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Brain Morphological Changes in Immature Mice after Perinatal Exposure to Cobalt Chloride

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Abstract

Over the last years, human activities have considerably increased the levels of cobalt (Co) in the environment. Cobalt overexposure is associated with serious health risks, especially in children. The aim of the present study was to examine the effects of perinatal Co treatment on the brain morphology in immature mice. Eighteen-day-old mice were subjected to cobalt chloride ($CoCl_2$) exposure 2-3 days prior to birth and during the postnatal period. The histopathological studies revealed substantial cerebral and cerebellar damage with features of neuronal necrosis compared to the age-matched healthy controls. In the cerebrum, the neurons, glial cells and the neuropil were affected, as well the Purkinje cells in the cerebellum.
The results are indicative of the enhanced susceptibility of the immature brain to the exposure of cobalt. They contribute to the elucidation of the neurotoxic potential of the metal and the related health risks in newborns and infants, especially in regions with cobalt pollution.

Key words: cobalt chloride, immature mouse brain, morphological changes

Introduction

Cobalt is a naturally-occurring trace element with a wide range of industrial and medical applications. It is essential to mammals and, being a key component of vitamin B12, cobalt is necessary for a variety of biological processes. Nevertheless, the inorganic form of the metal is toxic and excessive levels can induce various adverse health effects. The intense anthropogenic activities utilizing cobalt and its compounds result in extensive environmental pollution and human exposure. In daily life, humans are exposed to Co through inhalation, drinking water and food. Occupational exposure is also considered as an important route of uptake, as well as internal exposure through cobalt containing implants [11].

Cobalt toxicity has been documented in animal and human studies. The available literature data indicate that cobalt has also a neurotoxic potential. It has been shown

that Co can induce neural cell death, neurotransmitter deficits and inhibition of the synaptic transmission, reactive oxygen species generation and behavioural alterations [2]. Though, there are scanty data for the effects of perinatal Co exposure during late prenatal and early postnatal period on brain morphology of the offspring. According to Garoui et al. [4], their study has evaluated for the first time cerebral and cerebellar damages induced by Co treatment during late pregnancy and early postnatal periods in rats. The present study was undertaken to investigate the brain morphological changes in suckling mice after perinatal cobalt chloride exposure.

Material and Methods

Animal model

Mature ICR mice were purchased from the Experimental and breeding base for laboratory animals (EBBLA) - Slivnitza, Bulgaria, and left to acclimatize for a week prior to the initiation of experiment. Animals were maintained in the Institute's animal breeding facility at 23±2°C and 12:12 h light/dark cycle in individual standard hard-bottom polypropylene cages. The mice were fed a standard diet and had access to food ad libitum with strong control of the feeding regime. Pregnant mice were subjected to a daily dose of 75 mg/kg body weight cobalt chloride ($CoCl_2 \times 6H_2O$) 2-3 days before they gave birth and treatment continued until day 18 after delivery. The compound was dissolved and administrated with drinking tap water. The suckling pups were sacrificed by decapitation after etherization on day 18. Brains were excised, weighed and processed for histological studies. Age-matched mice obtaining regular tap water were used as a control group.

The experimental design was carried out in accordance with guidelines EU Directive 2010/63/EU for animal experiments.

Histological analysis

Brains from control and Co-exposed mice were fixed in Bouin fixative for 24 h and paraffin-embedded. Briefly, after fixation, the samples were dehydrated in a graded series of ethanol, cleared with xylene, impregnated in molten paraffin, embedded in fresh molten paraffin and sectioned into 5-μm thickness sections using a microtome. Subsequently, the sections were stained with hematoxylin and eosin (HE) and observed on a light microscope Leica DM 5000B (Leica Microsystems, USA).

Results

Our observations in controls showed normal architecture of the cerebral and cerebellar cortex. Studies on cerebral samples demonstrated intact neurons with round nuclei, centrally located within the perikaryon, and prominent nucleoli (**Fig. 1 a, b**). Morphologically normal glial cells and brain capillaries were also observed.

The cerebellar cortex was composed of three layers: the deep granular layer, the Purkinje cell layer and the superficial molecular layer (**Fig. 2 a, b**). The Purkinje cells (PC) were well differentiated and arranged in a single row of large neurons with pear-shaped perikaryon and large nucleus. A single binucleate PC in the granular layer was also observed, as well a few mononucleate PC with the same heterotopic location.

