INTERACTION OF THE COPPER COMPLEX Cu-2 WITH LIVER MONOOXYGENASES

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Complex compounds of copper with amino acids constitute a large group of compounds, some of which have antitumor activity. In particular, it has been shown that the preparation Cu-2 [6], which is currently undergoing preclinical study, has marked activity against several experimental tumors.

Superoxide dismutase activity of Cu-2 [6] led to the suggestion that this complex intervenes in processes of microsomal oxidation by liver monooxygenases, for we know that other low-molecular-weight analogs of superoxide dismutase (SOD) have a marked inhibitory action on this enzyme system. In the opinion of some workers, the reason for this is their superoxide dismutase activity [10, 12], for superoxide radicals play an important role in microsomal hydroxylation reactions [4, 7, 9, 11]. Meanwhile the inhibitory effect of chelate complexes of copper on microsomal metabolism has been attributed to their inactivating action on components of the electron transfer chain and, in particular, on cytochrome P-450.

With these data in mind, it was decided to study interaction of Cu-2 with liver monooxygenases in experiments in vivo and in vitro.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice aged 2-3 months. Cu-2 (Institute of Chemistry, Academy of Sciences of the Moldavian SSR), in doses of 5, 12, and 20 mg/kg, was injected once, intraperitoneally. The effect of Cu-2 on liver monooxygenase activity was determined in the experiments in vivo by recording the duration of sleep of the animals after intraperitoneal injection of hexobarbital in a dose of 60 mg/kg. The microsomal fraction of the mouse liver was isolated and its cytochrome P-450 concentration determined as described

TABLE 1. Effect of Complex Cu-2 on Duration of Hexobarbital Sleep (M ± m, n = 10)

Dose of Cu-2, mg/kg	Time after injection of Cu-2, days	Duration of sleep, min		Experiment/control	p
		experiment	control		
5	1	24,5±1,3	15,7±2,5	1,5	<0,05
	2	37,2±3,8	24,5±1,9	1,5	<0,02
12	1	33,0±1,4	15,7±2,5	2,1	<0,01
	2	56,0±1,6	24,5±1,9	2,3	<0,001
5	1	23,8±0,8	11,8±2,7	2,0	<0,002
	3	10,3±0,8	12,3±1,9	0,8	>0,05
12	1	29,8±3,1	11,8±2,7	2,5	<0,01
	3	15,0±1,7	12,3±1,9	1,2	>0,05
20	1	43,6±11,8	11,8±2,7	3,7	<0,002
12	1	41,4±1,7	21,8±2,9	1,9	<0,001
	2	23,0±1,1	16,6±3,7	1,4	<0,05
	3	20,8±1,2	18,9±3,1	1,1	>0,05
12	1	42,6±2,1	23,0±1,5	1,9	<0,002
	2	32,8±1,1	15,5±1,4	2,1	<0,001
	3	16,3±0,9	17,9±1,5	0,9	>0,1

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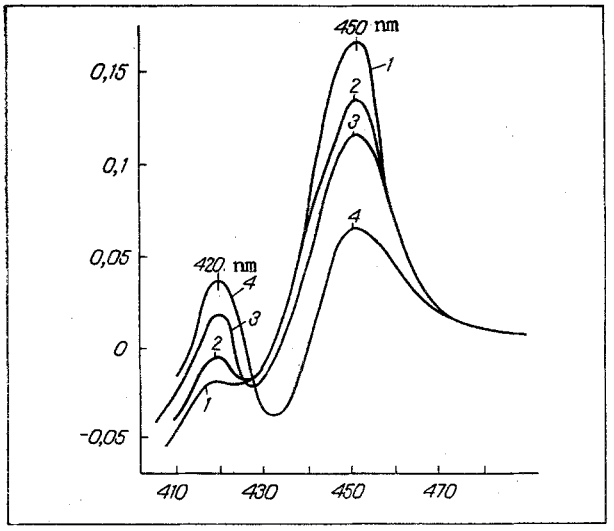

Fig. 1. Effect of Cu-2 on differential absorption spectrum of cytochrome P-450 in mouse liver microsomes. Abscissa, wavelength (in nm); ordinate, difference of optical density (in relative units). 1) Differential absorption spectrum of cytochrome P-450; 2-4) differential absorption spectrum of cytochrome P-450 after incubation of microsomes with different Cu-2 concentrations (8, 16, and 48 mM respectively).

previously [2]. The microsomes were incubated with 8, 16, 32, and 48 mM Cu-2 for 60 min at 4°C. Binding of the compound with cytochrome P-450 was recorded on a Hitachi-556 dual-beam spectrophotometer, using a four-cuvette scheme [8].

