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CHANGES IN RAT TESTIS AND SPERM COUNT AFTER ACUTE TREATMENT WITH SODIUM NITRITE

E. PAVLOVA, D. DIMOVA, E. PETROVA, Y. GLUHCHEVA and N. ATANASSOVA

Abstract

Sodium nitrite ($NaNO_2$) is an inorganic salt with various industrial applications. The adverse health effects of $NaNO_2$ in animals and humans are typically due to the formation of methemoglobin in the blood. This can lead to cyanosis and, at very high levels, death. Humans are constantly exposed to sodium nitrite through food and drinking water. The aim of the present study is to follow up the changes in rat testis and sperm count after acute treatment with $NaNO_2$. Four-month-old male Wistar rats were intraperitoneally injected with $NaNO_2$ at dose of 50 $mg.kg^{-1}$ body weight (distilled water for controls). Treated animals were sacrificed at different time intervals (1 h, 5 h, 24 h and 48 h) following the administration. Testes and epididymides were sampled, weighed and embedded in paraffin using routine histological practice. Spermatozoa were isolated from both vasa deferentia and counted. Preliminary histological observations of the testis of some experimental animals demonstrated disorganization of seminiferous epithelium and assemblance of undifferentiated germ cells in the luminal area of the tubules. Testis weight/body weight index was increased in the first hours after administration, which is probably due to higher seminal fluid volume. Statistically significant reduction in sperm count ranging between 480 $g.kg^{-1}$ (fifth hour) and 280 $g.kg^{-1}$ (48 h) was observed. These results may be associated with impaired hormonal balance and tissue anoxia - an adverse environment for germ cell development. In conclusion, acute treatment with $NaNO_2$ affects testis morphology, some weight indices and sperm count in mature rats.

Key words: rat testis, sodium nitrite, sperm count

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EFFECT OF COBALT ON MALE REPRODUCTIVE ORGANS DURING PUBERTY

M. MADZHAROVA, Y. GLUHCHEVA, E. PAVLOVA AND N. ATANASSOVA

Abstract

Cobalt is an essential oligoelement for mammals. It is not a cumulative toxin but chronic exposure induces negative effects on the organism. Data from the literature evidenced that in experimental animals cobalt impaired male reproductive organs and fertility when applied chronically. The aim of our study is to follow the effect cobalt on pubertal male progeny of female mice treated with cobalt in late pregnancy and during suckling period. Macroscopic parameters as weight of male reproductive organs and organ/body weight ratio were established. Significant reduction in body weight and 20% decrease (non significant) of testicular and epididymal weight as well as in testis/body weight index was found. The impact of cobalt on male progeny could be explained with transplacental route of exposure and with possible transfer of cobalt into mothers' milk. The negativ e effect of cobalt was not seen in mid puberty (day 25) with the exception of epididymal weight which was not compensated suggesting that epididymis is more sensitive to cobalt treatment. In conclusion, our data indicate that exposure to cobalt during perinatal and postnatal period affected body weight during puberty but not significantly reduced reproductive organs growth. However, negative impact of cobalt on later life could not be rule out and cobalt might be considered as possible risk factor for male reproductive health.

Keywords: male, puberty, reproductive organs, cobalt

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LOSS AND RECOVERY OF ANDROGEN RECEPTOR PROTEIN EXPRESSION IN THE ADULT RAT TESTIS FOLLOWING ANDROGEN WITHDRAWAL BY ETHANE DIMETHANESULFONATE