Perinatal exposure of suckling mice to cobalt chloride resulted in substantial brain morphological changes compared to the untreated control animals. The changes

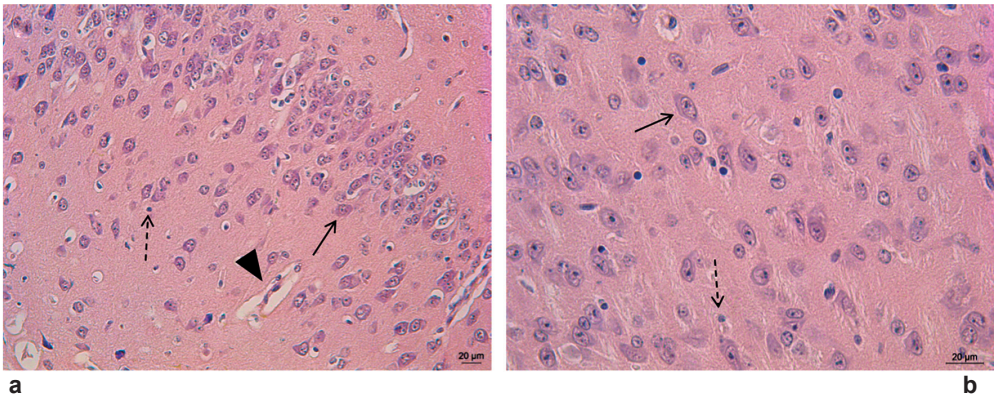

Fig. 1. Light microphotograph of the cerebral cortex of 18-day-old mice, hematoxylin-eosin staining. Untreated control, 200× (a), 400× (b): neurons (arrow) with normal nuclei, normal glial cells (dash arrow) and blood vessels (arrowhead).

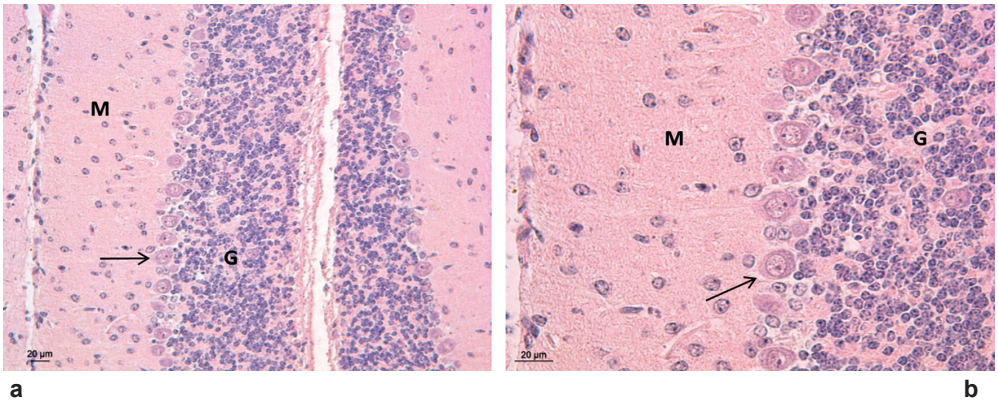

Fig. 2. Light microphotograph of the cerebellar cortex of 18-day-old mice, hematoxylin-eosin staining. Untreated control, 200× (a), 400× (b): different layers of the cerebellar cortex: molecular layer (M), Purkinje cell layer (arrow) and granular layer (G).

were accompanied by a significant decrease in the body weight of Co-exposed mice ($p<0.0002$). In contrast, brain weight index, calculated as organ-to-body weight ratio, was significantly increased by ~32.8% ($p<0.0001$) compared to the untreated age-matched control animals.

Histopathological studies in Co-treated mice demonstrated damaged cerebral and cerebellar cortices with features of neuronal necrosis compared to the untreated controls. In the cerebrum, abnormal basophilic neurons with atrophic shrunken perikarya and darkly stained pyknotic nuclei were present (**Fig. 1 c, d**). Prominent perineuronal and periglial spaces, as well as enlarged perivascular spaces and blood vessels with reactive endothelial cells were also observed. The neuropil appeared vacuolated.

