Effect of Radioprotector Indralin on Carboplatinum Hemotoxicity

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A decrease in carboplatinum hemotoxicity was detected in experiments on C57Bl mice treated with the drug in combination with indralin (urgent radioprotector). Carboplatinum in a dose of 125 mg/kg, injected intraperitoneally, caused 80-100% death; the median term of death was 6 days (3-17). Single oral dose of indralin (100 mg/kg) during the 1st min or 15 min after carboplatinum injection (125 mg/kg) increased animal survival by 40.0-46.7% by day 20 of the experiment primarily during the period of manifest hemotoxicity (days 7-17), indralin injected 1, 2, or 4 h after carboplatinum exhibited no chemoprotective effect.

Key Words: *carboplatinum; radioprotector; indralin; toxicity*

Clinical trials of radioprotector amifostine (chemoprotector intended for the protection of normal tissues during radiotherapy and for reduction of drug toxicity) are in progress; the results demonstrate high efficiency of the drug [12]. By the mechanism of action, amifostine prevents radiation and chemical damage to DNA by modifying the tertiary structure of the macromolecule and limiting the access to the most vulnerable sites of the DNA strand [13]. Catecholamines (norepinephrine) used repeatedly within 6 h reduce hemotoxicity of carboplatinum [11].

We investigated the chemoprotective activity of effective Russian urgent action radioprotector indralin [2] belonging to α_1(B)-adrenoagonists [1] and differing from amifostine by the mechanism of its action.

MATERIALS AND METHODS

Experiments were carried out on 100 C57Bl mice (22-30 g). The animals were kept 5 per cage on standard briquette fodder and water. The mice were intraperitoneally injected with 1% carboplatinum solution (Ebewe) in a toxic dose of 125 mg/kg (LD 80-100). Indralin was administered orally through a tube as a suspension in 0.3% starch in the optimal radioproptective dose of 100 mg/kg (0.5 ml/mouse) during the 1st min and 15 min, 1, 2, and 4 h after carboplatinum injection. The animals were observed for 20 days (period of carboplatinum hemotoxicity realization).

The effect of indralin on carboplatinum toxicity was evaluated by animal mortality over 20 days and by changes in their body weight and blood leukocyte counts 3, 7, 12, and 20 days after injection of carboplatinum. Blood for analysis was collected from the caudal vein.

The results were statistically processed using Statistica 6 software. The significance of results was evaluated using Fisher's exact test, Mann—Whitney *U* test, and Wilcoxon's paired test. Survival curves were analyzed using Kaplan—Meyer method (by Cox *F* test).

RESULTS

The time course of animal mortality from carboplatinum intoxication is presented in Fig. 1. By day

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20 after intraperitoneal injection of carboplatinum in a dose of 125 mg/kg animal mortality was 85%, mean life span 7.50±0.74 days, median life span 6 (4-11) days. Some animals (45%) died during the toxigenic phase of intoxication, the rest after 7-17 days (time of death from toxic injury to the hemopoietic system; Table 1). Single oral dose of indralin (100 mg/kg) during the 1st min or 15 min after injection of carboplatinum in the toxic dose reduced mouse mortality by 40-45% ($p<0.05$; Fig. 1) and in the presence of developing leukopenia promoted the increase (for 7 days) in leukocyte count ($5.80\pm0.63\times10^3$/μl, $p<0.05$) compared to $3.10\pm0.64\times10^3$/μl in the control (carboplatinum alone). The radioprotector administered 1, 2, or 4 h after carboplatinum had no effect on carboplatinum toxicity (Fig. 1).

Indralin decreased mouse mortality primarily during carboplatinum hemotoxicity period (after 7 days postinjection) and had virtually no effect on the time course of animal death during week 1 of experiment, on body weight loss, and leukocyte count on day 3 after carboplatinum injection (Table 1).

The adrenergic system is directly involved in the regulation of proliferative processes in the bone marrow [6,7,9,10]. Norepinephrine stimulates lymphopoiesis through high affinity α_1-adrenoreceptors, detected on the bone marrow lymphoid cells (pre-B cells) [8] and, presumably indirectly, suppresses proliferation of hemopoietic cells committed by the granulocytic macrophagic colony-stimulating factor (GM-CSF) [5,7,9], because these cells possess only low-affinity α_1-adrenoreceptors [8].

