REDUCING TOXICITY OF ADRIAMYCIN BY ITS COMBINED ADMINISTRATION WITH THE COPPER COMPLEX Cu-2

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Monochemotherapy is used in exceptional cases at the present time to treat maligant tumors. The principal method of treatment in polychemotherapy, which is usually considerably more effective, although this depends on the correct choice of combinations and modes of their administration.

The motivation behind the study of toxicity of adriamycin (AD) when used in combination chemotherapy with the new Soviet cytostatic Cu-2, a copper complex, was the obtaining of the data on the mechanism of action of these preparations. According to many investigators [4-7] the toxicity of AD but not its therapeutic action is attributable to the superoxide (O_2^-) and hydroxyl (OH^-) radicals of the antibiotic, generated during its metabolism. On the other hand, Cu-2 is a low-molecular-weight analog of superoxide dismutase [3], an enzyme which inactivates O_2^- and OH^- radicals and thereby reduces their biological activity.

We postulated that realization of the superoxide-dismutase properties of Cu-2 when used with AD may lead to selective reduction of toxicity of the antobiiotic, i.e., under certain conditions of administration a combination of these cytostatics may be promising. For the experimental testing of this hypothesis the order of administration of the cytostatics was chosen after a study of the effect of Cu-2 on liver mono-oxygenase (LMO), on whose activity depends the realization of the biological action of AD [2].

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice aged 2-3 months. AD (Farmitalia) and Cu-2 (Institute of Chemistry, Academy of Sciences of the Moldavian SSR) were injected intraperitoneally. The toxicity of AD was determined on the basis of the survival time of the animals, the number which died, and the leukocyte count in peripheral blood taken from the caudal vein. LMO activity after injection of Cu-2 was assessed on the basis of the duration of sleep after intraperitoneal injection of hexobarbital (HB). Each group consisted of 10 mice. The results were subjected to statistical analysis by the Fisher–Student method. Differences were considered to be significant at the $p \leq 0.05$ level.

EXPERIMENTAL RESULTS

As mentioned above, the first stage of the investigation was to determine the effect of Cu-2 on LMO, activity of which was assessed by the duration of HB-induced sleep. Stimulation of LMO is known to shorten sleep, whereas its inhibition potentiates the sedative effect of HB and the animals sleep longer. The dose of CU-2 in these experiments was chosen allowing for the results of preliminary experiments: after two injections of CU-2 with an interval of 50 min and in a total dose of 8 mg/kg, 20-30% of the animals died by the 4th-5th day, and the maximal tolerated total dose of Cu-2 was 7 mg/kg.

The results given in Table 1 show that 10 min after the second injection of 3.5 mg/kg of Cu-2, given 50 min after the first, the duration of HB-induced sleep was increased in the experimental animals by 50-100% compared with the intact control, evidence of a decrease in LMO activity, and in agreement with results obtained by the writers previously when using different doses of Cu-2 [1]. Since we know that during inhibition of LMO the toxicity of AD

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TABLE 1. Effect of Cu-2 on Duration of Hexobarbital Sleep in Mice (M ± σ)

Expt. No.	Duration of hexobarbital sleep (min)			p
	experiment	control	experiment/control	
1	36,0±2,2	22,0±1,4	1,6	<0,02
2	30,4±3,7	16,0±1,0	1,9	<0,01

Legend. Animals of experimental group received two injections each of 3.5 mg/kg Cu-2 with interval of 50 min. HB was injected 10 min after last injection of Cu-2. Controls received HB alone. Significance of differences determined between duration of HB-induced sleep in experimental and control groups.

TABLE 2. Effect of Cu-2 on Toxic Action of AD (M ± σ)

Expt. No.	Preparation	Length of survival of dying mice, days	Number of mice which died, % of initial	p
1	AD	6,1±0,9	100	<0,02
	AD + Cu-2	15,9±3,7	100	
2	AD	10,2±3,3	90	<0,02
	AD + Cu-2	38,0±7,8	40	
3	AD	13,0±3,5	100	<0,01
	AD + Cu-2	24,3±5,8	60	

Legend. In experiment 1 AD was injected in a dose of 25 mg/kg, in experiments 2 and 3 in a dose of 20 mg/kg; Cu-2 injected twice, in a dose of 3.5 mg/kg each time, with an interval of 50 min, starting 10 min after injection of AD. Significance of differences determined between length of survival of dying animals receiving AD and those receiving AD + Cu-2.