In the cerebellum, along with the intact PC with distinctly stained nucleus, shrunken and darkly stained necrotic PC with enlarged pericellular spaces were found

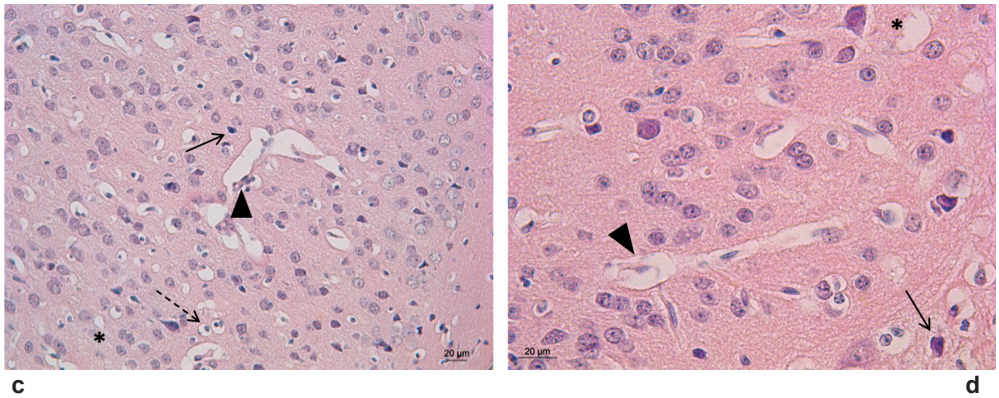

Fig. 1. Light microphotograph of the cerebral cortex of 18-day-old mice, hematoxylin-eosin staining. $CoCl_2$-exposed mice, 200× (c), 400× (d): neuronal necrosis characterized by many shrunken, darkly stained pyknotic neurons (arrow) with prominent perineuronal spaces; enlarged periglial spaces (dash arrow); dilated perivascular spaces and reactive endothelial cells (arrowhead) and vacuolation (asterisk).

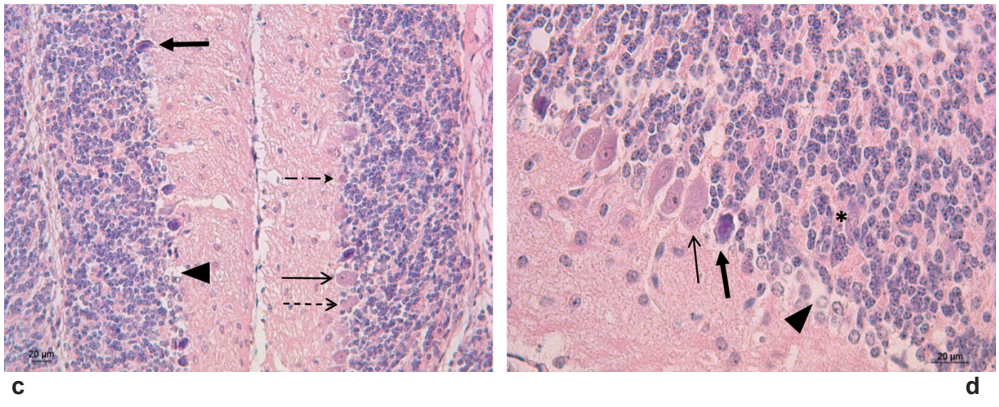

Fig. 2. Light microphotograph of the cerebellar cortex of 18-day-old mice, hematoxylin-eosin staining. $CoCl_2$-exposed mice, 200× (c), 400× (d): pyknotic PC (thick arrow), autolytic PC (dash arrow), PC with irregular shape (dash-dot arrow), loss of PC (arrowhead), binucleate PC (thin arrow), heterotopic PC in the granular layer (asterisk).

(**Fig. 2 c, d**). Some autolytic PC lacking nuclear staining and fading out of cytoplasm were observed. Our findings also demonstrated PC with irregular shape, loss of PC, a few binucleate PC, as well as PC in the granular layer.

Discussion

The results of the present study reveal that perinatal exposure of mice to $CoCl_2$ during late prenatal and early postnatal period affects the weight and the architecture of their brains. It is known that the developing central nervous system (CNS) is most sensitive to the influence of environmental factors namely throughout gestation and during the neonatal period. Moreover, the cerebellum is considered more vulnerable to neurotoxic agents than other brain regions [4, 5] and this is well demonstrated by our histopathological findings.

Besides neurotoxic, $CoCl_2$ is known as a hypoxia-mimicking agent which activates hypoxia-mediated signaling pathways. It is the most frequently used agent in both *in vivo* and *in vitro* experimental models for inducing chemical hypoxia [16]. Cobalt ions can substitute the iron ions in prolyl hydroxylases, the key enzymes in the regulation of oxygen homeostasis and the response to hypoxia. Thus, the enzyme activity is inhibited, leading to the accumulation of hypoxia-inducible factor-1α.

