Studies on the Acute Toxicity of the Antineoplastic Metal Chelate *trans*-Bis(salicylaldoximato)copper(II) in Rats

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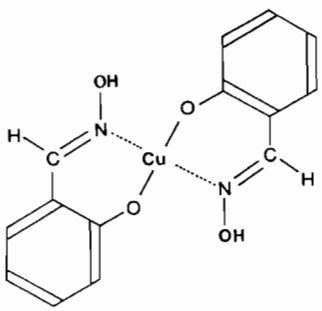
Abstract

The acute toxicity of the recently discovered antiproliferative and antineoplastic agent *trans*-bis(salicylaldoximato)copper(II) ($CuSAO_2$) has been studied in Wistar rats. When given perorally as a suspension, the compound had no acute lethal toxicity even at the dose of 5000 mg per kg body weight. The peroral LD_{50} value (in rats) of the corresponding free ligand, salicylaldoxime, is known to be only 400 mg/kg. The decreased toxicity of $CuSAO_2$, as compared to the free ligand, may at least in part be due to its lack of absorption. $CuSAO_2$ was found to be absorbed poorly, if at all, when given perorally. When $CuSAO_2$ was given intraperitoneally to Wistar rats, its absorption was found to be incomplete, and therefore no attempt was made to determine its intraperitoneal LD_{50} value.

Introduction

We have recently found a novel group of powerful antiproliferative agents, the parent compound of which is *trans*-bis(salicylaldoximato)copper(II) ($CuSAO_2$; I) [1]. Further, we have shown that this compound has significant, and in many cases even curative, antitumor activity *in vivo* against Ehrlich ascites carcinoma [2]. Because of these promising results, further studies on the compound and its derivatives are warranted. Data on the toxicity of $CuSAO_2$ and its derivatives are needed for further antitumor tests, and they will also be necessary if clinical trials on the compounds are to be initiated. Toxicological studies may also be of value in the elucidation of the mechanism of action of the compounds. Therefore, we have now studied the acute toxicity of $CuSAO_2$ in rats.



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Experimental

Materials and Methods

$CuSAO_2$ was prepared as previously described [3]. Methyl cellulose ('Tylose', purum MH 1000, medium viscosity) was obtained from Fluka AG, Buchs, Switzerland, and dimethyl sulfoxide (scintillation grade) from E. Merck, Darmstadt, Federal Republic of Germany.

Specific-pathogen free Wistar/Han rats were originally obtained from ZFV, Hannover, Federal Republic of Germany, and were maintained in half-barrier facilities at the Animal Center of the Faculty of Science of the University of Helsinki. At the beginning of the experiments, the animals were *ca.* 6–8 weeks old. The females used for p.o. experiments weighed 123 ± 29 g, and those used for i.p. experiments 105 ± 24 g. The males used for p.o. experiments weighed 171 ± 64 g, and those used for i.p. experiments 161 ± 50 g. After treatment, the animals were housed in a cubicle with a fotoperiod LL:DD 12:12 (darkness 20-08). The ambient temperature was 21 ± 2 °C and the relative humidity was 50%. The animals were given a standard laboratory diet

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(Ewos R3, sterilized by autoclaving) and water (filter sterilized) *ad libitum*. Before oral administration of drugs, the animals were fasted for 24 h. The animals were kept in Macrolon cages (size MIII), three in each cage. $CuSAO_2$ was administered between about 11.00 and 12.00 hours, by gastric intubation with a curved feeding needle in the case of oral doses.

$CuSAO_2$ is practically insoluble in water and most pharmacologically acceptable solvents. Thus, suspensions were used throughout the study. For p.o. administration, $CuSAO_2$ was suspended either in an aqueous solution containing 0.5% (*w/v*) of methyl cellulose (doses 40–850 mg/kg; injection volume 1.0 ml per animal) or in diluted dimethyl sulfoxide in 5% aqueous $NaHCO_3$ [4] (doses 700–5000 mg/kg; injection volume varied according to dose, being about 2–10 ml per animal). For i.p. administration, a suspension of $CuSAO_2$ was prepared by suspending 1000 mg of $CuSAO_2$ in 5 ml of ethanol and diluting the mixture with distilled water to the volume of 50 ml. In each test, a single dose was given. A clinical examination following the recommendations given in reference [4] was performed daily during the 14-day test period following the administration of $CuSAO_2$.

