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The Action of L-Cysteine in Acute Cobalt Chloride Intoxication

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A study in rats was made of the effects produced by L-cysteine on the acute toxicity of cobalt chloride given orally and intraperitoneally. The decrease in lethality was absolute for the different doses tested, except when the $CoCl_2$ was given orally and L-cysteine intraperitoneally in which only 40 % efficiency was obtained. No specially significant changes were observed in the blood parameters of the animals treated with the $CoCl_2$-cysteine complex after one week.

Significant differences were noted between serum parameters: glucose, triglycerides and cholesterol, measured in rats after twelve hours of receiving the $CoCl_2$-cysteine complex, compared with the same parameters measured when the $CoCl_2$ was given without complex.

Key words: Cobalt, Acute toxicity, Rat, L-Cysteine action.

Cobalt is a metal with numerous industrial uses and is also indispensable as an oligoelement, forming part of the vitamin B_{12}. However, it is able to cause considerable toxic action.

One of the most noticeable toxic effect produced by cobalt is in the heart (1, 2). There are several mechanisms which are able to cause a cardiotoxic effect. Cobalt reacts specifically with SH groups of dihydrolipoic acid, impeding the conversion of pyruvate into acetyl-CoA and α-cetoglutarate to succinyl-CoA in the tricarboxylic acid cycle (3, 12, 13).

Thus, it appears that the biological activity of cobalt is related to its capacity to form complexes with enzymes through its SH groups or also N-histidine (8, 10).

In previous studies, the acute toxicity some cobalt salts in rats by measuring their LD_{50} was determined (7); also the effects on lethality and some analytical parameters produced by EDTA, a chelate described as a cobalt antidote (5).

In this paper we study the effects which cysteine, a sulphured aminoacid abounding in mammalian organisms, can produce on acute cobalt toxicity when given by two means, orally and intraperitoneally, which is considered interesting bearing in mind that the reaction of cobalt with the SH group of aminoacids or enzymes has been shown *in vitro* (4, 12).

Finally, acute toxicity of the $CoCl_2$-cysteine complex was studied; also the variation on some blood parameters produced by treatment with the aforementioned complex, in relation to time, during the hours immediately after intoxication. It is during this phase when the greatest irregularities were observed with regard to the animals treated only with $CoCl_2 \cdot 6H_2O$, orally or intraperitoneally (6, 7, 11).

Materials and Methods

Five groups of female rats Sprague-Dawley were used, with 10 animals in each group, their weights ranging between 150 and 180 g. In order to measure blood parameters, 4 or 5 animals were used each time.

The $CoCl_2 \cdot 6H_2O$ and L-chlorhydrate of cysteine used were analytically pure and supplied by Merck.

Each animal was given in accordance with its weight, the corresponding volume of dissolved $CoCl_2 \cdot 6H_2O$ and cysteine, either orally or intraperitoneally, adjusting the proportion to 5 ml per kilo of weight. The syringes used were precision type with a capacity of 1 ml graded in hundredths of ml. Oral doses were given intragastrically. The intraperitoneal solutions were prepared in a buffer Tris/HCl 10 mM adjusted at pH 7.4.

The $CoCl_2$-cysteine complex was prepared using molar concentrations of cobalt and cysteine in a proportion of 1:3 (9), dissolved in the previous buffer at pH = 7.4.

The control animals received equivalent volumes of distilled water orally or from the buffer Tris/HCl 10 mM, pH = 7.4, intraperitoneally.

The different experiments carried out were as follows: Oral administration of $CoCl_2 \cdot 6H_2O$ 0.46 M [corresponding to the oral LD_{50} of the salt (7)] and immediately after L-cysteine 1.38 M (1:3), also oral.

Intraperitoneal administration of $CoCl_2 \cdot 6H_2O$ 0.036 M [corresponding to the LD_{50} i.p. of the salt (7)] immediately after L-cysteine 0.108 M, also intraperitoneally.

Oral administration of $CoCl_2 \cdot 6H_2O$ 0.46 M, and i.p. of L-cysteine 0.108 M, after 15 minutes.

Oral administration of the $CoCl_2$-cysteine complex prepared *in vitro* at concentrations of 0.46 M and 1.38 M respectively.

Intraperitoneal administration of the $CoCl_2$-cysteine complex prepared *in vitro* at concentrations of 0.036 M and 0.108 M respectively.

In every case, the animals were observed for seven days receiving food and water *ad libitum* during this time.

Four animals from experiments fourth and fifth, and four control animals were killed by decapitation after seven days, and the following blood parameters determined: hematocrit, hemoglobin, glucose, urea, total proteins, cholesterol, uric acid, creatinine, ALP, GOT and GPT.

