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Effects of Cobalt on Postnatal Development and Late Gestation in Rats Upon Oral Administration

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Four groups of pregnant Wistar rats, each of which consisted of 15 animals were administered 0, 12, 14 and 48 mg/kg/day of cobalt (II) chloride from the 14th day of gestation through 21 days of lactation. The offspring were observed for mortality, body weight, body and tail length and general symptomatology after 1, 4 and 21 days of nursing. The number of litters was higher for the control group. The survival ratios were also higher for the control group. Besides, a dose-dependent delay in the growth of the living young could be observed. No significant differences in organ weights in the animals killed 21 days after birth were observed. The blood parameters analysed did not show differences between the treated and control pups. Cobalt produced toxic effects on the mothers, affecting the late gestation as well as the postnatal development of the pups.

Key words: Cobalt, Oral administration, Pregnant rats, Reproduction.

It is a well known fact, that teratogenic effects of certain metals have resulted when the element was given to pregnant animals. Mercury, lead and cadmium have been known to be teratogenic in this respect for many years (5, 6, 11). Also, thallium, selenium and arsenic among other elements have been reported as teratogenic (2, 10, 15).

Cobalt is a trace element which causes diverse toxicity in experimental animals and man (1, 13). However, there is little information on the teratogenic effects of cobalt. This trace element was not a teratogenic threat in the golden hamster (7). In previous works, the acute and subacute toxicity of cobalt have been studied (4, 9). Also studies on semichronic toxicity were carried out when cobalt was given to rats in their drinking water (3).

In order to obtain an overall understanding of the toxicity of cobalt, in this paper, the effects of cobalt on late fetal

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development, labour and delivery, lactation, neonatal viability, and growth as well as development of the newborn have been studied.

Materials and Methods

Chemicals and animals. Cobalt as cobalt (II) chloride (Merck) was administered to female Wistar rats (250-300 g) supplied by Biocentre (Barcelona, Spain). The animals had free access to food (Panlab-diet, Barcelona, Spain) and tap water.

Biological studies. Rats in gestation day-14 were used. Previously, groups of five females and two males were kept in cages, and placed in a fully airconditioned facility where the temperature was regulated at 23 ± 2°C, and the humidity at 55 ± 5 %. Copulation was confirmed by the detection of spermatozoa in vaginal smears (2). Cobalt (II) chloride was given to four groups of 15 pregnant rats at doses of 0, 12, 24 and 48 mg/kg/day by gastric intubation from gestation day-14 through 21 days of lactation. The offspring were observed for mortality, normal body weight gain and general symptomatology after 1, 4 and 21 days of nursing plus gross external and internal examination at the conclusion of the study (14). Also, examination for external malformations was made. Body and tail length were measured on the same days (12).

After 21 days, all the offspring were sacrificed. After macroscopic examination, heart, lungs, spleen, liver and kidneys were removed and weighed. Previously, blood was collected from the arteriae femoralis. The following hematological analysis were carried out: hematocrit, by the hematocrit method using Clay-Adams centrifuge and hemoglobin measured as oxihemoglobin by means of a Coulter Electronic hemoglobinometer. In order to determine possible alterations on the liver and renal functions, GOT and GPT, bilirubin, total protein, urea, uric acid and creatinine were analysed. Also, the amounts of glucose and cholesterol were determined. Serum biochemical analyses were carried out with Hitachi automatic analyser (Model 400, Hitachi Ltd.) using classic techniques.

Statistical analysis. The significance of the differences in the results was determined by the t Student-Fischer test. A difference is considered to be significant when $P \leq 0.05$.

Results

Biological studies. Table I shows a summary of several data from rat pups nursed by cobalt-treated mothers during the period of lactation. Results are given after 1, 4 and 21 days of nursing. These results comprised number of litters, number of living young and dead young, male-female ratios and the average body weight by litter.

The number of litters was higher for the control group. Also, the ratios living young and dead young by litter for the treated animals showed significant differences with regard to the control group. These differences were more remarkable for the 48 mg/kg/day group ($P < 0.01$).

Lastly, a dose-dependent difference between the treated and the control animals can be seen in the average body weights by litter.

Table II shows the data in the days 1, 4 and 21 for pups being nursed by females receiving cobalt. Body weight, body length and tail length from the three groups showed significant decreases in both, males and females, compared with the control groups. These decreases were most noticeable for the 48 mg/kg/day group and were dose-dependent.

No external malformations were observed in any case.

Table I. *Summary of data from rat pups nursed by cobalt-treated mothers during a period of 21 days.* Mean values ± S.E. Statistical significance (Student Fischer's test): * $p < 0.05$; ** $p < 0.01$.

