EFFECTS OF $CoCl_2$ AND Co-EDTA ON TESTICULAR MORPHOLOGY AND SPERM COUNT IN MOUSE

Ekaterina Pavlova[a]*, Maria Madzharova[a], Donika Dimova[a],Yordanka Gluhcheva[a], Juliana Ivanova[b], and Nina Atanassova[a]

Keywords: Cobalt chloride, Cobalt-EDTA, *in vivo* model, mouse testis, sperm count

Cobalt (Co) is an essential trace element for mammals required for the synthesis of vitamin B_{12}. When cobalt was applied chronically it tends to accumulate in different organs and tissues that can induce pathological alterations. We focused our present study on the comparative effects of chronic exposure to cobalt chloride ($CoCl_2 \cdot 6H_2O$) or cobalt EDTA (Co-EDTA) on testis and sperm count in mice of different ages. Both compounds were given in drinking water water with doses per day of 75 mg/ kg or 125 mg/ kg, respectively. Treated animals were sacrificed at different time intervals (on days 18, 25, 45 and 60). The most obvious changes in testis morphology - depletion and retardations in germ cell development, disorganization of seminiferous epithelium and SCO (Sertoli-cell-only) tubules were observed on the studies of 45 and 60 days respectively. In early puberty, testis weight was reduced in similar extend (with 25%) after high doses of both Co compounds whereas in early maturity and adulthood this parameter was reduced significantly and more severe after exposure to high dose $CoCl_2$ as compared to Co-EDTA, probably due to the stability of the complex Co-EDTA and its weaker absorption. Concerning gonado-somatic index and sperm count in mature animals we found similar tendency of more adverse effects with high dose of $CoCl_2$. As a result of our work and data in literature we can conclude that the effects of chronic exposure to cobalt depend of the type of the compound used, dose, level, time duration as well as on the age of the animals under experiment. Cobalt could be considered as a possible risk factor for male fertility and health.

* Corresponding Author: Ekaterina Pavlova
Fax: +359 2 871 01 07
E-Mail: ebankova@yahoo.com
[a] Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria, Acad. Georgi Bonchev Str., bl. 25
[b] Faculty of Medicine, Sofia University "St. Kliment Ohridski" 1407 Sofia, Bulgaria, Kozjak 1.

Introduction

Cobalt is naturally occurring, relatively rare element of the earth's crust[1]. Sources of exposure to cobalt and inorganic cobalt compounds are both natural and anthropogenic[2]. Cobalt is an essential oligo-element for mammals involved as a constituent of vitamin B_{12} (cobalamin), mainly. Food and beverages represents the main source of cobalt for the general population[3]. By blood circulation cobalt could be delivered and subsequently accumulated in different organs like – liver, kidneys, hematopoietic organs, brain, reproductive organs, etc. Prolonged exposure to cobalt leads to different pathological alterations such as cardiomyopathy, impaired function of thyroid gland and liver. Cobalt has been shown to exert genotoxic and carcinogenic effects[4]. The crucial negative effects of cobalt on testis were rendered to its ability to induce conditions characterized with more or less decreased level of oxygen. Cobalt chloride is widely used pharmacological agent for inducing hypoxia. In the testis degenerative changes in adult seminiferous epithelium include vacuolation of Sertoli cells and germ cell nuclei reported by Elbetieha et al.[5] In rats cobalt chloride (with ^{58}Co tracer) complexed with histidine, lysine, glycylglycine, EDTA, casein, or glycine was absorbed less then free cobalt chloride. Ethylenediamine tertraacetic acid (EDTA) is a widespread organic pollutant. It is used as anticoagulant for blood samples and decalcifying agent in histopathology, in non-alcoholic beverages. EDTA is powerful antioxidant and due to its ability to bind metals it is used in chelation therapy[6].

Literature data show that oral exposure to cobalt compounds of adult animals causes reproductive and developmental alterations. We focused our study on the comparative effects of chronic exposure to $CoCl_2$ and Co-EDTA on testis morphology and sperm count in different ages of mice.

Materials and methods

Pregnant ICR mice in late gestation were subjected to cobalt chloride ($CoCl_2 \cdot 6H_2O$) or cobalt EDTA (Co-EDTA) treatment at daily doses of 75 mg kg^{-1} or 125 mg kg^{-1}, respectively, which continued until day 60 of the newborn pups. Cobalt compounds were applied via drinking tap water. Pure tap water was used as control. Animals were fed a standard diet and had access to food *ad libitum*. Mice were maintained in the institute's animal house at 23 ± 2 ºC and 12:12 h light-dark cycle in individual standard hard bottom polypropylene cages to insure that all experimental animals obtained the required dose of cobalt compounds.

