REVISTA ESPAÑOLA DE FISIOLOGIA, 40, 443-448, 1984

Treatment of Acute Cobalt Intoxication in Rats with L-Methionine

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(Received on May 14, 1984)

J. L. DOMINGO and J. M. LLOBET. *Treatment of Acute Cobalt Intoxication in Rats with L-Methionine.* Rev. esp. Fisiol., **40**, 443-448, 1984

The antidotal action of L-methionine in acute cobalt (II) chloride intoxication given orally or intraperitoneally to rats has been investigated in this paper. The doses of $CoCl_2$ (2.73 mmole/kg oral, 0.21 mmole/kg i.p.) are always above their LD_{50} for both means of administration, reaching during oral administration values above its LD_{95} (4.20 mmole/kg). The doses of L-methionine varied from 0.63 mmole/kg (i.p.) to 8.19 mmole/kg (orally).

L-methionine did not show a significant antidotal action (mortality rates) against the other sulphurous aminoacid: L-cysteine, which is considered an effective antidote. The administration of Co^{2+}-methionine chelates prepared *in vitro*, showed rates of 10 % mortality when given orally and 30 % when given intraperitoneally, against Co^{2+}-cysteine and Co^{2+}-N-acetylcysteine chelates with rates of 0 % mortality.

No significant functional changes were observed in the survivors killed seven days after administration in groups receiving L-methionine. Although L-methionine cannot be considered an effective antidote, it is likely to reduce partially the toxic effects of cobalt.

Key words: Cobalt, Acute toxicity, Rats, L-methionine, Antidotal action.

It is a well known fact that in cobalt intoxication a reaction of the metal with the SH groups of aminoacids or enzymes is produced (1,12). Cobalt also reacts specifically with SH groups of dihydrolipoic acid, impeding the conversion of pyruvate to acetyl coenzyme A (CoA) and α-cetoglutarate to succinyl-CoA in the tricarboxylic acid cycle. This may be the most probable mechanism of the cardiotoxic effect produced by cobalt (2, 3, 11).

In previous studies, the action that L-cysteine (4) and N-acetylcysteine (DOMINGO *et al.*, unpublished data), both sulphurous compounds, can have in acute cobalt intoxication has been

Abbreviations: i.p.: intraperitoneal; ALP: alkaline phosfatase; GOT: glutamic oxaloacetic acid transaminase; GPT: glutamic pyruvic acid transaminase.

investigated, via the presence of SH groups in their structures.

In this paper, the effects that L-methionine (the other sulphurous aminoacid common to mammalian organisms) can have in acute cobalt (II) poisoning have been studied. This is due to the fact that L-cysteine or L-methionine administrations inhibit the production of polycythemia, which is another of the most characteristic effects produced in cobalt intoxication (5). Theinhibition produced by L-methionine, however, was much lower when both aminoacids were given to rats during a semichronic oral intoxication by cobalt (6,8).

Materials and Methods

Animals and chemicals. Eight groups of male Sprague-Dawley rats bred by Biocentre (San Feliu de Codines / Barcelona) (200-250 g body weight) with 20 animals in each group were used. The animals had free access to food (Panlab diet) and tap water.

Cobalt used in the form of cobalt (II) chloride ($CoCl_2.6H_2O$) and L-methionine were analytically pure and supplied by Merck (Darmstadt, FRG).

Toxicological studies. To test the efficacy of L-methionine as an antidote to cobalt, various amounts of $CoCl_2.6H_2O$ were administered orally and intraperitoneally. Oral doses were dissolved in distilled water and were given intragastrically. Intraperitoneal doses were dissolved in 0.9 % saline. The dosages are specified in table I. Solution concentrations were adjusted so that a 250 g rat would receive 1 ml, and were administered at a pH of approximately 7.4.

Syringes were 1 ml disposable insulin syringes graded in hundredths of ml.

In every case, the animals were observed for seven days.

