mice receiving single dose of acrylonitrile in combination with verapamil, the proportion of aberrant cells significantly increased after 0.1 and 2.5 mg/kg intraperitoneal or 2.5 mg/kg intragastric verapamil (Table 2). In C57Bl/6 mice, similar effect of intraperitoneal verapamil compared to the positive control was observed at doses of 0.2, 0.4, and 2.5 mg/kg and compared to the negative control at 0.4 mg/kg (Table 4).

Repeated combined administration of acrylonitrile and verapamil significantly increased the number of aberrant cells: in BALB/c mice this increase was observed after 10 mg/kg intragastric verapamil, in C57Bl/6 mice — after 2.5 and 5 mg/kg (Table 3).

These data confirm the weak clastogenic effect of acrylamide and the absence of interstrain and quantitative differences after its single and repeated injections.

The clastogenic effect of acrylamide can be attributed to alkylating properties and its metabolite glycideamide [13] or to glycideamide-induced inhibition of some antioxidant enzymes [8]. The latter mechanism is of special importance, since disturbances in the antioxidant state of the organism are thought to play a leading role in the realization of clastogenic effects of different xenobiotics including alkylating agents [3]. However, similar effect of acrylamide on bone marrow cells in BALB/c and C57Bl/6 mice implies that this mechanism is not crucial for its mutagenic effect: these two strains are known to differ considerably in pro- and antioxidant parameters and sensitivity to prooxidant mutagens [4]. The factors

TABLE 3. Effect of Verapamil (V) on Clastogenic Effect of Acrylamide and Acrylonitrile in BALB/c and C57Bl/6 Mice after 5 Intraperitoneal Injections (*M*±*m*)

Mice	Group	Cell count	Number of chromosome aberrations per 100 cells			Percent of aberrant cells
			single fragments	paired fragments	exchanges	
BALB/c	Acrylamide, 50 mg/kg+V, mg/kg					
	0 (positive control)	1400	4.4	0.1	0.1	4.6±0.6
	2.5 (i.g.)[1]	500	7.4	0.2	0.2	7.4±1.2*
	5 (i.g.)	500	7.6	0.2	—	7.4±1.2*
	10 (i.g.)	500	3.4	—	—	3.2±0.8
C57Bl/6	0 (positive control)	1300	3.7	0.1	—	3.8±0.5
	2.5 (i.g.)	400	7.0	0.3	—	7.0±1.3*
	5 (i.g.)	500	4.6	—	—	4.4±0.9
	10 (i.g.)	500	3.2	—	—	3.2±0.8
BALB/c	Negative control	500	1.6	—	—	1.6±0.6
	Acrylonitrile, 10 mg/kg+V, mg/kg					
	0 (positive control)	500	2.2	—	—	2.2±0.7
	2.5 (i.g.)	500	2.2	—	—	2.2±0.7
	5 (i.g.)	500	3.4	—	—	3.4±0.8
	10 (i.g.)	500	4.0	—	—	4.0±0.9+
C57Bl/6	Negative control	500	2.0			2.0±0.6
	Acrylonitrile, 10 mg/kg+V, mg/kg					
	0 (positive control)	500	2.4	0.2	—	2.6±0.7
	2.5 (i.g.)	500	4.0	0.2	—	4.2±0.9+
	5 (i.g.)	500	4.0	0.2	—	4.2±0.9+
	10 (i.g.)	500	3.8	—	—	3.6±0.8

Note. [1]One cell showed multiple aberrations. Here and in Table 4: $p<0.05$ *compared with the positive and +negative controls, respectively.

TABLE 4. Effect of Verapamil (V) on Clastogenic Effect of Acrylonitrile (10 mg/kg, Intraperitoneally) in C57Bl/6 Mice after Single Administration (500 Cells, *M*±*m*)

Group	Number of chromosome aberrations per 100 cells			Percent of aberrant cells
	single fragments	paired fragments	exchanges	
Negative control	2.0	—	—	2.0±0.6
Acrylonitrile+V, mg/kg				
0 (positive control)	500	1.8	—	1.8±0.6
0.1	2.8	0.6	—	3.4±0.8
0.2	4.0	—	—	4.0±0.9*
0.4	4,8	—	—	4.8±1.0*+
2.5	3.6	0.4	—	3.8±0.9*
5	2.8	0.2	—	3.0±0.8
10	2.6	0.2	—	2.8±0.7
2.5 (i.g.)	2.2	—	—	2.2±0.7
5 (i.g.)	2.0	—	—	2.0±0.6
10 (i.g.)	0.8	0.2	—	1.0±0.4

determining the presence of the clastogenic effect of acrylamide in BALB/c and C57Bl/6 mice and its absence in CBA mice remain to be investigated.

The comutagenic effect of verapamil can be explained within the framework of the "accumulation theory" stating that calcium antagonists, including verapamil, inhibit cytotoxin excretion from cells which leads to their accumulation and enhanced mutagenesis [12]. Verapamil and its analogs are known to potentiate cytotoxic activity of some antitumor antibiotics [14]. According to a number of independent reports, membranes of cells resistant to antitumor therapy contain P-glycoprotein, which prevents passive transmembrane diffusion of drugs [9] or actively pumps them out of cells reducing their intracellular concentration [15]. Verapamil and its analogs can be considered as chemical inhibitors of P-glycoprotein [7]. Our findings agree with these data.

However, it can not be excluded that the discovered effect results from changes in microsomal enzymes and/or intracellular free radical processes, but these suggestions need experimental verification.

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