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Chronotoxicity of a Copper (II) Complex with a Linear Tetradentate Ligand in Mice

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Abstract

In vitro copper (II) complex presents antimetabolic effects. In this work, we have studied the *in vivo* seasonal toxic effects of copper (II), ligand (H₂L) and the complex [Cu(H₂L)(H₂O)₂]Cl₂·4H₂O in male Swiss mice. During spring, an i.p. injection of CuCl₂ in aqueous NaCl (9 g·l⁻¹) up to 0.05 μmol·kg⁻¹ b.w. (body weight) killed 60% of the rodents after 6 days. LD100 was up to 0.3 μmol·kg⁻¹; H₂L was well tolerated, while the complex was 30% lethal with 50 μmol·kg⁻¹. In autumn, mice were less sensitive to CuCl₂, and both ligand and complex were equally tolerated and this leads to the conclusion that, *in vivo*, chronotoxicities of copper (II) and complex in NaCl aqueous solutions are quite different in spring and autumn seasons.

Keywords: Swiss mice, copper (II), complex, chronotoxicity.

Introduction

Generally, *in vivo* effects studies do not take into account rhythmic changes in internal biologic susceptibility to drugs or chemical molecules, as well as the ethology of experimental animals. Mice and rats are rodents that are active in spring and summer and rest in autumn and winter. Many research workers such as Reinberg et al. (1991), Cambar et al. (1994), Touitou et al. (1994) or Bruguerolle (1994) related experiments on rodents and human beings on the subject of the moment of administration of drugs and their toxic effects. Cambar et al. (1994) showed that the same dose of mercuric chloride in intraperitoneal injection (i.p.) induced a different mortality rate when given in November and in February. In rats, a vitamin D₃ metabolite was more active during night than day on renal enzymatic markers, which presented annual variations

(Do et al., 1994, 1995). Effects of cobalt(II), 1,25-dihydroxy vitamin D₃ and their complex were quite different in spring and autumn (Do et al., 1996).

Our laboratory focused on studying chemical and/or physiological effects of transition metals, various organic ligands and of their mutual complexes. It has been reported that copper (II) is an anti-inflammatory metal and has some effects in carcinogenesis (Hall et al., 1996).

In our continuous investigations about the finding of new antimitotic metal complexes, we have synthesised a series of complexes of copper (II) and various polydentate ligands. Preliminary *in vitro* studies revealed that these compounds presented antimitotic effects towards cultured colon adenoma cells (not shown). It is therefore interesting to study their toxic effects in mice, in spring and autumn before further *in vivo* experiments, as knowledge of these administration times can coincide with best tolerance or effectiveness. We have chosen to perform our experiments during spring and autumn because these seasons correspond to important hormonal variations while winter and summer show hormonal stability. These data are useful in order to optimise both timing and dosage of any anti-mitotic molecule.

Material and Methods

Preparation of the compounds

Cupric chloride CuCl₂·2H₂O (Fluka, p.a.) was dissolved in aqueous 9 g·l⁻¹ sodium chloride (Fluka, p.a.). The ligand H₂L (N,N'-bis(2-hydroxyacetophenyl)-1,2-diaminoethane), presented in Figure 1 and the complex [Cu(H₂L)(H₂O)₂]Cl₂·4H₂O were prepared and purified as previously described (Djebbar-Sid et al., 1997). They were dissolved in aqueous sodium chloride.

Animals

Male animals used were Swiss mice from Debré (Saint Doulchard, France) weighing 18 to 25 g. They were chosen because of their regular weight gain and the absence of oestral cycle with changes in sexual hormonal status which could lead to errors in *in vivo* experiments. Animals had free access to both water and standard diet containing 0.8% Ca, 0.7% P and 3 IU·g⁻¹ vitamin D. They were placed at room temperature 20 ± 2°C and exposed to light/dark cycle 12 h/12 h (12L/12D) alternatively. The daily