Up to mid puberty (25 pnd) pups were influenced on $CoCl_2$ or Co-EDTA at first via transplacental routes (during pregnancy) and subsequently via mothers' milk (during suckling period). On 25 pnd male mice were separated in personal cages, the doses were calculated and authenticated once a week according to their weight. Male pups were sacrificed on 18 pnd (early puberty), 25 pnd (mid puberty), 45 pnd (early maturity) and 60 pnd (maturity) respectively.

Testes and epididymides were sampled, weighed and embedded in paraffin using routine histological practice. Spermatozoa were isolated from both vasa deferentia and counted using Buerker's chamber. Data were statistically processed using Student's t-test.

The animal experiments were performed in accordance with the animal protection guidelines approved by the Ethics Committee for Experimental Animal Use at IEMPAM, BAS.

Results

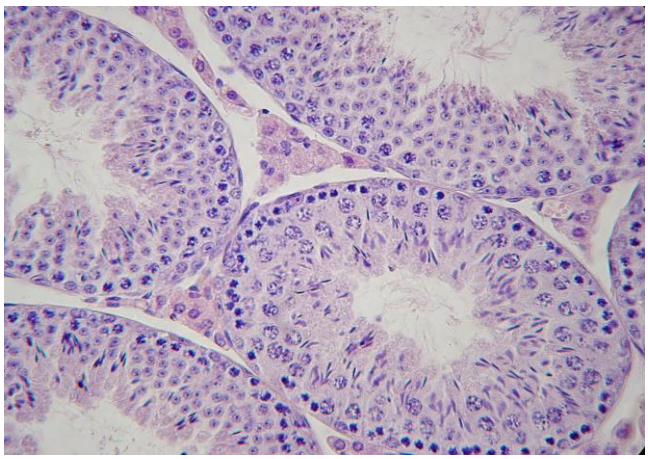
Histological evaluation of the testis

Histological sections of the testis were examined to determine whether the reduction in fertility of treated mice, reported in the literature, was in part due to a direct effect of cobalt on the structure of the testes. The most obvious changes in testis morphology we observed in early maturity and adulthood after treatment with high doses of both Co compounds. On day 45 spermatogenesis in the mouse is not completed but in control testis all the stages of spermatogentic cycle can be seen. Histological observation showed presence of mature spermatozoa and their release into the tubular lumen in stages VII-VIII of the cycle (Figure 1-A). On day 60 spermatogenesis is organized in 12 stages of classification by Clermont and Perey[7] and germ cells are arranged in 5-6 layers in seminiferous epithelium (Figure 2-A). After administration of high doses of $CoCl_2$ or Co-EDTA we found depletion of germ cells and retardation in germ cell development. Seminiferous epithelium was disorganized and undifferentiated germ cells were sloughed off in luminal area in many tubules (Figure 1-B, C; 2-B, C). SCO (Sertoli-cell-only) tubules were also seen (Figure 1-B; 2-C). In adult mice treated with high doses of both Co compounds we observed thinner seminiferous epithelium with less germ cells in the tubules. The diameter of seminiferous tubule was reduced probably due to impaired spermatogenesis.

