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ANTIDOTAL AND ANTITUMOR PROPERTIES OF COPPER SULFATE

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The antitumor agent cisplatin, which is a complex compound of platinum, is known to give rise to undesirable side effects. In particular, it disturbs renal function, inhibits hematopoiesis, and induces nausea and vomiting. Copper sulfate, injected subcutaneously into mice with Ehrlich's tumor, has been shown to reduce the nephrotoxicity of cisplatin, without reducing its antitumor activity [4]. It was therefore interesting to study the antidotal properties of copper sulfate when given internally, for this therapeutic agent cannot be given subcutaneously [1].

To discover whether copper sulfate can be used in clinical practice the antitumor activity of copper sulfate given internally, and its action on the antitumor effect and various side effects of cisplatin were investigated.

EXPERIMENTAL METHODS

Chemotherapeutic experiments were carried out on 50 C57Bl/6 mice, 90 (CBA × C57Bl)F_1 hybrid mice, 32 BALB/c mice, 40 (DBA × C57Bl)BDF_1 mice, and 50 SHK mice. The antidotal properties were studied on 50 noninbred rats and 125 F_1 hybrid mice. An aqueous solution of copper sulfate in 0.1-0.5% concentration was given internally in a single dose or 5 daily doses, within the dose range from 10 to 120 mg/kg (in doses of 60-120 mg/kg copper sulfate was given with milk to reduce its toxicity). Cisplatin was injected intraperitoneally in single doses of 8 mg/kg for rats and 6, 14, and 16 mg/kg for mice. The antitumor activity was studied against transplantable strains of tumors: Lewis epidermoid lung carcinoma (LLC) adenocarcinoma of the colon (ACACOL), mammary gland adenocarcinoma Ca 755, and Ehrlich's ascites tumor (EAT). The tumors were transplanted by the standard method [3]. Antitumor activity was estimated relative to two parameters for solid tumors: the percentage inhibition of tumor growth by volume and the increase in survival period of the animals in percent relative to the control, and for the ascites tumor, by the increase in duration of survival in per cent of the control. In toxicologic tests, the state of the animals was inspected, the number of animals which died and at what times was noted, the animals were weighted, and the leukocyte and erythrocyte counts determined in the peripheral blood of the rats on the 3rd and 5th days after injection of cisplatin. The protein concentration in the urine was determined by the sulfosalicylic acid test. The creatinine and urea concentrations

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TABLE 1. Antitumor Activity of Copper Sulfate

Strain of tumor	Dose, mg/kg	Number of injections	Beginning of treatment, days after transplantation of tumor	Inhibition of tumor growth, %		Increase in survival period, %
				days after transplantation of tumor		
				10—11	18—20	
LLC	10	1	7-	81	83	7
	60	1	1-	62	None	None
	80	1	2-	73	30	16
	120	1	1-	76	None	14
	20	5	2-	79	54	None
	20	5	7-	46	33	»
	30	5	2-	73	53	23
ACACOL	50	1	2-	31	None	None
	10	1	2-	81	63	»
	20	5	2-	66	None	»
Ca 755	50	1	1-	99,4	83	»
	10	1	1-	81	63	»
	10	1	7-	96	78	»
EAT	80	1	1-	Not determined		»
	20	5	1-	»	»	»

TABLE 2. Acute Toxicity of Cisplatin (DDP) Alone or in Combination with Copper Sulfate on Small Laboratory Animals

Preparation and interval between doses	Species of animals	Copper sulfate		Dose of DDP by intraperitoneal injection	Effect: number of dying/total number
		dose, mg/kg	mode of administration		
Copper sulfate (24 h) + DDP	Mice	10	Subcutaneously	14	1/10
Copper sulfate (24 h) + DDP	»	10	Internally	14	0/10
Copper sulfate + DDP (simultaneously)	»	10	»	14	6/10
DDP	»	—	—	14	7/18
Copper sulfate (24 h) + DDP	»	10	Internally	16	2/10
DDP	»	—	—	16	5/10
Copper sulfate (24 h) + DDP	Rats	10	Internally	8	0/5
DDP	»	—	—	8	2/5

in the rats' blood serum were measured on a "Greiner Elektronik" instrument (Switzerland) in the clinical biochemistry laboratory of the Center.

EXPERIMENTAL RESULTS

Antitumor Activity. Copper sulfate had an inhibitory action on growth of the solid tumors. Against the ascites tumor, copper sulfate in the doses and schedules used was ineffective (Table 1). The antitumor effect was independent of the schedule and size of the dose given, and in some cases the smallest dose (10 mg/kg) was more effective than doses close to MAD (80-120 mg/kg). Despite the high percentage of inhibition of tumor growth, copper sulfate did not give rise to any significant increase in the life span of the animals in a single case.

Antidotal Properties. Copper sulfate, injected subcutaneously or given perorally 24 h beforehand, reduced the toxicity of cisplatin (Table 2). Simultaneous administration of these compounds did not reduce the toxicity of cisplatin. In view of these results, the subsequent investigation was carried out with peroral administration of copper sulfate 24 h before injection of cisplatin.

Cisplatin reduced the peripheral blood leukocyte count of the rats by half compared with the control animals on the 3rd and 5th days of the experiment. Combined administration of copper sulfate and cisplatin did not induce this decrease in the blood leukocyte count. The peripheral blood erythrocyte count of the rats was unchanged after adminstration of a combination of cisplatin with copper sulfate. The change in body weight of the mice after receiving cisplatin preceded by copper sulfate did not differ from the change in this parameter in animals receiving the platinum compound alone. This fact is an indirect indication that copper sulfate does not reduce the toxic effect of cisplatin on the gastrointestinal tract.

The nephrotoxic action of cisplatin is known to reach a maximum on the 5th-6th day after injection of toxic doses of the compound, and to be reflected in an increase in the urea and creatinine concentrations in the blood serum and the appearance of protein in the rats' urine [2]. After preliminary administration of copper sulfate, disturbances of function of the urinary system were not observed after administration of cisplatin.

The study of the antitumor activity showed that preliminary administration of copper sulfate does not affect specific activity of cisplatin.

In the treatment of a developed Lewis tumor by cisplatin alone and in combination with copper sulfate, inhibition of tumor growth compared with the control on the 22nd day amounted to 40 and 45%.

These data on the antitumor activity of copper sulfate and its ability to reduce the nephrotoxicity and hematotoxicity of cisplatin provide a basis for the recommendation of this compound for clinical trials.

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