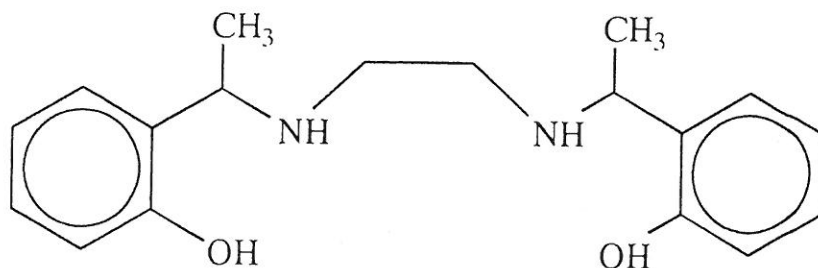


Figure 1. Chemical structure of the ligand.

cycle was inverted and the animals were kept in light during the night and in darkness during the day, allowing us to observe their active phase from 8:00 a.m. to 8:00 p.m.

Experiments with copper(II)

In addition to the control series, seven series of ten mice were used and each animal received a single i.p. injection (200 $\mu\text{L}/20\text{ g b.w.}$) between 10:00 and 12:00 a.m. On the 29th of April, rodents were treated as follows:

- Control series: animals received the vehicle, aqueous $9\text{ g}\cdot\text{l}^{-1}$ NaCl
- Series 1: $0.025\text{ }\mu\text{mol}\cdot\text{kg}^{-1}$
- Series 2: $0.050\text{ }\mu\text{mol}\cdot\text{kg}^{-1}$
- Series 3: $0.100\text{ }\mu\text{mol}\cdot\text{kg}^{-1}$
- Series 4: $0.150\text{ }\mu\text{mol}\cdot\text{kg}^{-1}$
- Series 5: $0.200\text{ }\mu\text{mol}\cdot\text{kg}^{-1}$
- Series 6: $0.300\text{ }\mu\text{mol}\cdot\text{kg}^{-1}$
- Series 7: $0.400\text{ }\mu\text{mol}\cdot\text{kg}^{-1}$

All animals were placed again at room temperature and 12L/12D during 7 days. The same protocol was used in autumn (29th of November).

Experiments with the ligand

Three series of ten mice, in addition to the control series, were used and each animal received a single i.p. injection (200 $\mu\text{L}/20\text{ g b.w.}$) between 10:00 and 12:00 a.m. On the 29th of April, rodents were treated as follows:

- Control series: animals received aqueous $9\text{ g}\cdot\text{l}^{-1}$ NaCl
- Series 1: $1\text{ }\mu\text{mol}\cdot\text{kg}^{-1}$
- Series 2: $25\text{ }\mu\text{mol}\cdot\text{kg}^{-1}$
- Series 3: $50\text{ }\mu\text{mol}\cdot\text{kg}^{-1}$

The same protocol was used in autumn (29th of November).

Experiments with complex

Three series of ten mice and the control series were used and each animal received a single i.p. injection (200 $\mu\text{L}/20\text{ g b.w.}$) between 10:00 and 12:00 a.m. On the 29th of April, rodents were treated as follows:

- Control series: animals received the vehicle
- Series 1: $1\text{ }\mu\text{mol}\cdot\text{kg}^{-1}$
- Series 2: $25\text{ }\mu\text{mol}\cdot\text{kg}^{-1}$
- Series 3: $50\text{ }\mu\text{mol}\cdot\text{kg}^{-1}$

All animals were placed again at room temperature and 12L/12D during 7 days. The same protocol was used in autumn (29th of November).

Results

In spring, NaCl and copper (II) chloride at $0.025 \mu\text{mol}\cdot\text{kg}^{-1}$ (b.w.) did not harm mice under study as shown in Table 1. But $0.050 \mu\text{mol}\cdot\text{kg}^{-1}$ (b.w.) killed 20% six days after the injection, then about $0.100 \mu\text{mol}\cdot\text{kg}^{-1}$ (b.w.) was a lethal dose 60% (LD 60). A dose up to $0.300 \mu\text{mol}\cdot\text{kg}^{-1}$ (b.w.) was lethal after 24h (LD 100). According to our experiments, LD 50 was found to be between 0.050 and $0.100 \mu\text{mol}$.