Finally, the $CoCl_2$-cysteine complex (1:3 molar) was given orally to 20 animals at concentrations 0.315 M of $CoCl_2$ and 0.945 M of cysteine, [corresponding to 3/4 LD_{50} of the oral $CoCl_2$ (7)], determining the following parameters: hematocrit, hemoglobin, total proteins, glucose, triglycerides, cholesterol and total lipids in groups of five animals at 1, 2, 3 and 12 hours after administration.

The same experiment was carried out by injecting the $CoCl_2$-cysteine complex (1:3 molar) intraperitoneally 0.027 M and 0.081 M [corresponding to 3/4 LD_{50} of the $CoCl_2$ i.p. (7)], determining the same parameters at 30 minutes, 1, 3 and 12 hours after injection. These times were selected to be able to compare with the results obtained in previous studies (7).

The blood parameters was determined: hematocrit, by the microhematocrit method, using Clay-Adams centrifugue Mod. Adams-Autocrit Centrifugue; hemoglobin measured as oxihemoglobin with a Coulter Electronic hemoglobinometer; the serum parameters were analized using classic analytical techniques, by means of Technicon multichannel analyzer Mod. SMA-12, with the exception of total lipids that were determined according to sulphophosphovanillin reaction.

The significance of the differences in the results was determined by the t Student-Fischer test.

Results

Table I contains the results from the five experiments aforementioned, showing that, with the exception of the oral administration of the $CoCl_2$ and intraperitoneal administration of cysteine in which the effective decrease in the planned lethality was 40 %, this was 100 % in all remaining cases.

In table II there are comparisons between the different blood parameters of the animals treated with the $CoCl_2$-cysteine complex oral and i.p. and the control animals.

And last, table III shows comparisons among analytical parameters measured 12 hours after giving the $CoCl_2$-cysteine complex (oral and i.p.), or after giving $CoCl_2$ freely (oral and i.p.).

Glucose was increased significantly when $CoCl_2$ was given orally, while the oral administration of $CoCl_2$-cysteine complex did not give any significant variation.

Triglycerides showed a significant increase when $CoCl_2$ was given freely; results obtained for both oral and intraperitoneal administration. By administration of intraperitoneal complex, triglycerides even decreased significantly during the same period.

Cholesterol was significantly below the control values, except when $CoCl_2$ was given orally. In this case, the results showed a significant increase.

Table I. *Effects of L-cysteine in acute $CoCl_2.6H_2O$ intoxication.*
Animals treated per group, 10. Animals survivors seven days after treatment.

Group	$CoCl_2.6H_2O$ M	L-Cysteine M	Animals Survivors
1	0.46 oral	1.38 oral	10
2	0.036 i.p.	0.108 i.p.	10
3	0.46 oral	0.108 i.p.	7
4	0.46	1.38	10
		Complex oral	
5	0.036	0.108	10
		Complex i.p.	
Control oral	0.46 (LD_{50})	—	5
Control i.p.	9.036 (LD_{50})	—	5

Table II. *Comparison among some analytical parameters in the control animals and those treated with $CoCl_2$-cysteine complex (oral and i.p.), after seven days.*
Mean values ± S.E. The $CoCl_2$-cysteine complex was prepared 1:3 molar at concentrations of $CoCl_2$ corresponding to the oral and intraperitoneal LD_{50} of the salt respectively.

Parameter	Control	$CoCl_2$-Cysteine complex oral	$CoCl_2$-Cysteine complex i.p.
Hematocrit %	31.6 ± 1.14	36.3 ± 4.03	36.5 ± 1.29**
Hemoglobin g/100 ml	13.2 ± 0.19	12.8 ± 1.49	13.0 ± 0.44
Glucose mg/100 ml	125 ± 7.9	140 ± 9.4	140 ± 7.5*
Urea mg/100 ml	27.3 ± 1.97	39.3 ± 2.79***	35.4 ± 3.07**
Total proteins g/100 ml	4.8 ± 0.08	5.2 ± 0.22	5.5 ± 0.21**
Cholesterol mg/100 ml	56 ± 4.5	50 ± 7.5	53 ± 8.1
Uric acid mg/100 ml	6.3 ± 0.53	5.1 ± 0.29**	4.8 ± 0.41**
Creatinine mg/100 ml	0.5 ± 0.10	0.4 ± 0.10	0.4 ± 0.14
ALP U/l	46 ± 4.8	39 ± 5.1	37 ± 4.9*
GOT U/l	301 ± 32.9	308 ± 17.1	256 ± 32.9
GPT U/l	59 ± 8.8	44 ± 5.6*	41 ± 5.5*

Statistical significance (Student-Fischer's test): *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; Degrees of freedom = 6.

