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ANTITUMOR AND TOXIC PROPERTIES OF LIPOSOMES CONTAINING cis-DICHLORODIAMINOPLATINUM

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Several complex compounds of platinum, including cis-dichlorodiaminoplatinum (CDP), possess marked antitumor activity, probably due to their interaction with DNA [3, 4, 9]. However, the practical application of platinum complexes is made difficult by their high toxicity; the principal factor limiting the use of effective doses of the compound is a disturbance of renal function [9]. It was shown recently [11] that the distribution of therapeutic substances in the organs and tissues of a recipient can be modified by incorporating them into phospholipid vesicles or liposomes.

It was decided to study how the antitumor and toxic properties of platinum complexes were modified as a result of their incorporation into liposomes. For this purpose liposomes of different phospholipid composition, containing CDP, were obtained and their action tested on mice with a solid Crocker's sarcoma.

EXPERIMENTAL METHOD

Phosphatidylcholine isolated from egg yolk by the method [7], phosphatidylserine from bovine brain [2], lysophosphatidylcholine obtained from egg phosphatidylcholine with the aid of phospholipase A_2 [8], and cholesterol recrystallized from absolute ethanol were used. The purity of the phospholipids was verified immediately before the experiment by thin-layer chromatography on silica-gel in a chloroform–methanol–water mixture (65:25:4) [1]. Lipid phosphorus was determined spectrometrically [10].

Liposomes were obtained from pure phosphatidylcholine and from mixture of phosphatidylcholine with phosphatidylserine (85:15), phosphatidylcholine with lysophosphatidylcholine (93:7), or phosphatidylcholine with cholesterol (93:7); all ratios are molar.

To prepare liposomes a solution of phospholipids in an organic solvent (benzene or chloroform–methanol in the ratio of 2:1) was evaporated to dryness on a rotary vaporizer. The lipid film was dispersed in physiological saline containing a saturated solution of CDP. The dispersion was sonicated for 1 min by means of the UZDN-1 generator (output frequency 22 kHz). The resulting solution was centrifuged at 500g for 10 min to remove solid particles and subjected to exhaustive dialysis against physiological saline to remove any CDP not taken up. The total concentration of lipids in the dispersion was 10%. The resulting CDP liposomes contained 10 μg platinum/mg lipid phosphorus.

Crocker's sarcoma (S-180) was transplanted subcutaneously into noninbred albino mice weighing 18-20 g. The CDP preparations were injected intravenously as a single dose into the caudal vein of the mice 48 h after transplantation of the tumor. The animals were killed by decapitation on the 4th or 12th days after injection of the preparation. The percentage inhibition of tumor growth, determined relative to the mean weight of the tumors, was used as criterion of antitumor activity of the preparations; their toxic action was assessed by the change in body weight of the animals, changes in the spleen, and the total peripheral blood leukocyte count.

To study the distribution of CDP in the experimental animals, tissues (spleen, liver, kidney, muscle) were removed and dried and ground into a fine powder. Platinum was determined by Bankovskii's method.

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TABLE 1. Antitumor and Toxic Effects of CDP when Administered Dissolved in Physiological Saline and Incorporated into Phosphatidylcholine Liposomes (M ± m)

Group of animals	Mode of administration and dose	Weight of tumor, g	Inhibition of growth of tumor, %	Loss of weight of animals, g	Weight of spleen, g	Leukocyte count, thousands
		4-th DAY				
1	CDP in physiological saline, 10 mg/kg	0,32±0,05	75±5	17±3	0,17±0,06	10±3
2	CDP in liposomes, 10 mg/kg	0,9±0,1	25±10	3±3	0,25±0,06	16±3
3	CDP in liposomes, 20 mg/kg	0,13±0,05	89±5	12±3	0,20±0,06	16±3
4	Liposomes	1,2±0,2	0	0±3	0,35±0,06	20±3
5	Control	1,2±0,2	—	6±3	0,32±0,06	27±3
		12-th DAY				
1	CDP in physiological saline, 10 mg/kg	1,5±0,3	73±5	18±3	0,21±0,06	23±3
2	CDP in liposomes, 10 mg/kg	4,8±2	13±10	10±3	0,35±0,06	27±3
3	CDP in liposomes, 20 mg/kg	1,6±0,3	71±5	10±3	0,26±0,06	30±7
4	Liposomes	5,4±2	0	6±3	0,47±0,06	49±10
5	Control	5,5±2	—	7±3	0,32±0,06	43±10

Legend. Results of one of four experiments are given; number of animals in each experiment 8-13; difference between results of individual experiments not more than 15%.

