SOME CHARACTERISTICS OF THE MECHANISM OF ACTION OF COPPER (II) COMPLEXES WITH α-AMINO ACIDS

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In 1979 antitumor activity of copper (II) glycinato-L-serinate (Cu-1) was discovered. The character of the chemical structure and spectrum of antitumor action, and also the effectiveness of combined administration of Cu-1 and sarcolysin [3] suggested that this complex has a different mechanism of action from that of alkylating agents.

The aim of this investigation was to study the effect of Cu-1 on DNA synthesis in the tumor cell compared with the action of sarcolysin and of cis-diamminedichloroplatinum (II — DDP). The ability of Cu-1 and its structural analog Cu-2 to inhibit the superoxide radical O_2^-, its so-called superoxide dismutase-like (SODL) activity, also was investigated. The SODL effect was studied because, in several publications, great importance is attached to the role of the $O_2^- \rightleftarrows$ superoxide dismutase (SOD) system in carcinogenesis and it is suggested that the antitumor and SODL activities of certain antitumor agents are interconnected [1, 6]. This hypothesis is based on the fact that SOD activity, specifically inhibiting O_2^-, is reduced in the tumor cell. Restoration of this activity in various ways and, in particular, by injecting into the cell substances performing the function of SOD, may be one way of achieving an antitumor effect [1, 6, 7]. On the basis of the supposed difference in the mechanisms of action of copper (II) complexes with α-amino acids and of alkylating agents, the antitumor activity of Cu-2 when given in conjunction with DDP, was studied.

EXPERIMENTAL METHOD

The effect on DNA synthesis in tumor cells was studied on a model of ascites hepatoma 22a in C_3HA mice. The degree of the effect of Cu-1 on DNA synthesis was compared with that of DDP and sarcolysin. The preparations for testing were dissolved in physiological saline and injected into mice intraperitioneally in a single dose after intraperitoneal inoculation of the tumor on the 6th day, in doses adequate as regards toxicity: Cu-1 20 mg/kg, DDP 6 mg/kg, and sarcolysin 7 mg/kg. The mice were killed, 3, 6, 9, and 24 h after injection of the preparations. The DNA precursor [^{14}C]thymidine (specific activity 5×10^7 cpm) was injected into the mice 30 min before sacrifice. After sacrifice ascites fluid was collected from the mice. Isolation and treatment of the tumor cells and nucleic acid fractionation were carried out as described previously [2]. Specific activity of DNA was expressed in cpm/mg. The effectiveness of the preparations was judged from inhibition of incorporation of ^{14}C thymidine into DNA in the course of 24 h. The SODL activity of Cu-1 and Cu-2 was studied in a test system by the method in [5]. The principle of the method is based on the ability of certain substances, like SOD, to bind O_2^- specifically. The formation of O_2^- in this system was initated by the addition of xanthine oxidase to a cuvette containing 3 ml of substrate-buffer solution at pH 10.2, containing 0.05 M calcium carbonate, 10^{-4} M xanthine, and 2.5×10^{-5} M Nitro-blue tetrazolium (nitro-BT). O_2^- formation was judged by the increase in optical density of the sample as a result of reduction of nitro-BT. The reaction kinetics was followed by means of a Cary 219 recording Φ spectrophotometer (Varian, USA) at a wavelength of 560 nm and a temperature of 25°C. To determine SODL activity the substances for testing were dissolved in deionized water and added to the cuvette in different concentrations. The SODL

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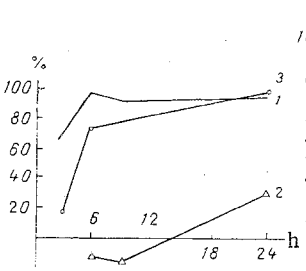

Fig. 1

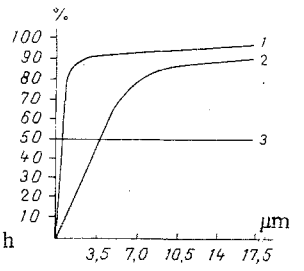

Fig. 2

Fig. 1. Inhibition of DNA synthesis (in %) in ascites hepatoma 22a cells in C_3HA mice by 6 mg/kg DDP (1), 20 mg/kg Cu-1 (2), and 7 mg/kg sarcolysin (3).

Fig. 2. Inhibition of reaction of reduction (in %) of nitro-BT by Cu-1 at pH 10.2 (1) and 7.4 (2). 3) Line for computation of K. Curves for Cu-2 and Cu-1 are analogous. 1 mg/ml = 3.5 μM.