Hematocrit, hemoglobin, total proteins and total lipids scarcerly showed significant differences between the animals treated with the $CoCl_2$-cysteine complexes and the animals treated with $CoCl_2$.

Discussion

The results show that L-cysteine is an effective antidote to the lethal action of cobalt, as the number of treated animals surviving one week for each of the different administrations of $CoCl_2(LD_{50})$, was 100 %, except if the metal was given orally and the cysteine was given intraperitoneally after about 15 minutes, when effectiveness was reduced to 40 %. Probably this is due to a problem of cobalt and cysteine absorption times, as previous tests showed that the intraperitoneal administration of cysteine immediately after oral cobalt ingestion (LD_{50}) and at theoretical concentrations for the formation of the complex *in vivo* produces immediate death in the animals. However, with the intraperitoneal concentrations of cysteine used, if the time for the administration of the aminoacid was delayed too long it was unable to avoid the lethal action of cobalt taking place due to its rapid absorption.

Nevertheless, cysteine proved to be totally efficient when given as a complex *in vitro* with $CoCl_2$ for both methods of administration. The analytical controls carried out in the surviving animals seven days after treatment with the $CoCl_2$-cysteine complexes scarcely showed any significant variations with the exception of: an increase in urea and decrease in the uric acid for oral administration; and an increase in the hematocrit, urea and total proteins; together with a decrease in uric acid for intraperitoneal administration; these results being taken as a whole do not enable any precise conclusions to be reached.

The increases in significant parameters related to lipid metabolism during the twelve hours subsequent to intoxication with $CoCl_2$, scarcely appeared when the $CoCl_2$ is forming complex with

Table III. *Analytical parameters measured 12 hours after giving the $CoCl_2$-cysteine complex (oral and i.p.) or $CoCl_2$ freely. Comparison with the control animals.*

Mean values ± S.E. Amounts of $CoCl_2$ in parentheses. The $CoCl_2$-cysteine complex was prepared 1:3 molar, with the same concentrations of cobalt as in the free administration.

Parameter	Control	$CoCl_2$ (3/4 LD_{50}) Oral	$CoCl_2$-Cysteine complex oral	$CoCl_2$ (3/4 LD_{50}) i.p.	$CoCl_2$-cysteine complex i.p.
Hematocrit %	31.8 ± 2.17	33.0 ± 1.67	32.4 ± 1.52	32.5 ± 1.30	33.8 ± 0.84
Hemoglobin g/100 ml	12.0 ± 0.75	13.3 ± 1.50	13.2 ± 0.49	12.2 ± 0.65	13.1 ± 0.68
Total proteins g/100 ml	4.9 ± 0.73	5.0 ± 0.21	4.8 ± 0.79	5.6 ± 0.38**	4.6 ± 0.45
Glucose mg/100 ml	166 ± 18.5	181 ± 7.1**	152 ± 13.1	146 ± 12.8	165 ± 16.5
Triglycerides mg/100 ml	63 ± 15.6	98 ± 11.5***	68 ± 10.7	88 ± 7.5***	44 ± 3.1*
Cholesterol mg/100 ml	82 ± 14.0	102 ± 7.4**	59 ± 5.5**	53 ± 6.7***	64 ± 12.0*
Total lipids mg/100 ml	244 ± 27.0	220 ± 19.4	210 ± 20.0	204 ± 15.8	200 ± 14.6*

Statistical significance (Student-Fischer's test): *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Degrees of freedom = 8.

cysteine. These increases are provoked by the action of cobalt (11) and the differences observed confirm in any case the role of cysteine in its block.

Consequently, the results obtained show a substantially different behaviour for $CoCl_2$ when given freely or forming a complex with L-cysteine. The previous formation of the complex *in vitro* did not evidence any lethality, and when the complex is formed directly *in vivo*, also its lethality is shown to be considerably reduced; always according to the experimental conditions mentioned.

Resumen

Se estudian en rata los efectos de la L-cisteína sobre la toxicidad aguda producida por el cloruro de cobalto administrado oral e intraperitonealmente. La disminución de la letalidad es absoluta para las distintas dosis ensayadas, excepto cuando el $CoCl_2$ se administra oralmente y la L-cisteína intraperitonealmente en que sólo se alcanza un 40 % de eficacia. No se observan alteraciones especialmente significativas en los parámetros sanguíneos de los animales que recibieron el complejo $CoCl_2$-cisteína, al cabo de una semana de su administración.

La glucosa, los triglicéridos y el colesterol, medidos a las doce horas de la administración del complejo $CoCl_2$-cisteína registran diferencias significativas con respecto a los mismos parámetros medidos tras la administración del $CoCl_2$ sin acomplejar.

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