Blood analyses. At the end of the experiment, four animals of each group were killed by decapitation, and the following blood parameters were investigated: hematocrit, hemoglobin and glucose, all three due to their close relation with cobalt intoxication: total protein, ALP, GOT, GPT, urea, uric acid and creatinine as a measure of the liver and renal functions.

Methods of clinical chemistry and hematology were described earlier (4).

Statistical analysis. The results of the experiments were analyzed using quantal response methodology. The significance of the differences in the results of blood parameters was determined by the t Student-Fischer test. A difference is considered to be significant when $P \leq 0.05$.

Results

Biological studies. The results of the different experiments carried out to test efficacy of L-methionine as an antidote in cobalt intoxication are shown in table I.

The results of the first and the second experiments show the effects of the free administration of cobalt given orally or intraperitoneally at dosages lightly above the LD_{50} of the salt (7), 2.73 mmole/kg oral and 0.21 mmole/kg i.p. The mortality rate for the oral administration was 55 % (group 1) and for the i.p. administration was 65 % (group 2).

In the third experiment the oral dosage of $CoCl_2$ was the same (2.73 mmole/kg) and L-methionine was administered intraperitoneally 5 minutes later (1.26 mmole/kg, corresponding 6-fold to the i.p. LD_{50} ot the $CoCl_2$). This dose was chosen due to the relatively low absorption rate of cobalt when taken orally (11). Under these conditions the mortality was reduced to 25 % (group 3).

Table I. *Effects of the administration of L-methionine upon the acute mortality of rats after oral or i.p. administration of $CoCl_2$.*

Group	$CoCl_2.6H_2O$ (mmole/kg)	Route	L-methionine (mmole/kg)	Route	Survivors/Total[a]
1	2.73	oral	0	—	9/20
2	0.21	i.p.	0	—	7/20
3	2.73	oral	1.26	i.p.[b]	15/20
4	0.21	i.p.	0.63	i.p.[c]	12/20
5	4.20	oral	2.52	i.p.[c]	1/20
6	2.73	oral[d]	8.19	oral[d]	18/20
7	0.21	i.p.[d]	0.63	i.p.[d]	14/20
control	0	—	0	—	20/20

a: Survivors seven days after administration. b: i.p. injection 5 minutes after administration of cobalt. c: i.p. injection immediately after administration of cobalt. d: chelate prepared *in vitro*.

In the fourth experiment, the i.p. dosage of $CoCl_2$ was 0.21 mmole/kg and L-methionine was given immediately by i.p. administration the dosage being increased 3-fold to 0.63 mmole/kg. The mortality compared with the second experiment was reduced to 40 % (group 4).

In the fifth experiment, the oral dose of $CoCl_2$ was increased to 4.20 mmole/kg. In our laboratory, this dose of $CoCl_2$ killed 100 % of the animals (7). Immediately L-methionine was given intraperitoneally [2.52 mmole/kg corresponding 12-fold to the i.p. LD_{50} of the $CoCl_2$ (7)]. The administration of L-methionine did not reduce the mortality (95 %, group 5).

Lastly, in the sixth and seventh experiments cobalt-methionine chelates prepared *in vitro* were given orally and intraperitoneally at doses of $CoCl_2$: 2.73 mmole/kg oral and 0.21 mmole/kg i.p. and amounts of L-methionine corresponding 3:1 to those of $CoCl_2$: 8.19 mmole/kg and 0.63 mmole/kg. In the oral administration, mortality was only 10 % (group 6) against 55 % in the first experiment. For the i.p. administration (group 7) mortality was 30 % against 65 % in the second experiment.

In all the cases described, the deaths appeared within 72 hours as is habitual in acute cobalt intoxication (7,10).

Table II summarizes the results obtained for L-cysteine, N-acetylcysteine and L-methionine for acute cobalt (II) chloride intoxication.