TABLE 2. Platinum Content (in mg/kg) in Body Tissues of Intact Animals 15 min after Injection of CDP

Organs	Dose of CDP			
	8 mg/kg		16 mg/kg	
	physiological saline	liposomes	physiological saline	liposomes
Muscles	6	4	8	5
Liver	6	16	14	20
Spleen	9	75	9	80
Kidneys	22	14	42	13

TABLE 3. Total Platinum Content in Liver, Spleen, and Kidneys (in % of injected dose)

Dose of CDP, mg/kg	Mode of administration	
	physiological saline	phosphatidylocholine liposomes
	15 MIN AFTER INJECTION	
8	11	24
16	12	17
	2 h AFTER INJECTION	
8	9	28
16	8	16

EXPERIMENTAL RESULTS

After administration of CDP in phosphatidylcholine liposomes in low doses (10 mg/kg) protection was observed with respect to both animal and tumor, as shown by a reduction in the toxic and antitumor action of CDP (Table 1, groups 1 and 2). However, phosphatidylcholine lysosomes protect the animal against the action of large doses of the preparation which are lethal if administered dissolved in physiological saline, and at the same time, they enable much of its antitumor effect to be preserved. For instance, after injection of CDP liposomes in a dose of 20 mg/kg, which is lethal for injection of the preparation in solution, a marked antitumor effect was obtained, accompanied by only slight toxic effects on the animal as a whole (Table 1, group 3). The protective action of phosphatidylcholine liposomes on the recipient was manifested particularly clearly on the 2nd-4th days after injection, i.e., at times when its toxic action was maximal. At these times injection of CDP dissolved in physiological saline, in a dose of 20 mg/kg, caused death of all the animals, whereas when the compound was given in liposome form all the animals survived.

Comparison of the action of CDP in a dose of 20 mg/kg in phosphatidylcholine liposomes (group 3) with the action of half the dose (10 mg/kg) in physiological saline (group 1) shows that in the first case, with a higher percentage of inhibition of tumor growth, both the general toxic action (as judged by loss of weight of the animals) and the specific toxic action of the preparation on lymphoid tissues (judging from the weight of the spleen and the peripheral blood leukocyte count) was reduced. The same tendency as regards protection of the animals was observed also on the 12th day.

To study differences in the distribution of "free" and liposomal CDP in the body the platinum concentration was determined in individual tissues of intact animals. As Table 2 shows, 15 min after injection of liposomes containing the preparation the platinum concentration in the liver and spleen was much higher than when CDP was given in solution, whereas the concentration of platinum injected in lysosome form in the muscles and, in particular, in the kidneys was significantly less than when the preparation was given in physiological saline. In the case of the liposome preparation the platinum level in the kidneys was independent of dose, whereas when CDP was injected dissolved in physiological saline the

platinum concentration in the kidneys increased proportionally to the dose. With the passage of time (2-4 h after injection of the liposomes) the platinum concentration in the kidneys rose a little, whereas in the other tissues it was almost unchanged. Meanwhile, for the liposome preparation the total content of platinum in the kidneys, liver, and spleen of the intact animals was 2 to 3 times higher, only 15 min after injection, than when the same quantity of CDP was injected in physiological saline (Table 3). This suggests that CDP, injected in physiological saline, is excreted more rapidly from the body than the liposome form of the compound, and the decrease in toxicity of the latter is connected with the fact that liposomes remain impermeable for CDP for a long time after their injection.

To shed light on the effect of the phospholipid composition of the liposomes on biological activity of CDP, liposomes containing other lipid components than phosphatidylcholine were tested – lysophosphatidylcholine, phosphatidylserine, and cholesterol. These experiments showed that the addition of a small quantity of lysophosphatidylcholine (up to 3%) to the phosphatidylcholine did not change the therapeutic or toxic action of CDP. In this case, however, the leukopenia induced by the compound when injected in physiological saline was prevented. In the case of phosphatidylcholine liposomes containing 15% of phosphatidylserine the antitumor effect was approximately the same as when the compound wad administered in liposomes made from phosphatidylcholine alone. However, under these circumstances the general toxic effect, expressed as loss of weight of the animals, was almost doubled. Meanwhile phosphatidylserine-containing liposomes induce a significant protective protective effect relative to the lymphatic system, to judge from the very small change in the weight of the spleen compared with the control. Phosphatidylcholine liposomes containing 10% of cholesterol protect the recipient against the lethal action of high doses of the compound (16-20 mg/kg). In this case, however, inhibition of tumor growth was only two-thirds of that found in the case of liposomes of phosphatidylcholine alone.

The results of this investigation on the whole show that CDP in liposomes exerts a marked inhibitory action on growth of Crocker's sarcoma and, at the same time, is less toxic than the free CDP injected into animals dissolved in physiological saline. As a result of this, the use of phosphatidylcholine liposomes enables the dose of the compound to be increased while at the same time, reducing its over-all toxic effect. Considering existing data on the increased affinity of phospholipid liposomes for tumor tissues [5], it seems likely that the liposome form of administration of complex platinum compounds could prove interesting for antitumor therapy.

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