In autumn, the cupric solution was less effective. Lethal dose LD 100 was up to $0.400 \mu\text{mol}$. These results proved that CuCl_2 was toxic to mice, more toxic in spring-time than during autumn (see Tables 1 and 2). The ligand and its complex were well tolerated by rodents with respect to our experimental conditions. But, in spring, the complex killed 30% of animals. Our experiments indicated once more, that CuCl_2 loses or modifies its toxicity when it is complexed by the present ligand or other molecules (Jamali et al., 1998).

Discussion

This work shows that in Swiss mice, tolerances of aqueous CuCl_2 were quantitatively different in April as compared to November for rodents kept under constant conditions. In Debré Laboratories, rodents are kept under artificial light and dark (12L/12D) at $20 \pm 2^\circ\text{C}$, so we maintained our animals under the same conditions, as indicated above in experimental section.

To Cambar et al. (1994), Reinberg et al. (1991), the experiments under constant parameters (e.g., light, temperature) with laboratory animals during many seasons

Table 1. Toxic effects of CuCl_2 in aqueous $\text{NaCl } 9\text{g}\cdot\text{l}^{-1}$ in spring.

Doses ($\mu\text{mol}\cdot\text{kg}^{-1}$ b.w.)	Living animals after injection (days)						
	1	2	3	4	5	6	7
Control	10	10	10	10	10	10	10
0.025	10	10	10	10	10	10	10
0.050	10	10	10	10	10	8	8
0.100	8	5	5	5	5	4	4
0.150	5	4	4	4	4	2	2
0.200	3	3	3	2	2	1	1
0.300	0	0	0	0	0	0	0
0.400	0	0	0	0	0	0	0

Table 2. Toxic effects of the complex in aqueous NaCl 9 g·l⁻¹ in spring and autumn.

Doses ($\mu\text{mol}\cdot\text{kg}^{-1}$ b.w.)	Living animals after injection (day)						
	1	2	3	4	5	6	7
Control (spring)	10	10	10	10	10	10	10
1 (spring)	10	10	10	10	10	10	10
25 (spring)	10	10	10	10	10	10	10
50 (spring)	10	10	10	8	7	7	7
Control (autumn)	10	10	10	10	10	10	10
1 (autumn)	10	10	10	10	10	10	10
25 (autumn)	10	10	10	10	10	10	10
50 (autumn)	10	10	10	10	10	10	10

showed that their seasonal rhythms were not altered. The discovery of *per* gene enhanced the hypothesis that most biologic rhythms are genetically programmed. Pevet (2000) showed that these seasonal rhythms are regulated by a hormone (melatonin) secreted by pineal gland in mammals by the way of membrane receptors connected to a G-protein.

Few papers showed seasonal chrono-effectiveness of drugs. This may be due to the size and the cost of such experiments (Cambar et al., 1994). Our toxic effect studies, dealing with periodic changes of activity of cupric chloride and the complex could be correlated with previous works. According to Levi (1987) tolerance of adriamycine in rodents was maximal in autumn and less in spring. Brugerolle et al. (1988) demonstrated that the acute toxicity of phenobarbital in mice varies during the year: the compound is more toxic in January and it is well supported in July, like complex, which is more toxic in spring than in autumn.

We can explain the chronesthesia of CuCl_2 and the studied complex on the basis of biovariability. All living beings present biovariability (Cambar et al., 1987) regulated by melatonin (Pevet, 2000), so they could react differently to the same drugs when injected during various seasons in the same year. This has been explicitly shown in the cases of other molecules while observing the variations of their kidneys and brain tolerances (Levi, 1987; Cambar et al., 1987; Dorian et al., 1987; Do et al., 1996).

For further experiments, we will have to take into account the importance of seasonal changes, as well as the circadian variations of tolerances toward toxic molecules. We might hypothesise that circadian acrophases and bathyphases of the structural and functional parameters of rodents responsible for the susceptibility resistance cycle change along the year. This is a result of an endogenous circumannual rhythm along a twelve-month period based on melatonin influence.

Further studies are now in progress to test copper (II) chloride, free ligand and the complex in mice presenting induced colorectal cancer the effects. The above results show that their effects should be followed during different seasons of the year in order to determine both time for lower toxicity and time for higher therapeutically activity.

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