NINA ATANASSOVA, YVETTA KOEVA, MARIANA BAKALSKA, EKATERINA PAVLOVA, BOYCHO NIKOLOV AND MICHAIL DAVIDOFF

Abstract

Androgens are especially important for the maintenance of spermatogenesis in adulthood and the experimental withdrawal of testosterone (T) production by ethane dimenthanesulfonate (EDS) is a valuable tool for studying androgen-dependent events of spermatogenesis. The aim of the present study was to investigate the specific changes in immunoexpression of androgen receptor (AR) in the testis in relation to degeneration and regeneration of Leydig cell (LC) population and seminiferous epithelium. Immunohistochemistry for AR and 3P-hydroxysteroid dehydrogenase (3P-HSD) as well as TUNEL assay for apoptosis were performed on testicular sections of control and EDS-treated rats. Serum LH and T levels were measured by RIA. Our results revealed a total loss of AR immunoexpression from the nuclei of Sertoli (SCs), LCs and peritubular cells during the first week after EDS administration and that coincided with severe drop in T levels. Two weeks after EDS administration, the AR expression was recovered in these cells but normal stage-specificity in SCs was replaced by uniform intensity of AR immunostaining at all the stages of the spermatogenic cycle. The stage-specific pattern of androgen expression in SCs with a maximum at stages VII-VIII appeared 5 weeks after treatment. LC immunoreactivity for 3 P-HSD at different time points after EDS administration correlated with values of T concentration. The maximal germ cell apoptosis on day 7 was followed by total loss of elongated spermatids 2 weeks after EDS treatment. Regeneration of seminiferous epithelium 3 weeks after EDS administration and onwards occurred in tandem with the development of new LC population indicated by the appearance of 3P-HSD-positive cells and gradual increase in T production. The specific changes in AR after EDS including their loss and recovery in Sertoli cells paralleled with degenerative and regenerative events in Leydig and germ cell populations, confirming close functional relationship between Sertoli, Leydig and germ cells.

Key words: Androgen receptor, Sertoli cells, Leydig cells, Spermatogenesis, Testis, EDS

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LOSARTAN SUPPRESSES THE KAINATE-INDUCED CHANGES OF ANGIOTENSIN AT1 RECEPTOR EXPRESSION IN A MODEL OF COMORBID HYPERTENSION AND EPILEPSY

DIMITRINKA ATANASOVAA, JANA TCHEKALAROVAA, NATASHA IVANOVA, ZLATINA NENCHOVSKA, EKATERINA PAVLOVA, NINA ATANASSOVA, NIKOLAI LAZAROV

Abstract

Aims: Experimental and clinical studies have demonstrated that components of renin-angiotensin system are elevated in the hippocampus in epileptogenic conditions. In the present work, we explored the changes in the expression of angiotensin II receptor, type 1 (AT1 receptor) in limbic structures, as well as the effect of the AT1 receptor antagonist losartan in a model of comorbid hypertension and epilepsy. Main methods: The expression of AT1 receptors was compared between spontaneously hypertensive rats (SHRs) and Wistar rats by using immunohistochemistry in the kainate (KA) model of temporal lobe epilepsy (TLE). The effect of losartan was studied on AT1 receptor expression in epileptic rats that were treated for a period of 4 weeks after status epilepticus. Key findings: The naive and epileptic SHRs were characterized by stronger protein expression of AT1 receptor than normotensive Wistar rats in the CA1, CA3a, CA3b, CA3c field and the hilus of the dentate gyrus of the dorsal hippocampus but fewer cells were immunostained in the piriform cortex. Increased AT1 immunostaining was observed in the basolateral amygdala of epileptic SHRs but not of epileptic Wistar rats. Losartan exerted stronger and structure-dependent suppression of AT1 receptor expression in SHRs compared to Wistar rats. Significance: Our results confirm the important role of AT1 receptor in epilepsy and suggest that the AT1 receptor antagonists could be used as a therapeutic strategy for treatment of comorbid hypertension and epilepsy.

Keywords: AT1 receptors, Losartan, Kainate, Spontaneously hypertensive rat, Wistar rat

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COBALT ACCUMULATION AND IRON-REGULATORY PROTEIN PROFILE EXPRESSION IN IMMATURE MOUSE BRAIN AFTER PERINATAL EXPOSURE TO COBALT CHLORIDE

EMILIA PETROVA, EKATERINA PAVLOVA, ALEXEY A. TINKOV, OLGA P. AJSUVAKOVA, ANATOLY V. SKALNY, PAVEL RASHEV, IVELIN VLADOV, YORDANKA GLUHCHEVA