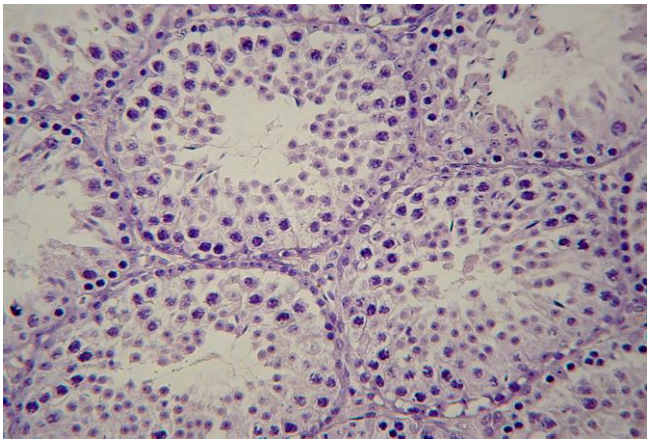
Quantitative data

Changes in the testes weight (TW) are indicative for altered spermatogenesis. In early puberty (d18) we found more severe effect of low dose of $CoCl_2$ compared to Co-EDTA that was significant whereas high doses of both compounds induced similar reduction of 25% compared to control TW (Figure 3). On day 45 and day 60 after administration of 75 mg/kg of $CoCl_2$ and Co-EDTA, TW remained in normal range and no difference was found between both groups. In contrast to puberty, during maturity and in adulthood $CoCl_2$ -125 mg kg^{-1} induced significantly more damaging alterations in TW (respectively 35% and 30% lower than control on days 45 and 60) compared to Co-EDTA (15% reduction than control value on day 60, that is not significant). Simultaneously, gonado-somatic index (ratio TW to body weight) in late ages was also considerably more sensitive to $CoCl_2$ as compared to Co-EDTA (data not shown).

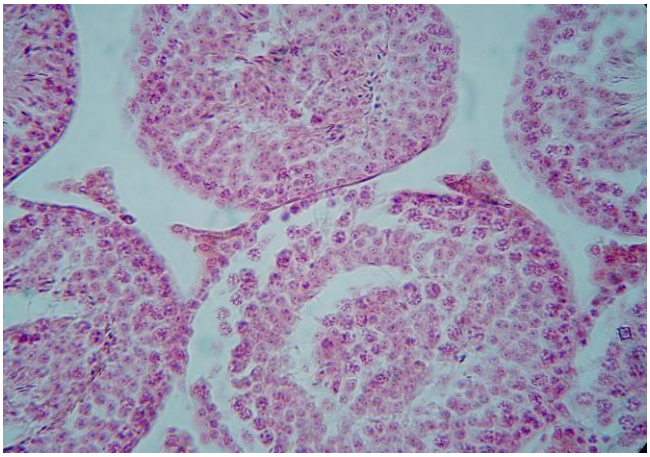
Evaluation of sperm count accumulated and isolated from vas deferens in maturity and adulthood demonstrated a wide deviation in this parameter in all of the experimental groups. Low doses of both Co compounds induced similar changes of sperm count on day 45, whereas high dose of $CoCl_2$ lead to 70% reduction of the investigated parameter compared to 35% decrease after Co-EDTA (Figure 4). Difference between the effects of high doses of both compounds was significant on day 60.



(A) Control



(B) $CoCl_2$ – 125 mg kg^{-1} d^{-1}



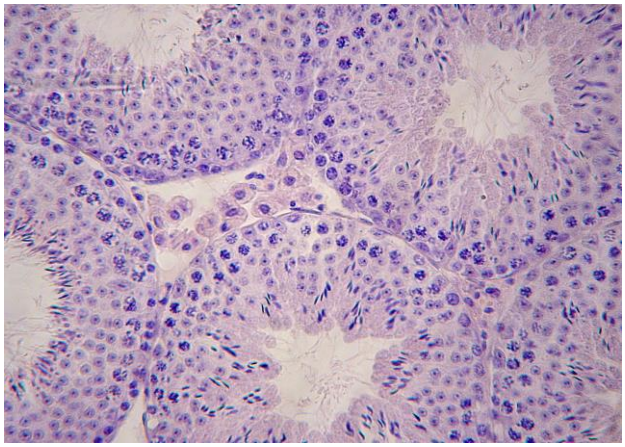
(C) Co-EDTA – 125 mg kg^{-1} day^{-1}

Figure 1. Morphology of the seminiferous tubules on testis cross sections of 45-day old mice. HE, x 400. Depletion of germ cells and retardations in germ cell development after treatment with high doses of $CoCl_2$ or Co-EDTA (125 mg kg^{-1} d^{-1}) was observed.