Day	Dose levels (mg/kg/day)	N.° of litters	N.° of living young	N.° of dead young	Dead / Living ratio (× 100)	Male / female ratio	Living young / litter	Dead young / litter	Average body weight / litter
1	0	12	120	4	3.33	0.93	10.0 ± 3.4	0.3 ± 0.9	74.8 ± 19.2
	12	5	64	3	4.68	1.06	12.8 ± 1.1	0.5 ± 0.9	80.9 ± 14.4
	24	6	56	7	12.50	0.93	9.3 ± 4.4	1.2 ± 1.5	54.8 ± 27.7
	48	7	60	15	25.00	1.00	8.6 ± 4.2	2.1 ± 1.7*	57.4 ± 17.0
4	0	12	114	6	5.26	1.00	10.7 ± 2.2	0.5 ± 0.4	110.8 ± 21.2
	12	5	61	3	4.92	1.10	12.1 ± 1.1	0.6 ± 0.4	111.5 ± 12.2
	24	6	51	5	9.80	0.88	8.5 ± 3.9	0.6 ± 0.3	80.8 ± 38.5*
	48	7	30	30	100.00	1.30	4.3 ± 5.4**	4.3 ± 3.7**	86.1 ± 14.2
21	0	12	106	8	7.55	1.02	8.8 ± 3.6	0.3 ± 0.2	392.2 ± 102.5
	12	5	57	4	7.02	1.10	11.8 ± 2.4	0.8 ± 0.6	357.1 ± 62.6
	24	6	44	7	15.91	0.90	8.3 ± 4.3	1.2 ± 2.4	279.0 ± 52.4**
	48	7	29	1	3.45	1.23	4.1 ± 2.0**	0.1 ± 0.0**	245.7 ± 16.8**

Table II. *Average body weight, body length and tail length of rat pups nursed by cobalt-treated mothers.* Mean values ± S.E. In parentheses the number of animals studied. Statistical significance (Student-Fischer's test): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Day	Dose levels (mg/kg/day)	Body weight (g)		Body length (mm)		Tail length (mm)	
		Males	Females	Males	Females	Males	Females
1	0	7.18 ± 1.24 (41)	6.61 ± 1.16 (43)	53.8 ± 0.5	50.9 ± 0.5	18.2 ± 0.3	17.4 ± 0.3
	12	5.68 ± 0.69 (33)***	5.69 ± 1.02 (31)***	49.1 ± 0.3**	49.5 ± 0.3	15.7 ± 0.1***	15.8 ± 0.2**
	24	5.61 ± 1.13 (27)***	6.04 ± 0.82 (29)**	50.1 ± 0.4*	48.3 ± 0.3**	16.6 ± 0.2***	16.9 ± 0.2
	48	5.34 ± 1.17 (26)***	5.56 ± 1.08 (28)***	47.2 ± 0.4**	47.5 ± 0.3***	15.3 ± 0.2***	15.1 ± 0.1***
4	0	10.82 ± 2.14 (38)	10.00 ± 2.09 (39)	64.1 ± 0.5	61.3 ± 0.5	25.4 ± 0.4	25.3 ± 0.4
	12	9.03 ± 0.87 (30)***	8.78 ± 1.03 (29)**	62.1 ± 0.3*	61.3 ± 0.3	24.2 ± 0.2	24.1 ± 0.3
	24	8.64 ± 1.19 (27)***	8.75 ± 0.94 (29)**	62.0 ± 0.3*	60.9 ± 0.3	24.3 ± 0.2	24.5 ± 0.2
	48	8.65 ± 0.43 (18)***	8.83 ± 0.77 (18)**	59.8 ± 0.3***	58.5 ± 0.2*	22.7 ± 0.3***	23.0 ± 0.2*
21	0	43.06 ± 8.07 (35)	41.24 ± 8.59 (35)	109.6 ± 0.9	106.8 ± 0.8	75.8 ± 1.3	73.3 ± 1.2
	12	30.75 ± 7.97 (30)***	29.82 ± 7.34 (28)***	101.4 ± 0.7***	100.1 ± 0.7***	70.0 ± 0.6*	70.8 ± 0.6
	24	26.69 ± 5.12 (23)***	27.33 ± 4.28 (28)***	94.7 ± 0.9***	95.8 ± 0.9***	67.9 ± 1.2***	65.2 ± 1.1**
	48	25.70 ± 3.22 (14)***	28.73 ± 6.65 (13)***	91.6 ± 0.8***	88.3 ± 0.7***	59.1 ± 0.9***	56.6 ± 0.8***