Astract

Developing brain is very sensitive to the influence of environmental factors during gestation and the neonatal period. The aim of the study is to assess cobalt and iron accumulation in the brain as well as changes in the expression of iron-regulatory proteins transferrin receptor 1, hepcidin, and ferroportin in suckling mice. Perinatal exposure to cobalt chloride increased significantly cobalt content in brain tissue homogenates of 18-day-old (d18) and 25-day-old (d25) mice inducing alterations in brain iron homeostasis. Higher degree of transferrin receptor 1 expression was demonstrated in cobalt chloride-exposed mice with no substantial changes between d18 and d25 mice. A weak ferroportin expression was found in 18-day-old control and cobalt-treated mouse brain. Cobalt exposure of d25 mice resulted in increased ferroportin expression in brain compared to the untreated age-matched control group. Hepcidin level in cobalt-exposed groups was decreased in d18 mice and slightly increased in d25 mice. The obtained data contribute for the better understanding of metal toxicity impact on iron homeostasis in the developing brain with further possible implications in neurodegeneration.

Keywords: Cobalt, Iron, Immature brain, Transferrin receptor 1, Hepcidin, Ferroportin

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IMPACT OF ENVIRONMENTAL ENDOCRINE DISRUPTORS ON SPERMATOGENESIS IN RAT EXPERIMENTAL MODEL

M. MADZHAROVA, E. PAVLOVA, N. ATANASSOVA, R. SHARPE

Abstract

The human being is exposed to a great variety of endocrine disruptors (EDs), widely spread in environment and they are potential risk for male reproductive health and fertility. The present study aimed to investigate the influence of EDs with estrogenic activity on testis development and function. Seven experimental groups were used and rats were treated neonatally with different oestrogen active compounds (diethylstilbestrol /DES/, Bisphenol-A /Bis-A/, octylphenol /OP/ and genistein). The oestrogen effect was evaluated by using a complex system of functional and morphological criteria. Data showed dosedependent effect of DES (inhibitory for medium and high doses and stimulatory for low dose) on the testis during puberty and in adulthood. Genistein affects negatively but temporary spermatogenesis in puberty, while Bis-A and OP has slight stimulatory effect. No significant effect was observed in the adulthood neither for genistein treated group nor for animals injected with Bis-A and OP. In conclusion potent synthetic estrogens are serious risk factor for male reproductive health while there is slight possibility for human to be exposed on industrial estrogens to such high doses that could affect male fertility. However, accelerative effect of industrial oestrogrens on onset of puberty should be interpreted carefully because such effect could have consequences in later life.
Keywords: endocrine disruptors, environment, spermatogenesis

Comptes rendus de l'Acad´emie bulgare des Sciences, Tome 67, No 3, 2014

NEONATAL EXPOSURE TO PHYTOESTROGENS-INDUCED ELEVATION OF DIFFERENT TESTIS PARAMETERS IN ADULT WISTAR RATS AND COMMON MARMOSET MONKEYS

EKATERINA PAVLOVA, NINA ATANASSOVA, RICHARD SHARPE

Abstract

In Western countries, many infants are fed with formula milk instead of being breastfed. Although feeding with formula milk was restricted initially to cow's milk-based formulae (SMA), within the last half century it has become common in some parts of the world for infants to be fed with soy formula milk (SFM). That provokes intense debate whether or not feeding with SFM may impair the reproductive development and function of these male infants. In this respect the aim of our work was to elucidate whether the phytoestrogens might have long-term consequences in terms of reproductive functions in adulthood of soy formula-fed male infants. We compared the effects of neonatal exposure of male rats to genestein with data from common marmoset monkeys (Callithrix jacchus) in which co-twin males were fed during the neonatal period with either SMA or SFM. Testicular development and function was assessed in adult males. Our findings showed that testicular weight (TW), lumen of seminiferous tubules as well as Sertoli cell (SC) number were increased in animals on soy-rich diet of both species whereas germ (GC) number was elevated only in marmosets but not in rats. Gonadotrophin levels (both LH and FSH) are known to be elevated in soy-fed males that could explain the increase of SC-, GC number and, respectively the TW. Although soy-rich food does not exert dramatically adverse reproductive consequences, these "positive" changes should be interpreted carefully especially when they interfere with the hormonal balance and in this regard growth and/or development of the organism.