TABLE 1. Antitumor and Toxic Effect of Combined Administration of Cu-2 and DDP in CBA Mice with Transplantable Carcinoma of the Cervix Uteri

Preparation	Dose, mg/kg	Inhibition of tumor growth, % day after treatment			Mean weight of spleen, mg	Mortality, %
		7-th	16-th	29-th		
Control	—	—	—	—	186(212—160)	0
Cu-2	9	50	44	15	180(208—152)	0
DDP	4	48	42	19	160(203—117)	0
Cu-2	18	100	85	35	120(189—51)	17*
DDP	8	72	91	46	116(148—54)	17*
Cu-2 + DDP	9+4 (simultaneously)	10	81	50	260(508—202)	0
Cu-2 + DDP	9+4 (after 6 h)	98	73	13	140(182—98)	33*
DDP + Cu-2	4+9 (after 6 h)	98	76	52	180(233—127)	0

Legend. *$P \leqslant 0.05$.

effect also was determined at pH 7.4, corresponding to the pH of the liquid media of the body. The SODL activity of the preparations was judged from the value of the inhibition constant (K_i), namely the concentration (in micromoles) causing 50% inhibition of the reaction. The combined action of Cu-2 and DDP was studied on male CBA with transplantable carcinoma of the cervix uteri RShM-5, inoculated subcutaneously into the flank. Treatment began 48 h after inoculation of the tumor. Doses of the substances appropriate as regards toxicity were used: Cu-2 9 mg/kg, DPP 4 mg/kg. The preparations were dissolved in physiological saline and injected intraperitoneally as a single dose into the animals of the experimental groups. The control group consisted of untreated mice inoculated with the tumor. The number of mice in the experimental groups was eight and in the control group ten. The antitumor effect was assessed as inhibition of tumor growth, calculated relative to the mean volumes of the tumor in the experimental animals compared with the controls over a period of time. Toxicity was recorded as the percentage of mice which died and changes in the mean weight of the spleen (in milligrams compared with the control). The antitumor effect and toxicity were compared with these effects in groups of mice treated with double doses of the preparations compared with those used in the combination. Statistical significance of the results was calculated by the Fisher—Student t test, using Tippet's coefficient [4] at $P \leqslant 0.05$.

EXPERIMENTAL RESULTS

Cu-1 inhibited DNA synthesis by 30% 24 h after injection, whereas DDP and sarcolysin inhibited DNA synthesis by more than 50-80% after only 3 h, and this effect lasted for 1 day (Fig. 1). The results are evidence of differences in the mechanism of action of the copper complex and known alkylating agents DDP and sarcolysin on DNA synthesis. Parallel with absence of direct inhibition of DNA synthesis in the compounds Cu-1 and Cu-2, high SODL activity also was found (Fig. 2); both compounds, moreover, exhibited the SODL effect in the same

range of concentrations: K_i at pH 10.2 was 0.18 μM (0.05 mg/liter), and at pH 7.4 it was 3.7 μM (1.1 mg/liter), as a result of which the curves in Fig. 2 show the effects of the two preparations at different pH values. With an increase in concentration in both cases almost total inhibition of the reaction was obtained: at 7.4 in concentrations of 5 to 20 mg/liter, corresponding to a range of therapeutic doses of these compounds of 5-20 mg/liter. The results suggest that the SODL effect of these compounds can also be realized *in vivo*. Results obtained by combined administration of Cu-2 and DDP are given in Table 1. In the groups of animals receiving the compounds separately, in doses used in the combination, borderline inhibition of tumor growth (50-48%) was observed on the 7th day after injection of the preparations. No toxic manifestations were observed in this case. Separate administration of the two compounds in twice the above doses gave considerable potentiation of the antitumor effect (100% inhibition of tumor growth) at this time of observation, but the duration of the effect was found to be only 16 days after treatment. Meanwhile, death of 17% of mice was observed in these groups with an increase in weight of the spleen in the case of DDP administration by 45% compared with the control. In animals receiving the combination therapy, the antitumor effect was enhanced by summation on the 7th day (98-100%). Furthermore, in the mice receiving Cu-2 simultaneously with or 6 h after DDP inhibition of tumor growth remained at a significant level (over 50%) until the 29th day after treatment. In the case of simultaneous administration of the compounds, an increase in weight of the spleen was observed compared with the control and with its value in animals receiving double doses of the compounds. No mice died in this group. The investigations thus showed that Cu-1 does not induce direct inhibition of DNA synthesis in the tumor cell, unlike DDP and sarcolysin. Cu-1 and its structural analog Cu-2 posses high SODL activity and exhibit it under conditions close to those pertaining *in vivo*, in concentrations corresponding to doses giving an antitumor effect. Combined administration of Cu-2 and DDP leads to summation of their antitumor effect without any increase in toxicity.

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