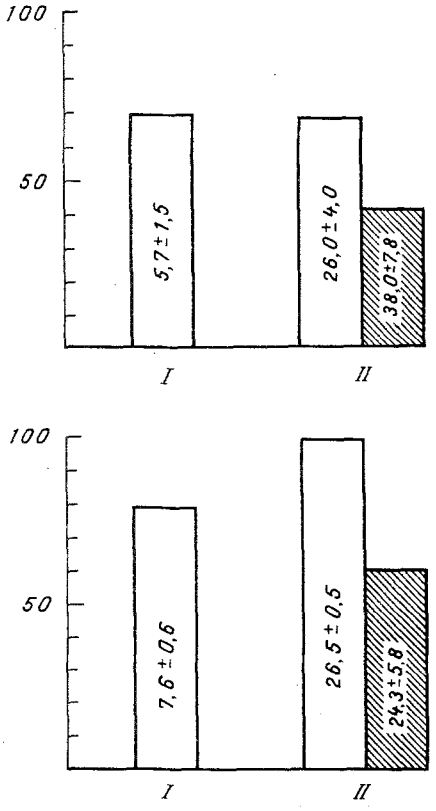

Fig.1. Effect of Cu-2 on early and delayed toxicity of AD in mice. Abscissa: I) early and II) delayed toxicity of AD (mean survival time of dying animals). Ordinate; number of animals which died (in % of initial number). When calculating the number of animals dying through early toxicity the number of mice in the group used in the experiment was taken at 100%, but in the analysis of delayed toxicity the number of mice surviving 14 days was taken as 100%. Unshaded columns – AD, shaded – AD + Cu-2.

is increased [2], and this may cancel out the postulated antitoxic effect of the superoxide-dismutase activity of Cu-2, we concluded that preliminary injection of Cu-2 is ineffective and we studied the effect of Cu-2 on the toxicity of AD when given after AD, for at the moment of realization of LMO inhibition by Cu-2 (60 min after its injection) metabolic conversions of AD in the liver are virtually finished.

The results of experiments to assess the toxicity of AD when two injections each of 3.5 mg/kg of Cu-2 were given with an interval of 50 min (the first injection was given 10 min after AD) are given in Table 2. In the first experiment in which a highly toxic dose of AD (25 kg/kg), exceeding LD_{100}, was given the toxicity of the antibiotic was reduced after injection of Cu-2, as shown by an increase in the survival time of the dying animals. When

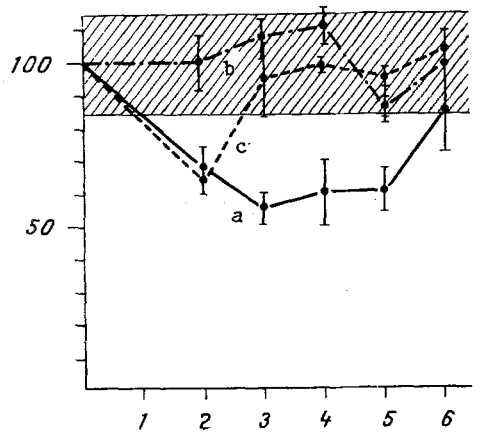

Fig. 2. Effect of Cu-2 on peripheral blood leukocyte count after injection of AD. Abscissa: days after injection of AD; ordinate: peripheral blood leukocyte count (in % of control). a) AD (15 mg/kg); b) AD + Cu-2; c) Cu-2. V) Fluctuations of leukocyte count in blood of animals receiving AD and AD + Cu-2 at different times of observation: 2nd day) > 0.5; 3rd day) = 0.05; 4th day) < 0.05; 5th day) < 0.01; 6th day) > 0.5.

AD was injected in a dose of 20 mg/kg (LD_{100}), the reduction of toxicity of the antibiotic after injection of Cu-2 was reflected in an increase in the survival time and a decrease in the number of dying animals compared with the corresponding values for the group of mice receiving AD alone.

In two experiments (2 and 3) death of the animals from AD poisoning was observed in the course of 27-30 days after injection of the antibiotic. In this case toxicity can be conventionally divided into early (the first 14 days) and delayed (more than 14 days) after injection of AD. The results of differential analysis of the effect of Cu-2 on early types of AD toxicity are given in Fig. 1. Clearly the manifestations of early toxicity of AD were completely abolished by Cu-2. Meanwhile delayed toxicity was only partially reduced, i.e., the main contribution of Cu-2 to the protective action revealed is its ability to prevent toxic damage developing in the early period after administration of AD.

In the study of the effect of Cu-2 on hemotoxicity of AD in a dose of 15 mg/kg, assessed on the basis of the peripheral blood leukocyte count, a decrease in toxicity of the antibiotic also was observed. The results of one such experiment are given in Fig. 2. Clearly the severity and duration of the AD-induced leukopenia were less than after injection of the antibiotic together with Cu-2.

To summarize the results it can be concluded that not only does summation of the toxic effects of the two cytostatics not take place, but as a result of their combined administration the toxicity of AD, the most toxic of the preparations, is reduced. Consequently, combined administration of Cu-2 and AD may prove to be very promising, although a final decision must await the study of the mutual influence of the cytostatics on their therapeutic effect. Nevertheless, even at this stage of the research the results are interesting because they demonstrate the fruitfulness of a rational combination of cytostatics based on knowledge of their mechanism of action.

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