Key words: marmoset, rat, phytoestrogens, spermatogenesis

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CHRONIC EXPOSURE TO COBALT COMPOUNDS – AN IN VIVO STUDY

YORDANKA G. GLUHCHEVA, VASIL N. ATANASOV, JULIANA M. IVANOVA, EKATERINA H.PAVLOVA

Abstract

An in vivo experimental model for testing the effects of long-term chronic treatment with cobalt(II) compounds – cobalt chloride (CoCl2) and cobalt-EDTA (Co-EDTA) on mice at different stages of development was optimized. Pregnant mice and their progeny were treated with daily doses of 75 or 125 mg kg-1 body weight until postnatal day 90. The compounds were dissolved in regular tap water. Mice were sacrificed on days 18, 25, 30, 45, 60 and 90 after birth, which correspond to different stages of their development. Altered organ weight indices (calculated as a ratio of organ weight to body weight) of spleen, liver and kidneys, were found depending on the type of compound used, dose, duration of treatment, and the age of the animals. The results also showed significant accumulation of cobalt ions in blood plasma, spleen, liver and kidneys of the exposed mice. More Co(II) was measured in the organs of the immature mice (day 18, 25 and 30 pnd) indicating that they were more sensitive to treatment.

Keywords: Cobalt chloride, Cobalt EDTA, Mice, Blood plasma, Spleen, Liver, Kidney

Comptes rendus de l'Acad'emie bulgare des Sciences, Tome 69, No 11, 2016

EXPRESSION OF TESTICULAR ANGIOTENSIN CONVERTING ENZYME (tACE) IN EXPERIMENTAL CONDITION OF ANDROGEN DEFICIENCY IN RAT

NINA ATANASSOVA, EMILIA LAKOVA, EKATERINA PAVLOVA, DONIKA DIMOVA, YVETA KOEVA

Abstract

Testicular ACE is expressed in spermatids and spermatozoa being essential for fertilization. Experimental model of EDS treatment is valuable tool for investigation of androgen regulation of spermatogenesis. The current paper aimed to study the expression pattern of tACE in germ cells of androgen deficient rat testes. Such study would provide new data about androgen regulation of tACE. Expression of rat tACE started and reached maximal expression in androgen dependent stage VIII of spermatogenic cycle suggesting androgen regulation of tACE. Our current data suggested that depletion and recovery of elongating spermatids after EDS treatment occurred in stage-specific pattern. Application of tACE as a marker for postmeiotic stages of spermatogenesis provided a new tool for investigation of germ cell differentiation under pathological and experimental conditions. In particular, tACE can be recommended for precise visualization and evaluation of spermatid loss that is not optional by routine histological technique.

Key words: testicular ACE, spermatogenesis, androgens, rat

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COMPARATIVE ASSESSMENT OF THE EFFECTS OF SALINOMYCIN AND MONENSIN ON THE BIODISTRIBUTION OF LEAD AND SOME ESSENTIAL METAL IONS IN MICE, SUBJECTED TO SUBACUTE LEAD INTOXICATION