Our findings for significantly lower body weight of mice born to mothers exposed to $CoCl_2$ are in agreement with the results obtained in hypoxia models. For example, Minior et al. [14] and Tong et al. [19] demonstrate that chronic hypoxia during late pregnancy caused a significant reduction in the body weight of rat pups. The authors also report reduced brain size, indicating a developmental retardation via the activation of cell death pathways. In fact, fetal and postnatal brain damage due to hypoxia is well documented and is reported to depend on the duration, intensity of oxygen deprivation and age of the fetus and the pup [12]. In contrast, we estimated increased brain weight and brain weight index in Co-exposed suckling mice. A similar tendency of brain weight increase is associated with cerebral edema in many neurological conditions (hemorrhagic stroke, ischemic stroke, traumatic brain injury, brain tumors, etc.) [7].

Cobalt is among the elements actively transported across the placenta and the breast milk, causing temporary or permanent brain injury in the offspring [4, 9]. In the present study, the histopathological manifestation of Co-induced brain damage in the suckling mice is well demonstrated. The changes include vacuolation in the neuropil and different neural cell types affected by the exposure. Some cortical neurons appeared shrunken with pyknotic nuclei and enlarged perineuronal spaces. Increased periglial and perivascular spaces were also observed, as well reactive capillary endothelial cells. Our findings are in agreement with the results of Mohamed et al. [15], obtained for the adult $CoCl_2$-exposed rat cerebrum. In contrast, the study of Garoui et al. [4] in $CoCl_2$-exposed suckling rats indicated a normal structure of the treated cerebrums.

The Purkinje cells in our study showed a heterogeneous pattern of changes. They appeared shrunken with pyknotic nucleus, autolytic with no nucleus and no distinct cellular morphology, irregular in shape. Areas with loss of PC were also observed. Similar findings in the cerebellum were reported by Garoui et al. [4]. Their study was performed on 14-day-old rats, and at this postnatal age, the cerebellar cortex is still consisted of four layers. The authors observe markedly developed external granular layer which is suggested to be an indication of a delayed migration of granular cells towards the molecular layer and the internal granular layer.

Our histological examination in $CoCl_2$-exposed suckling mice showed some PC present in the granular layer. Since we have found a single PC with this heterotopic location in the untreated mice, it may be due to defective migration that causes the Purkinje cells to end-up trapped in the white matter tracts or granular layer [10]. In the mice born to $CoCl_2$-treated mothers, Purkinje cells in the granular layer could be a result of the exposure, as the same heterotopic location has been reported following prenatal exposure to X-radiation [3].

Moreover, binucleate PC were observed both in control and Co-exposed suckling mice. It has been shown that under normal physiological conditions, although extremely rarely, migration and fusion of bone marrow-derived stem cells with neuronal cells, predominantly Purkinje cells, occurs [20]. Paltsyn and Komissarova [18] suggest that the appearance of the second nucleus is a form of physiological and reparative regeneration of Purkinje cells. Because of the low frequency of Purkinje cell fusion under normal physiological conditions, some authors hypothesize that its role is negligible and fusion is a transient event [8, 17]. Magrassi et al. [13] suggest that this cell fusion represents a physiological phenomenon to introduce young nuclei or functional genes in

aged or degenerating cells. In fact, binucleate PC are more frequently demonstrated in old and sick mammals, therefore their occurrence is considered a compensatory mechanism for the age-related or pathogenic loss of Purkinje cells. Inflammation and other pathological conditions in rodents and humans have been shown to promote migration and infiltration of bone marrow-derived stem cells to the site of brain injury [8]. Based on the above, the observed binucleate PC in $CoCl_2$-treated suckling mice in our study may be suggested as a sequel of the exposure to the toxic metal.

One of the major mechanisms of heavy metal toxicity is the generation of oxidative stress [6]. Garoui et al. [4] have demonstrated development of oxidative stress and impairment of defense systems even in the cerebrum and cerebellum of suckling pups born to Co-exposed pregnant and lactating rats.

The brain histopathological findings in the present investigation are not surprising since the developing CNS is extremely sensitive to disruption. The vulnerable period extends from the beginning of organogenesis to the postnatal period. Unlike humans, the cerebellum in rodents is relatively immature at birth and the postnatal period is characterized by intense neuronal proliferation, migration and differentiation [1]. Therefore, Co exposure during this critical period may have a deleterious impact on the structure and maturation of the brain.

Conclusions

Exposure of mice to CoCl2 during late prenatal and early postnatal period affects body and brain weight and provokes cerebral and cerebellar histopathological changes. Our data are indicative of the enhanced susceptibility of the immature brain to the exposure of cobalt. The results contribute to the elucidation of the neurotoxic potential of the metal and the related health risks in newborns and infants, especially in highly industrialized areas.

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