Table III. *Relative organ weight of rat pups nursed by cobalt-treated mothers.* Results are presented as arithmetic means of 12 animals in each group ± S.E. and expressed in g per 100 g of body weight. Statistical significance (Student Fischer's test): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Dose levels (mg/kg/day)	0	12	24	48
Males				
Heart	0.535 ± 0.085	0.489± 0.075	0.626 ± 0.277	0.544 ± 0.086
Lungs	1.387 ± 0.702	0.892 ± 0.143**	1.189 ± 0.354	1.053 ± 0.148
Spleen	0.437 ± 0.144	0.322 ± 0.054	0.359 ± 0.220	0.288 ± 0.101
Liver	4.120 ± 0.394	3.400 ± 1.163***	3.681 ± 0.807	3.157 ± 0.394***
Kidneys	1.288 ± 0.157	1.074 ± 0.205**	1.321 ± 0.294	1.327 ± 0.128
Females				
Heart	0.504 ± 0.053	0.460 ± 0.056	0.527 ± 0.108	0.543 ± 0.066
Lungs	1.254 ± 0.442	1.015 ± 0.179	1.234 ± 0.490	1.081 ± 0.141
Spleen	0.485 ± 0.128	0.287 ± 0.060***	0.346 ± 0.107***	0.302 ± 0.086***
Liver	4.091 ± 0.444	2.938 ± 0.425***	3.486 ± 0.652***	3.389 ± 0.453***
Kidneys	1.254 ± 0.182	1.107 ± 0.251*	1.273 ± 0.186	1.367 ± 0.163

Organ weights. Table III shows the relations organ weight/body weight of the pups killed after a period of 21 days. In males, the relative weights of the livers were lower than those of the control group. In females, as well as the liver, the relative weights of the spleens were inferior. However, in both cases, there were no dose-dependent differences between the treated and the control animals as regards absolute and relative organ weights.

Blood analyses. Enzyme activities (GOT and GPT) and amounts of bilirubin and total protein in the plasma showed no significant differences between the control and the treated animals, whose mothers received cobalt. Also, the concentrations of uric acid, urea and creatinine in plasma were within the normal range for all the groups, as well as those of glucose and cholesterol. Nor did hematocrit and hemoglobin show significant differences between treated and control groups.

Discussion

The above mentioned results, show remarkable differences in the late gestation and postnatal development of rats whose mothers received cobalt from the 14th of gestation through 21 days of lactation.

With regard to the gestation, an inferior number of litters as well as of living young can be seen for the offspring whose mothers received cobalt. Furthermore, body weight, body length and tail length were inferior for these animals.

On the other hand, the postnatal development was also remarkably affected. Body weight, body length and tail length were always higher for the control group. An important dose-dependent difference could be observed.

In all the groups, the non-surviving pups died for the most part during the first four days of nursing. This result indicates that the non-surviving animals could not live out the first period of their life. Nevertheless, most of the animals which overcame this period were able to survive.

Another noticeable fact is that the surviving animals after 21 days of lactation showed scarcely any significant differences in relative organ weights. The decreases observed are not significant according to the criterion «organ weight/body weight» (8), and are due to a reduction in the growth.

The results of blood parameters did not show significant increases in hematocrit and hemoglobin in the pups whose mothers received cobalt. The increases in hematocrit and hemoglobin are an important response to cobalt (13). Enzyme activities (GOT and GPT) and concentrations of bilirubin, total protein, uric acid, urea and creatinine in plasma were within the normal range for all the groups. Therefore, no alterations on the liver and renal functions could be recorded.

In previous studies (3, 4), toxic signs which were apparent in male and female rats receiving cobalt at dosages of 24 and 48 mg/kg/day (which correspond to 1/20 and 1/10 of the oral LD_{50} of the $CoCl_2$ (9)) have been described. These toxic signs could not be observed in the pups. Then, we can conclude that cobalt administered to pregnant rats from the 14th day of gestation and during the period of lactation, produces toxic effects on the mothers, which have a repercussion on the late gestation. Besides, a delay in growth on the living young is observed. Nevertheless, after a 21-day period, no toxic signs can be seen in the survivors.

Resumen

Se estudia en 60 ratas Wistar gestantes, distribuidas en cuatro grupos, el efecto del $CoCl_2$ (0, 12, 24 y 48 mg/kg/día) administrado desde el día 14 de gestación hasta el día 21 de lactancia. Se observa en las proles: mortalidad, peso del cuerpo, longitudes del cuerpo y de la cola, así como el aspecto general en los días 1, 4 y 21. El número de camadas y los porcentajes de supervivencia de las crías son más elevados para el grupo control y existe un retraso en el crecimiento de las crías supervivientes dependiente de la concentración. No se registran diferencias significativas en los pesos de los órganos de las crías sacrificadas a los 21 días de edad. Los parámetros sanguíneos analizados no presentan diferencias entre las crías del grupo control y las de los grupos cuyas madres reciben $CoCl_2$. El cobalto, produce efectos tóxicos sobre las madres, los cuales afectan la gestación en su última fase, así como el posterior desarrollo de las crías.

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