JULIANA IVANOVA, YORDANKA GLUHCHEVA, DONIKA DIMOVA, EKATERINA PAVLOVA, SONJA ARPADJAN

Abstract

In this this study, we present a comparative assessment of the effects of two polyether ionophorous antibiotics(monensin and salinomycin) on the concentrations of lead (Pb), cooper (Cu), zinc (Zn) and iron (Fe) in thekidneys, spleen, liver and brain of Pb-intoxicated animals. Our data demonstrated that the intoxicationof ICR male mice with Pb salt resulted in a significant accumulation of Pb in all studied organs of themice compared to the untreated control animals. The biodistribution of the toxic metal was in the orderkidneys > spleen > liver > brain. The treatment of the Pb-intoxicated animals with tetraethylammoniumsalts of monensic and salinomycinic acids significantly decreased the concentration of the toxic metalion compared to the toxic control. The effect varied in the interval 38% (for kidneys) to 52% (for brain)compared to the toxic control group (Pb). The tetraethylammonium salt of salinomycinic acid was moreeffective in reducing the Pb concentration in the brain of the Pb-treated mice compared to monensin.Pb-intoxication did not affect significantly the Zn endogenous concentration compared to the normalvalues. The treatment of ICR male mice with Pb-salt decreased the Cu concentration in the spleen andincreased the Cu concentration in the liver compared to the untreated control animals. The detoxificationof the Pb-intoxicated mice with tetraethylammonium salts of salinomycinic and monensic acids restoredthe Cu concentration in the spleen, but did not affect the Cu levels in the liver. The Pb-intoxication ofthe ICR mice resulted in a significant decrease of the Fe-concentration in the spleen and liver comparedto the untreated control animals. The administration of the tetraethylammonium salts of salinomycinicand monensic acids to the Pb-treated animals restored the levels of Fe in both organs.

Keywords: Pb intoxication, Monensin, Salinomycin, Chelation therapy, *In vivo* models, Trace elements

Endocrinology, February 2019, 160(2):387–398

TUMOR SUPPRESSOR PTEN REGULATES NEGATIVELY SERTOLI CELL PROLIFERATION, TESTIS SIZE, AND SPERM PRODUCTION IN VIVO

YASMINE NEIRIJNCK, FRANÇOISE KUHNE, CHLOE MAYERE, EKATERINA PAVLOVA, PAULINE SARAROLS, MICHELANGELO FOTI, NINA ATANASSOVA and SERGE NEF

Abstract

The IGFs are the major intratesticular factors regulating immature Sertoli cell proliferation and are, therefore, critical to establish the magnitude of sperm production. However, the intratesticular source of IGF production and the downstream signaling pathway mediating IGF-dependent Sertoli cell proliferation remain unclear. Single-cell RNA sequencing on mouse embryonic testis revealed a robust expression of Igf1 and Igf2 in interstitial steroidogenic progenitors, suggesting that IGFs exert paracrine actions on immature Sertoli cells. To elucidate the intracellular signaling mechanism that underlies the proliferative effects of IGFs on immature Sertoli cells, we have generated mice with Sertoli cell-specific deletion of the Pten gene, a negative regulator of the phosphatidylinositol3 kinase (PI3K)/AKT pathway, alone or together with the insulin receptor (Insr) and the IGF1 receptor (Igf1r). Although ablation of Pten appears dispensable for Sertoli cell proliferation and spermatogenesis, inactivation of Pten in the absence of Insr and Igf1r rescued the Sertoli cell proliferation rate during late fetal development, testis size, and sperm production. Overall, these findings suggest that IGFs secreted by interstitial progenitor cells act in a paracrine fashion to promote the proliferation of immature Sertoli cells through the IGF/PTEN/PI3K pathway.

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THE IMPACT OF PERINATAL COBALT CHLORIDE EXPOSURE ON EXTRAMEDULLARY ERYTHROPOIESIS, TISSUE IRON LEVELS, AND TRANSFERRIN RECEPTOR EXPRESSION IN MICE

YORDANKA GLUHCHEVA, EKATERINA PAVLOVA, EMILIA PETROVA, ALEXEY A. TINKOV, OLGA P. AJSUVAKOVA, MARGARITA G. SKALNAYA, IVELIN VLADOV, ANATOLY V. SKALNY