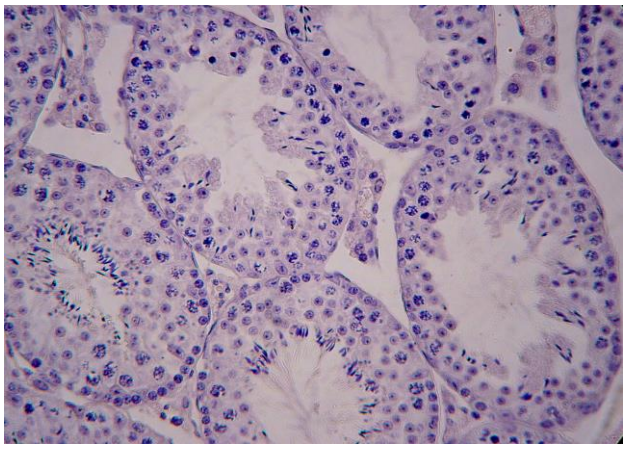
 DOI: 10.17628/ECB.2012.1.387

Discussion

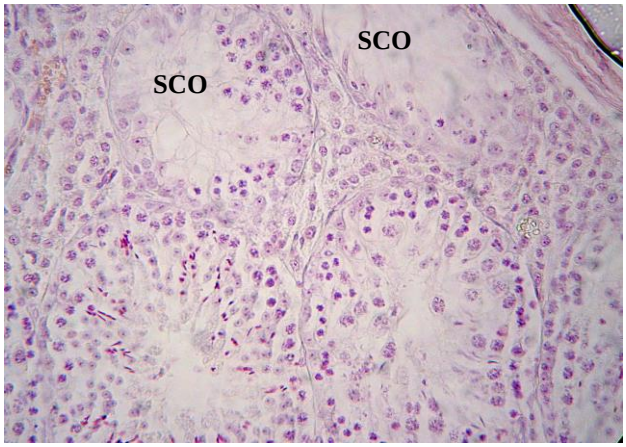
The experimental results showed that treatment with $CoCl_2$ and Co-EDTA induced a lot of abnormalities in testis morphology that could later affect male fertility. Testicular histology of mature and adult animals was not different in mice treated by both Co compounds.



(A) Control



(B) $CoCl_2$ – 125 mg kg^{-1} day^{-1}



(C) Co-EDTA – 125 mg kg^{-1} day^{-1}

Figure 2. Morphology of the seminiferous tubules on testis cross sections of 60-day old mice. HE, x 400. Depletion of germ cells and disorganization of seminiferous epithelium after treatment with high doses of $CoCl_2$ or Co-EDTA (125 mg kg^{-1} day^{-1}) was observed. Presence of SCO (Sertoli-cell-only) areas.

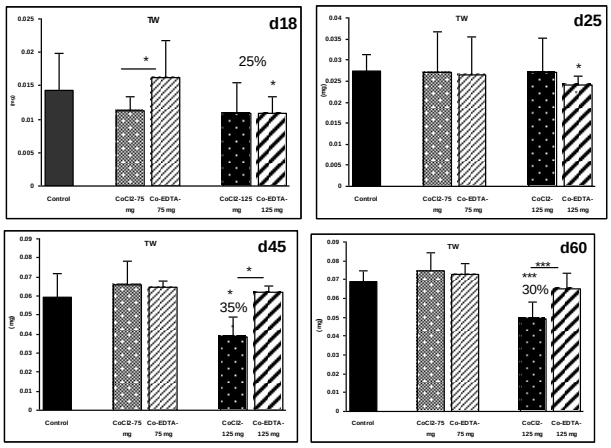


Figure 3. Changes in testis weight (TW) in different stages of development in mouse after treatment with $CoCl_2$ or Co-EDTA at two daily doses of 75 mg kg^{-1} or 125 mg kg^{-1}.

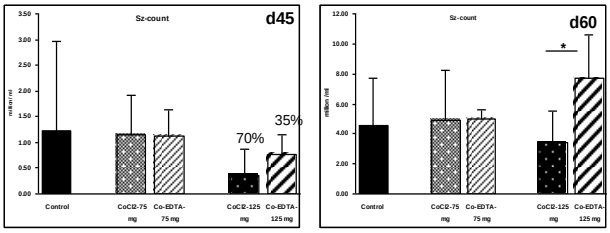


Figure 4. Changes in spermatozoa count in early and late maturity (day 45 and 60) in mouse after treatment with $CoCl_2$ or Co-EDTA at two daily doses of 75 mg kg^{-1} or 125 mg kg^{-1}.