Only when cobalt was given orally

Table II. *Comparisons of the mortality rates (%) for three antidotes for acute cobalt (II) chloride intoxication.*

Mole ratio of antidote to cobalt is 3:1 with cobalt (II) chloride hexahydrate given at the level of LD_{50} (oral or i.p.) or greater.

Test	Antidote		
	L-cysteine	N-acetylcysteine	L-methionine
1. $CoCl_2$, oral antidote, i.p.[b]	30	30	25[a]
2. $CoCl_2$, i.p. antidote, i.p.[c]	0	30	40
3. Chelate[d] Co^{2+}-antidote oral	0	0	10
4. Chelate[d] Co^{2+}-antidote i.p.	0	0	30

a: mole ratio of L-methionine to cobalt is 6:1. b: i.p. injection between 5 and 15 minutes after administration of cobalt. c: i.p. injection immediately after administration of cobalt. d: chelate prepared *in vitro*.

Table III. *Investigations into the blood of treated rats and control rats seven days after the administration of cobalt. (Mean values ± S.E.).* Statistical significance (Student-Fischers's test): $^{*}P < 0.05$; $^{**}P < 0.01$. Degrees of freedom = 6. The different groups are the same as in table I, and have been described in the text.

PARAMETER	CONTROL	group 1	group 2	group 3	group 4	group 6	group 7
Hematocrit %	42.3 ± 0.96	45.5 ± 1.02**	46.5 ± 1.31**	39.5 ± 1.29**	41.8 ± 3.50	39.8 ± 0.96*	40.0 ± 1.41*
Hemoglobin g/100 ml	14.5 ± 0.46	15.2 ± 0.22	15.5 ± 0.31	13.4 ± 0.43	14.1 ± 0.82	13.4 ± 0.43	13.4 ± 0.76
Glucose mg/100 ml	130 ± 9.6	93 ± 12.6*	144 ± 8.9	143 ± 21.4	133 ± 12.7	132 ± 12.0	129 ± 10.3
T. Proteins g/100 ml	4.9 ± 0.46	5.4 ± 0.19	4.8 ± 0.15	5.7 ± 0.17*	4.7 ± 0.22	5.1 ± 0.22	5.2 ± 0.54
ALP U/l	47 ± 12.8	47.0 ± 18.7*	22.2 ± 6.3	28.4 ± 8.4	41.8 ± 15.2	34.8 ± 13.3	44.5 ± 4.8
GOT U/l	107 ± 22.7	94 ± 12.3	92 ± 6.9	88 ± 13.2	83 ± 15.4	99 ± 10.0	91 ± 12.3
GPT U/l	22 ± 3.4	16 ± 1.9	26 ± 4.1	16 ± 1.7	14 ± 1.6*	19 ± 4.5	16 ± 1.7
Urea mg/100 ml	30.5 ± 1.73	51.3 ± 6.60*	33.0 ± 7.12	50.5 ± 4.79*	35.8 ± 2.21	60.06 ± 11.8**	49.5 ± 5.80*
Uric acid mg/100 ml	1.4 ± 0.31	0.6 ± 0.91*	1.2 ± 0.60	1.9 ± 0.29	0.4 ± 0.05*	1.0 ± 0.21	0.9 ± 0.26
Creatinine mg/100 ml	0.3 ± 0.46	0.4 ± 0.08	0.4 ± 0.06	0.5 ± 0.08	0.4 ± 0.01	0.5 ± 0.05	0.5 ± 0.06

and the chelating agents intraperitoneally after 5 minutes, the mortality rate was lower for L-methionine (25 %, test 1).

The most striking feature of these results, is the fact that also the administration of the chelates Co^{2+}-methionine produced relatively important mortality rates.

Hematological investigations and liver and renal functions. Table III shows comparisons between the analytical parameters of the treated animals and the control animals after seven days. A significant increase of hematocrit ($P < 0.01$) can be seen in the animals receiving only $CoCl_2$ freely. A significant decrease ($P < 0.01$) can also be seen in the third group. In the animals receiving the chelates significant decreases ($P < 0.05$) can be seen.