Abstract

The objective of the present study was to elucidate the effect of perinatal cobalt chloride (CoCl2) exposure on extramedullary erythropoiesis in suckling mice in relation to iron (Fe) content and transferrin receptor (TfR) expression. Pregnant ICR mice were subjected to a daily dose of 75 mg CoCl2/kg body weight 2–3 days prior and 18 days after delivery. Co exposure significantly increased erythrocyte count (RBC), and reduced the erythrocytic parameters mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in the offspring. Total iron-binding capacity (TIBC) was decreased while bilirubin values were ~ 1.2-fold higher in the metal-exposed mice. Perinatal CoCl2 treatment also induced pathohistological changes in target organs (spleen, liver, and kidneys) as altered organ weight indices, leukocyte infiltration, abundant Kupffer cells in the liver, increased mesangial cellularity, and reduced capsular space in the kidney. CoCl2 administration induced significant 68-, 3.8-, 41.3-, and 162-fold increase of Co content in the kidney, spleen, liver, and RBC, respectively. Fe content in the target organs of CoCl2-treated mice was also significantly elevated. Immunohistochemical analysis demonstrated that TfR1 was well expressed in the renal tubules, hepatocytes, the red pulp, and marginal zone of white pulp in the spleen. TfR2 showed similar expression pattern, but its expression was stronger in the spleen and liver samples of Co-treated mice compared with that of the untreated controls. The results demonstrate that exposure to CoCl2 during late pregnancy and early postnatal period affects body and organ weights and alters hematological and biochemical parameters, iron content, and TfR expression in target organs

Keywords: Cobalt, Iron, Suckling mice, Hematological indices, TfR

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ttps://doi.org/10.1007/s11356-020-10960-4

AMELIORATIVE EFFECTS OF DEFERIPRONE AND TETRAETHYLAMMONIUM SALT OF SALINOMYCINIC ACID ON LEAD-INDUCED TOXICITY IN MOUSE TESTES

EKATERINA PAVLOVA, IRENA PASHKUNOVA-MARTIC, MARTIN SCHAIER, EMILIA PETROVA, YORDANKA GLUHCHEVA, PETER DORKOV, THOMAS H. HELBICH, BERNHARD KEPPLER, GUNDA KOELLENSPERGER, JULIANA IVANOVA

Abstract

In this study, we compare the effects of deferiprone (Def) and tetraethylammonium salt of salinomycinic acid (Sal) on lead (Pb)-induced toxicity in testes of Pb-exposed mice. Mature male ICR mice were allocated into four groups as follows: untreated control mice (ctrl)—received distilled water for 4 weeks; Pb-exposed mice (Pb) - subjected to 14-day Pb (II) nitrate administration at dose 80 mg/kg body weight (b.w.); Pb + Def group - Pb-exposed mice, treated with 20 mg/kg b.w. Def for 2 weeks; and Pb + Sal group - Pb-intoxicated mice, treated with 16 mg/kg b.w. Sal for 14 days. The results demonstrated that Pb exposure significantly increased blood and testicular Pb concentrations, decreased testicular calcium (Ca) content, significantly elevated testicular levels of magnesium (Mg), zinc (Zn), and selenium (Se) but did not significantly affect the endogenous contents of phosphorous (P) and iron (Fe) compared with untreated controls. Pb intoxication induced disorganization of the seminiferous epithelium. Def or Sal administration reduced blood Pb and testicular Pb concentrations in Pb-exposed mice compared with the Pb-intoxicated group. Mg, Zn, and Se concentrations in testes of Pb-exposed mice, treated with Def or Sal, remained higher compared with the untreated controls. Sal significantly increased testicular P concentration compared with untreated controls and significantly elevated the testicular Ca and Fe concentrations compared with the toxic control group. Both chelating agents improved testicular morphology to a great extent. The results demonstrate the potential of both compounds as antidotes for treatment of Pb-induced impairment of male reproductive function.

Keywords: Deferiprone, Salinomycin, Lead, Male reproductive system, Mice, Essential elements

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EFFECT OF ACUTE SODIUM NITRITE INTOXICATION ON SOME ESSENTIAL BIOMETALS IN MOUSE SPLEEN