After chronic exposure to cobalt Bitner et al.[8] and Elbetieha et al.[5] reported structural changes in the testis including necrosis and degeneration of seminiferous epithelium. Our data support the findings of Lukac et al.[9] about sloughing of germ and Sertoli cells and shrinkage of the seminiferous tubules. Formation of empty spaces within the epithelium was also described (SCO areas). In the literature, morphometric analysis was reported to reveal significant decrease in relative volume of seminiferous epithelium in cobalt treated animals, whereas the relative volume of interstitium was significantly increased. Larger interstitium is probably due to increased Leydig cell (LC) volume that could predict elevated testosterone (T) levels. Pedigio et al.[10] presumed that cobalt interferes with local regulatory mechanisms in testosterone synthesis. Experimental treatment with cobalt influenced LC steroidogenesis - serum T-levels were significantly increased, while FSH and LH serum levels remained normal. Besides possible indirect effect on spermatogenesis cobalt readily crosses the blood-testis barrier. Subsequently, a direct cytotoxic effect of cobalt on spermatogenic and Sertoli cells is possible[11]. Mollenhauer et al.[12] suggested that testicular degeneration was not a primary response to cobalt but the testes become hypoxic due to both the blockage of veins and arteries by red blood cells and to the changes in permeability caused by thickening of basal lamina.

In early puberty (d18) we observed similar effect of high doses of $CoCl_2$ and Co-EDTA on testis weight whereas in maturity and adulthood $CoCl_2$ affects more severe this

 DOI: 10.17628/ECB.2012.1.387

parameter compared to Co-EDTA. Our finding is probably due to the stability of the complex Co-EDTA and its weaker absorption. Bitner et al.[8] and Elbetieha et al.[5] reported decreased weight of testes and epididymides while weight of seminal vesicles and preputial glands was significantly increased. It has been noted that the size and activity of secondary sex glands is clearly influenced by a variety of steroid hormones. Duration of cobalt exposure is very important for the subsequently induced abnormalities and for the following period of recovery[4]. Probably, the observed effects in adult animals are due to accumulated Co in the organism and its effect on testis morphology and TW.

Our data for reduced sperm count were in good agreement with previous studies of the rodent testis after chronic Co intoxication[10]. Impairment in this parameter was dose-dependent and it increased as the concentration of cobalt increased. More pronounced were the changes in sperm count after high dose of $CoCl_2$. These findings suggest that the effects of cobalt (II) depend on the type of compound used and on stability of its complex. Time duration and age of the experimental animals are also important.

In conclusion, our data and findings indicate that exposure to cobalt during perinatal and postnatal period affected testis morphology in mature and adult animals; reduced TW in pubertal and mature animals and decreased epididymal sperm count. The effects of $CoCl_2$ were more pronounced as compared to Co-EDTA induced alterations.

Acknowledgements

This work was supported by grants No DO 02 – 351/2008 and DMU 03/25 for Young scientists from the Bulgarian National Science Fund.

References

[1]Gal, G., Hursthouse, A., Tatner, P., Stewart, F., Welton R., *Environ. Intern.* **2008**, *34*(6), 821.

[2]World Health Organization. *In: Concise International Chemical Assessment Document* **2006**, *69***,** 13.

[3]Lauwerys, R., Lison, D. *Sci. Tot. Environ.* **1994**, *150*(1-3), 1.

[4]Madzhariva, M., Pavlova, E., Atanassova, N. *Acta Morphol. Anthropol.* **2010**, *16*, 113.

[5]Elbetieha, A., Al-Thani, A. S., Al-Thani, R. K., Darmani, H., Owais, W. *J. Applied Biol. Sci.* **2008,** *2*(1), 1.

[6]Gluhcheva, Y., Atanassov, V., Zhorova, R., Madzharova, M., Ivanova, J., Mitewa, M. *Boitechnol. & Biotechnol. Eq.* **2010**, *24*, 311.

[7]Clermont, Y., Perey, B. *Am. J. Anat.* **1957**, 100 (2), 241.

[8]Bitner, A. M., Pedigo, N. G., Katz, R. P., George, W. J. *Reprod. Toxicol*. **1992**, *6*(1), 41.

[9]Lukac, N., Massanyi, P., Zakrewski, M., Toman, R., Cigankova, V. Stawarz, R., *J. Environ. Sci Health Part A*, **2007**, *42*, 389.

[10]Pedigo, N. G., George, W. J., Anderson, M. B. *Reprod. Toxicol.*, **1988**, *2*(1), 45.

[11]Corrier, D. E., Mollenhauer, H. H., Clark, D. E., Hare, M. F., Elissalde, M. H. *Vet. Pathol.* **1985**, *22*, 610.

[12]Mollenhauer, H. H., Corrier, D. E., Clark, D. E., *Cell Pathol.*, **1985**, *49*(3), 241.

Received: 15.10.2012.
Accepted: 01.11.2012.