Observing the hepatic and renal parameters, significant changes ($P < 0.05$) in urea and uric acid can be seen in the animals receiving $CoCl_2$ orally. These changes could suggest an effect only on the renal function in these animals. This has been described earlier (4, DOMINGO *et al.*, unpublished data).

With regard to the other groups, the differences between the treated and control animals scarcely showed any significance.

In the fifth group there are no results since only one animal survived seven days.

Discussion

The results indicate that L-methionine is apparently lower than the other sulphurous aminoacid (L-cysteine) in its effectivity in preventing death from oral or i.p. administration of $CoCl_2$ (4).

Under similar experimental conditions, the mortality rates of rats was higher in the case of L-methionine being used as an antidote. Only when $CoCl_2$ was given orally (2.73 mmole/kg) followed by the i.p. administration of L-methionine (1.26 mmole/kg), the results of both aminoacids were seen to be almost equal. Another noticeable observation is the fact that the administration of the chelates Co^{2+}-methionine showed remarkable mortality rates. In previous studies, the administration of Co^{2+}-cysteine chelates had reduced the mortality rates to zero (4). Also when N-acetylcysteine, which was not considered to be an effective antidote in acute cobalt (II) intoxication, was administered forming a previous chelate with Co^{2+}, the mortality rate was reduced to zero (DOMINGO *et al.*, unpublished data).

These differences found in the present study between L-cysteine and L-methionine (and even with N-acetylcysteine) as antidotes in acute cobalt (II) intoxication can be explained if we take into consideration the lower stability constants from Co^{2+}-methionine chelate ($\log K_1 = 4.12$, $\log K_2 = 3.44$) as compared with Co^{2+} -cysteine chelate ($\log K_1 = 9.31$, $\log K_2 = 7.60$) (9). Besides, a probably greater difficulty in the formation of the chelate itself, can be corroborated through the relatively important rate of mortality which appeared during the administration of the theoretical chelate prepared *in vitro*.

However, with the exception of the fifth experiment (LD_{95} of the $CoCl_2$), in the other experiments L-methionine showed a certain effectivity as an antidote in acute cobalt intoxication. In addition, the absence of functional changes, could possibly suggest that L-methionine takes part in the process of detoxication.

Nevertheless, due to the small reduction in the mortality rate of rats suffering from acute cobalt poisoning, L-methionine cannot be suggested as an effective antidote for acute cobalt intox-

ication, despite the fact that it could partly reduce the toxic effects, and always according to the experimental conditions mentioned in this paper.

Resumen

Se estudia en ratas la acción de la L-metionina como antídoto en la intoxicación aguda por cloruro de cobalto (II) administrado oral e i.p. Las dosis de $CoCl_2$ (2,73 mmole/kg oral, 0,21 mmole/kg i.p.), son siempre superiores a los valores de sus DL_{50} para ambas vías, llegando en la administración oral hasta 4,20 mmole/kg, valor por encima de la DL_{95}. Las dosis de L-metionina varían desde 0,63 mmole/kg (i.p.) a 8,19 mmole/kg (oral).

La L-metionina no muestra una significativa acción antidotal comparada con el otro aminoácido azufrado: L-cisteína, el cual se considera un antídoto eficaz. La administración de quelatos Co^{2+} -metionina preparados *in vitro*, registra índices de mortalidad del 10 % (quelato oral) y 30 % (quelato i.p.), frente a índices de mortalidad del 0 % en la administración de los quelatos Co^{2+} -cisteína y Co^{2+} -N acetilcisteína.

No se observan alteraciones funcionales significativas en los animales supervivientes sacrificados a los siete días de la intoxicación en ninguno de los grupos que reciben L-metionina. Probablemente, la L-metionina aún sin poder ser considerada un antídoto eficaz, puede reducir parcialmente los efectos tóxicos del cobalto.

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