EMILIA PETROVA, YORDANKA GLUHCHEVA, EKATERINA PAVLOVA, IVELIN VLADOV, TSVETOMIL VOYSLAVOV, JULIANA IVANOVA

Abstract

Background and aim: Sodium nitrite ($NaNO_2$) is an inorganic salt with numerous applications in a variety of industries, as well as in medicine. Nevertheless, exposure to high levels of $NaNO_2$ is toxic for animals and humans. Sodium nitrite intoxication is shown to decrease the activity of major antioxidant defence enzymes which is dependent on the maintenance of specific ion equilibrium. The aim of the present study was to investigate the effect of acute $NaNO_2$ intoxication on the content of the essential metals iron (Fe), calcium (Ca) and zinc (Zn) in mouse spleen. Methods: Mature male ICR mice were divided into four groups and subjected to acute $NaNO_2$ exposure by a single intraperitoneal injection of 120 mg/kg body weight. Animals in each group were sacrificed at certain time interval after treatment (1 h, 5 h, 1 day and 2 days). Spleens were excised and processed for atomic absorption spectrometry analysis of Fe, Ca and Zn content. Results: At the first hour after treatment, a decrease in Fe and Ca levels was observed. One day following $NaNO_2$ administration, Zn concentration reached its lowest value and Ca levels remained lower, compared to the untreated controls. In contrast, Fe concentration increased on the first and second day after treatment. Conclusion: The results of the present study demonstrate that acute $NaNO_2$ intoxication provokes changes in the endogenous levels of Fe, Ca and Zn in mouse spleen. These findings suggest disruption of the ionic balance and impact on the activity of antioxidant defence enzymes

Keywords: Sodium nitrite, Mouse spleen, Iron, Calcium, Zinc

Biological Trace Element Research, (2021), 199:588–593

ALTERATIONS IN BLOOD METABOLIC PARAMETERS OF IMMATURE MICE AFTER SUBCHRONIC EXPOSURE TO COBALT CHLORIDE

IVELIN VLADOV, EMILIA PETROVA, EKATERINA PAVLOVA, ALEXEY A. TINKOV, OLGA P. AJSUVAKOVA, ANATOLY V. SKALNY, YORDANKA GLUHCHEVA

Abstract

The wide use of cobalt (Co) in food, industry, and medical devices requires full elucidation of its biological effects on tissues and organs. The aim was to assess serum metabolic alterations in immature mice after subchronic exposure to $CoCl_2$. Pregnant ICR mice were subjected to a daily dose of 75 mg cobalt chloride/kg body weight ($CoCl_2x6H_2O$) 2–3 days before they gave birth, and treatment continued until days 25 and 30 after delivery. The compound was dissolved in and obtained with regular tap water. ICP-DRC-MS analysis showed significantly elevated serum Co^{2+} and diverse alterations in metabolic parameters of 25- and 30-day old pups after exposure to $CoCl_2$. Cholesterol and urea levels were significantly elevated in day 25 mice while HDL-C and LDL-C were reduced. In day 30, Co-exposed mice LDL-C and triglycerides were significantly increased while the total cholesterol level remained unchanged. Alkaline phosphatase was significantly reduced in day 25 Co-exposed mice. Blood glucose level of Co-exposed mice remained close to the untreated controls. Total protein content was slightly increased in day 30 mice. Co exposure reduced albumin content and albumin/globulin ratio but increased significantly globulin content. Co administration showed strong correlation with cholesterol, urea, and HDL-C in both day 25 and 30 mice. Inverse correlation was found with alkaline phosphatase and albumin for day 25 and with triglycerides, globulin, and total protein content in day 30 Co-exposed mice. Subchronic $CoCl_2$ exposure of immature mice induced significant changes in key metabolic parameters suggesting possible further disturbances in energy metabolism, osteogenesis, and reproduction.
Keywords: Cobalt, Cholesterol, Urea, Alkaline phosphatase, Serum proteins, Immature mice

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N, N-DIMETHYLACETAMIDE, AN FDA APPROVED EXCIPIENT, ACTS POSTMEIOTICALLY TO IMPAIR SPERMATOGENESIS AND CAUSE INFERTILITY IN RATS