Results and Discussion

Peroral Administration

In a preliminary experiment, 14 different dose levels, ranging from 40 to 850 mg/kg, were tested. Each dose was given p.o. to one female and one male Wistar rat. All of the animals survived to the end of the experiment. Many animals had constipation, usually *ca.* 7–10 days after the administration of $CuSAO_2$. Four animals temporarily had mucous faeces. Tachypnea and cardiovascular signs (tachycardia, arrhythmia) as well as cyanosis of the tail were also seen in some cases, but the significance of these symptoms is questionable, since they were not dose-dependent.

Another experiment was also performed, in which eight different doses ranging from 700 to 5000 mg/kg were given to a total of 21 rats. The highest dose was given to a total of six rats (three males and three females). All of the animals survived to the end of the experiment. A visual inspection of the faeces revealed that at the highest doses probably most, if not all, of the $CuSAO_2$ administered was contained in the faeces as green deposits.

The above results clearly indicate that $CuSAO_2$ has no acute lethal toxicity in Wistar rats even at the dose of 5000 mg/kg when given p.o. as a suspension. DeWitt *et al.* have shown that the p.o. LD_{50} value of the free ligand of $CuSAO_2$, *i.e.* salicylaldoxime, is 400 mg/kg [5]. Thus, when given p.o., the free ligand is much more toxic than the copper(II) chelate. The above results also indicate that the absorption of $CuSAO_2$ after p.o. administration is far from complete. Possibly, $CuSAO_2$ is not absorbed at all. The reason for this may well be the very low solubility of $CuSAO_2$ in water. The poor absorption is probably one of the main reasons for the decreased p.o. toxicity of $CuSAO_2$, as compared to the free ligand.

Intraperitoneal Administration

A preliminary i.p. toxicity test was also performed, in which 11 different dose levels, ranging from 40 to 400 mg/kg, were tested. Each dose was given i.p. to one female and one male rat. With the exception of two males (doses 300 mg/kg and 350 mg/kg) that died within 48 h, all animals survived to the end of the experiment. Constipation (often *ca.* 7–11 days after the administration of $CuSAO_2$) was common, and at the highest doses, the faeces were mucous. Cardiovascular signs, tachypnea and cyanosis of the tip of the tail were again encountered. At the highest doses, hair loss and apathy were also seen. Necropsies of the two rats that were found dead revealed that the absorption of $CuSAO_2$ had not been complete. Instead, there were deposits of it on the peritonea. The peritoneal cavities of both rats contained several millilitres of fluid, the lungs were bleak and spotty, and the pancreas of one of the animals was dark brown. (In another study, we have found that after i.p. administration of the 4-hydroxy derivative of $CuSAO_2$ to rats, the pancreas or tissue close to it almost invariably becomes dark brown, obviously because of the accumulation of the compound or its dissociation products [6].) Both animals had lost weight (10 and 12 g) before death, and both had hemorrhage under the nails.

Because of the inadequate absorption of $CuSAO_2$, a more detailed i.p. toxicity study was not considered meaningful and no attempt was made to determine the LD_{50} value of the compound. Although no LD_{50} values are available, a comparison of the present results with those obtained in our previous studies [2, 7] indicates that, when given i.p., $CuSAO_2$ is far less toxic in Wistar rats than in NMRI mice.

Because of the solubility problems and the questionable absorption associated with the use of $CuSAO_2$, we have performed an i.p. toxicity study on the 4-hydroxy derivative of $CuSAO_2$, namely *trans*-bis(2,4-dihydroxybenzaldoximato)copper(II), which has better solubility characteristics than the parent compound. In that study, we have also used the methods of histology and clinical chemistry. The results will be reported elsewhere [6].

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