EXPERIMENTAL RESULTS

On the 1st-2nd days after injection of 5 mg/kg of Cu-2 the duration of sleep was increased by 1.5-2 times, and after injection of 12 mg/kg of Cu-2 it was lengthened by 2-2.5 times compared with intact animals. The duration of sleep of animals of the control and experimental groups 72 h after injection of Cu-2 in the above-mentioned doses was virtually identical (Table 1). The duration of sleep of mice of the experimental group was lengthened by 3.7 times compared with the control 24 h after injection of Cu-2 in a toxic dose (20 mg/kg), causing death of 100% of the animals 72 h after injection.

To discover the possible mechanism of the inhibitory action of Cu-2 on the liver monooxygenases, the effect of the complex on cytochrome P-450 was studied in experiments in vitro. On incubation of microsomes with Cu-2, a decrease in the cytochrome P-450 content and the appearance of an enzymically inactive form of the hemoprotein, namely cytochrome P-420, were observed; the quantity of cytochrome P-420 was increased with an increase in the dose of the complex (Fig. 1). This is evidence of inactivation of cytochrome P-450 in the presence of the complex and it explains its inhibitory action on this enzyme system. On the addition of Cu-2 to the intact microsomes, the formation of a complex of Cu-2 with cytochrome P-450 could not be recorded on the basis of the appearance of a differential spectrum. Considering the data given above on inactivation of cytochrome P-450 in the presence of Cu-2, similar experiments were carried out with the microsomal fraction, stabilized with a 20% solution of glycerol. Under these conditions spectral changes of type I were recorded: the appearance of a differential spectrum with maximum of absorption at 383 nm and with a minimum at 418 nm (Fig. 2a). The spectral binding constant of Cu-2 with cytochrome P-450 was 330 μM (Fig. 2b).

The results show that the complex Cu-2, which is a low-molecular-weight analog of SOD, in near-therapeutic doses causes inhibition of liver monooxygenases, one cause of which is inactivation of cytochrome P-450. Meanwhile, in stabilized microsomes Cu-2 is bound with cytochrome P-450 as a typical type I substrate, indicating that it may undergo metabolism with the aid of this enzyme. Thus the character of interaction of Cu-2 with liver monooxygenases in vivo may depend on the concentration of the preparation. With high concentrations, Cu-2 can inhibit this enzyme system, but in low concentrations, which do not inactivate cytochrome P-450, it may act as its substrate. For this reason, in our opinion, when this compound is handed over for clinical trials, among the other characteristics of Cu-2 its inactivating

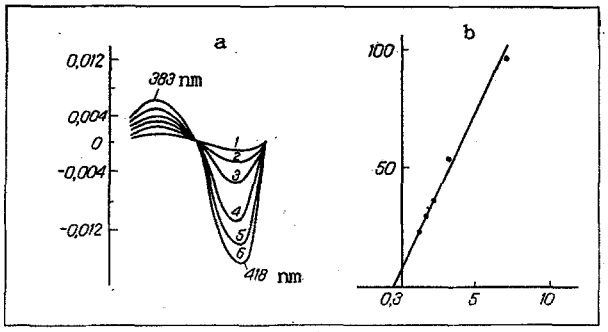

Fig. 2. Differential spectra of microsomes (a) in the presence of Cu-2 and determination of the spectral binding constant (K_b = 330 μM) of Cu-2 with cytochrome P-450 (b). a: 1-6) 8, 16, 32, 48, 56, and 80 μM Cu-2, respectively. Remainder of legend as to Fig. 1; b: abscissa, reciprocal of Cu-2 concentration (in $M \times 10^{-4}$); ordinate, reciprocal of difference in absorption at 383 and 418 nm.

effect on cytochrome P-450 must be noted. This fact should be taken into account during comparison of schemes and schedules of polychemotherapy, including preparations metabolized by monooxygenases. For example, it was shown previously that during inhibition of this enzyme system, the therapeutic action of adriamycin [3], cyclophosphamide, and other therapeutic agents is reduced [1]. It is also desirable to study changes in the toxicity and therapeutic action of Cu-2 in the presence of inhibition of liver monooxygenases, for preparations metabolized by monooxygenases, but at the same time, inhibiting this enzyme system, if administered frequently may modify not only the action of other drugs, but also the manifestation of their own biological activity.

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