NUPUR KHERA, CHAFIK GHAYOR, ANNA K. LINDHOLM, EKATERINA PAVLOVA, NINA ATANASSOVA, FRANZ E. WEBER

Abstract

N, N-Dimethylacetamide is an FDA approved solvent widely used in pharmaceutical industry to facilitate the solubility of lipophilic, high molecular weight drugs with poor water solubility. However, the cytotoxic effects of DMA raises the concern about its use in clinical applications. In the present study, we address the effect of DMA on spermatogenesis. Male Sprague Dawley rats were injected intraperitoneally for 8 weeks, once a week at a dose of 862 mg/kg. Analysis of reproductive parameters revealed that DMA treated animals exhibit spermatid formation defects within the testis describing the characteristics of oligozoospermia. A subsequent decrease in epididymal sperm concentration along with distortion of sperm morphology was observed. The mitochondrial and microtubule organization in the sperm is considerably modified by DMA. This disrupts the sperm kinetics thus decreasing the total and progressive sperm motility. Finally, DMA treatment resulted in loss of fertility. Our results indicate that exposure to DMA has a negative impact on spermatogenesis and leads to infertility in male rats by inhibiting the post meiotic stages of sperm development. Therefore, the use of DMA in humans must be closely monitored.

Keywords: N N-Dimethylacetamide, Reproductive toxicology, Male infertility

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REVERSIBLE CONTRACEPTIVE POTENTIAL OF FDA APPROVED EXCIPIENT N, N-DIMETHYLACETAMIDE IN MALE RATS

KHERA NUPUR, GHAYOR CHAFIK, LINDHOLM ANNA K, PAVLOVA EKATERINA, ATANASSOVA NINA, WEBER FRANZ

Abstract

Development of an effective male contraceptive agent remains a challenge. The present study evaluates the potential of N, N-Dimethylacetamide (DMA), a FDA approved excipient as a male contraceptive agent. Male Sprague Dawley rats injected with DMA for a period of 8 weeks (one injection per week) showed a significant alteration of reproductive parameters. Furthermore, DMA treated animals showed complete infertility in a dose dependent manner, as no pups were born despite proper mating between females and DMA treated males. However, stopping the DMA treatment for a period of 8 weeks (after the initial treatment) restored the reproductive parameters to normal. Moreover, the fertility was resumed to normal as pups were born in the groups where DMA treatment was halted after initial DMA treatment. All these changes had no effect on the level of reproductive hormones FSH, LH and testosterone. Taken together, our results indicate that DMA acts in a reversible and non-hormonal manner to achieve contraception in rats. Therefore, repurposing the use of DMA could lead in a short time to an inexpensive and safer male contraceptive option.

Keywords: BET inhibitor, bromodomain (BRD), excipient, male contraception, rat, reproduction

Food and Chemical Toxicology, *Volume 149, March 2021, 111973*

PERINATAL AND EARLY-LIFE COBALT EXPOSURE IMPAIRS ESSENTIAL METAL METABOLISM IN IMMATURE ICR MICE

ANATOLY V. SKALNY, YORDANKA GLUHCHEVA, OLGA P. AJSUVAKOVA, EKATERINA PAVLOVA, EMILIA PETROVA, PAVEL RASHEV, IVELIN VLADOV, ROZA A. SHAKIEVA, MICHAEL ASCHNER, ALEXEY A. TINKOV

Abstract

The objective of the present study was to assess the impact of cobalt (Co) exposure on tissue distribution of iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn), as well as serum hepcidin levels in immature mice (18, 25, 30 days). Pregnant mice were exposed to 75 mg/kg b.w. cobalt chloride ($CoCl_2 \times 6H_2O$) with drinking water starting from 3 days before delivery and during lactation. At weaning (day 25) the offspring were separated and housed in individual cages with subsequent exposure to 75 mg/kg b.w. $CoCl_2$ until 30 days postnatally. Evaluation of tissue metal levels was performed by an inductively coupled plasma-mass spectrometry (ICP-MS). Serum hepcidin level was assayed by enzyme linked immunosorbent assay (ELISA). Cobalt exposure resulted in a time- and tissue-dependent increase in Co levels in kidney, spleen, liver, muscle, erythrocytes, and serum on days 18, 25, and 30. In parallel with increasing Co levels, $CoCl_2$ exposure resulted in a significant accumulation of Cu, Fe, Mn, and Zn in the studied tissues, with the effect being most pronounced in 25-day-old mice. Cobalt exposure significantly increased serum hepcidin levels only in day18 mice. The obtained data demonstrate that Co exposure may alter essential metal metabolism *in vivo*.

Keywords: Cobalt, Iron, Copper, Hepcidin, Toxicity