TABLE 1. Effect of Indralin (100 mg/kg) on Toxic Manifestations during the Toxigenic and Somatogenic Phase of Carboplatinum Poisoning (125 mg/kg)

Parameter	Group	
	control (carboplatinum)	experimental (carboplatinum+indralin)
Survival		
on day 20	15.0 (6/40)	56.0$^+$ (14/25)
on day 6	55.0 (22/40)	68.0 (17/25)
Body weight, g		
initially	26.70±0.63	27.60±0.59
day 3	23.30±0.75*	24.50±0.56*
day 7	22.70±0.95*	24.70±0.84*
Blood leukocytes, 10^3		
initially	13.40±0.77	12.30±1.55
day 3	8.00±0.77*	7.20±0.66*
day 7	3.10±0.64*	5.80±0.63^x

Note. The ratio of survivors to total number of mice is given in parentheses. *$p<0.01$ compared to initial level (paired Wilcoxon's test); $^+p<0.05$ compared to control group (Fisher's exact test); $^xp<0.05$ compared to control group (Mann—Whitney *U* test).

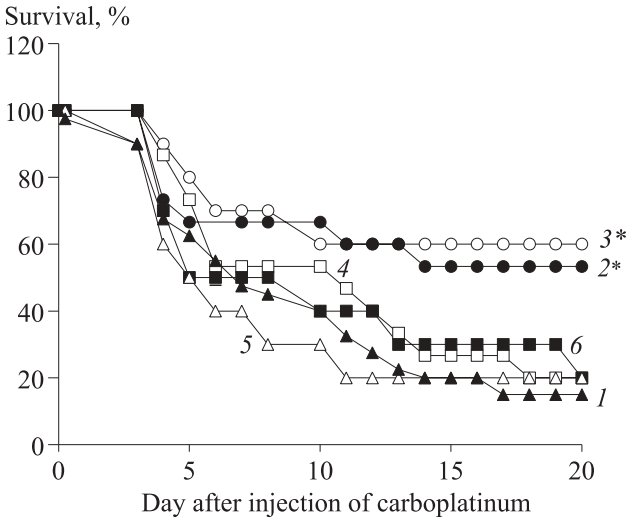

Fig. 1. Time course of mouse death from intoxication with carboplatinum (125 mg/kg) alone (*1*; *n*=40) or in combination with indralin (100 mg/kg) received 1-2 min (*2*; *n*=10), 15 min (*3*; *n*=15), 1 h (*4*; *n*=15), 2 h (*5*; *n*=10), and 4 h (*6*; *n*=10) after carboplatinum. *n*: number of animals; *$p<0.05$ compared to *1*.

Repeated dose of catecholamine within 6 h reduced carboplatinum hemotoxicity [11]. The animals received norepinephrine 1 h before, immediately before, and 2 and 4 h after carboplatinum. Suppression of the proliferative activity of myeloid cells under the effect of norepinephrine could reduce the risk of toxic damage to cell DNA during the most sensitive phase of the mitotic cycle. Chemoprotective effect of norepinephrine was significantly reduced by prasocine (α_1-adrenoblocker), but was not suppressed completely [11].

In our study effective reduction of carboplatinum toxicity was attained with a single dose of α_1-adrenoagonist, but only if it was used during the early period after the cytostatic, while later (during 4 h) it was ineffective. The period of pharmacological effect of indralin is just 1 h, while carboplatinum circulates in the blood during several hours; half-life period of irreversible platinum-protein compound is 5 days, and it is therefore most likely that the mechanism of chemoprotective effect of α_1-adrenoagonist is not confined to its effect on the proliferation of GM-CSF committed hemopoietic cells.

Indralin (direct α_1-adrenomimetic) treatment leads to activation of ribonucleotide reductase in the bone marrow and spleen; this enzyme catalyzes incorporation of ribonucleoside-5-diphosphate into DNA molecule through deoxyribonucleoside triphosphate synthesis. Indralin activates ribonucleotide re-

ductase within 30 min, this activation persisting during 3 days [3]. These changes play an important role in effective reparation of cell DNA damage [4] and can be due to the favorable effect of indralin during the period of carboplatinum hemotoxicity.

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