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Disturbance of aerobic metabolism accompanies neurobehavioral changes induced by nickel in mice

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ABSTRACT

The oral ingestion of soluble nickel compounds leads to neurological symptoms in humans. Deficiencies in aerobic metabolism induced by neurotoxic stimulus can cause an energy crisis in the brain that results in a variety of neurotoxic effects. In the present study, we focused on the aerobic metabolic states to investigate whether disturbance of aerobic metabolism was involved in nickel-induced neurological effects in mice. Mice were orally administered nickel chloride, and neurobehavioral performance was evaluated using the Morris water maze and open field tests at different time points. Aerobic metabolic states in the cerebral cortex were analyzed at the same time points at which neurobehavioral changes were evident. We found that nickel exposure caused deficits in both spatial memory and exploring activity in mice and that nickel was deposited in their cerebral cortex. Deficient aerobic metabolism manifested as decreased O_2 consumption and ATP concentrations, lactate and NADH accumulation, and oxidative stress. Meanwhile, the activity of prototypical iron–sulfur clusters (ISCs) containing enzymes that are known to control aerobic metabolism, including complex I and aconitase, and the expression of ISC assembly scaffold protein (ISCU) were inhibited following nickel deposition. Overall, these data suggest that aerobic metabolic disturbances, which accompanied the neurobehavioral changes, may participate in nickel-induced neurologic effects. The inactivation of ISC containing metabolic enzymes may result in the disturbance of aerobic metabolism. A better understanding of how nickel impacts the energy metabolic processes may provide insight into the prevention of nickel neurotoxicity.

1. Introduction

Nickel is a widely distributed environmental pollutant. In terms of nickel exposure through oral routes, soluble nickel compounds appear to be a greater concern in terms of acute health effects than insoluble nickel compounds (Coogan et al., 1989). Soluble nickel compounds are usually absorbed as a divalent nickel ion, Ni (II), and distributed into various organs, including the brain (Borg and Tjalve, 1989; Ishimatsu et al., 1995). Acute nickel poisoning accidents were observed to cause neurologic symptoms, including weakness, headache, giddiness, and lassitude (Daldrup et al., 1983; Sunderman et al., 1988). Some chelating agents that are conventionally applied as scavengers during nickel carbonyl intoxication, including tetraethylthiuram disulfide and sodium diethyldithiocarbamate, can cause nickel redistribution in the body and elevate the nickel burden in the brain (Nielsen and Andersen, 1994). Although nickel exposure has been linked to a variety of adverse nervous system effects (Gordon and Stead, 1986; Liapi et al., 2011; Marchetti and Gavazzo, 2003; Nation et al., 1985), the mechanism underlying nickel-induced neurologic symptoms is not well understood.

The brain is a heavy energy consumer. It represents only 2% of the total body weight but receives 15% of the cardiac output and is responsible for 20% of total oxygen consumption and 25% of total glucose utilization in the body (Magistretti et al., 1993). Under normal conditions, the majority of glucose in the body is oxidized through aerobic metabolism to fuel neurologic function. The ratio of oxygen consumed to carbon dioxide formed in the brain is close to 1.0, and the ratio of oxygen to glucose utilization is approximately 6.0 (Schurr and Rigor, 1998). Pathological states

Abbreviations: MWM, Morris water maze; ATP, adenosine triphosphate; NAD/NADH, nicotinamide adenine dinucleotide; HIF-1α, hypoxia inducible factor-1 alpha; OXPHOS, oxidative phosphorylation; ISC, iron–sulfur cluster; ISCU, iron–sulfur cluster scaffold protein; MDA, malondialdehyde; SOD, superoxide dismutase; ROS, reactive oxygen species.

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that alter aerobic metabolism in the brain, such as ischemia, hypoxia, and hypoglycemia, promptly induce brain dysfunction and neurologic syndromes (Rosenthal et al., 2001; Schurr, 2002). Some exogenous stimuli also manifest their neurotoxicity by blocking neurologic energy flux (Sandhir et al., 2010; Struzynska et al., 1997). Nickel influences cellular energy metabolism in multiple ways. Nickel may cause the accumulation of hypoxia-inducible-factor-1 alpha (HIF-1α) by inhibiting HIF prolyl hydroxylases, thereby triggering hypoxic responses in cells, which are characterized by decreased oxygen consumption and increased glycolysis (Davidson et al., 2006). Ni (II) also inhibits oxidative phosphorylation (OXPHOS) by inactivating Fe-containing enzymes (Chen and Costa, 2006). Our previous study found that nickel exposure induced mitochondrial dysfunction in cerebral neurons, which may have prevented energy generation (He et al., 2011; Xu et al., 2010). Therefore, nickel may interfere with energy metabolism, causing the neurologic energy crisis, and may cause neurologic syndromes.

Iron–sulfur proteins, which are characterized by the presence of iron–sulfur clusters (ISCs), play important roles in OXPHOS (NADH dehydrogenase, succinate CoQ reductase, and CoQ-cytochrome c reductase) and the Krebs cycle (aconitase). ISC assembly and utilization disturbances not only hamper energy metabolism but also promote ROS generation by promoting abnormal mitochondrial electron transfer (Napoli et al., 2006). The ISC assembly scaffold protein (ISCU) functions as a scaffold on which ISCs are assembled, after which the clusters are transferred to various apoproteins for utilization (Kim et al., 2012). The suppression of ISCU expression leads to low OXPHOS rates by inactivating ISC-containing enzymes (Chen et al., 2010). ISC metabolism and ISCU regulation are associated with cellular iron homeostasis (Tong and Rouault, 2006), which can be disturbed by nickel (Chen et al., 2005). The levels of ISC-containing enzyme activity and ISCU expression may determine aerobic metabolic states under nickel exposure.

Thus, the purpose of this study was to investigate the states of aerobic metabolism in cerebral cortex of mice when nickel-induced neuro-behavioral changes emerged. Our results indicated that the suppression of OXPHOS that originated from ISC-containing enzyme inactivation and ISCU down-regulation accompanied a neurologic energy crisis and participated in the neurotoxicity of nickel.

2. Materials and methods

2.1. Animals and treatments

Inbred Kunming male mice weighing 25 ± 3 g were obtained from the Animal Center of the Third Military Medical University (Chongqing, China) and housed in an environmentally controlled animal room. Animal breeding and tissue collection were performed in accordance with China's laws on animal experimentation and the guide to the care and use of laboratory animals. Nickel chloride ($NiCl_2{\cdot}6H_2O$) (Sigma–Aldrich, USA) was dissolved in sterile H_2O and administered by gavage. For the neurobehavioral tests, the mice were divided into 3 dose groups that were administered 0, 5, or 50 mg of nickel (as $NiCl_2{\cdot}6H_2O$) per kg of body weight. In all other assays, the mice were divided into 6 groups (0, 5, and 50 mg exposure at 3 h post-treatment; 0, 5, and 50 mg exposure at 24 h post-treatment). The treated mice were given free access to food and water throughout the experimental period.

2.2. Neurobehavioral tests

The Morris water maze test was carried out according to the procedures developed by Morris (Steele and Morris, 1999). The MWM module of TM-Vision behavioral test system (Chengdu TME Technology, China) was consisted of a circular pool (130 cm in diameter, 50 cm deep), a circular escape platform (10 cm in diameter, 25 cm height), and a computer-controlled camera. The water was filled to the pool at depth to over the escape platform, opaque with milk powder and maintained at 25 ± 2 °C. The ambient stimuli, such as the camera and its cable, marks of staring points, etc. around the pool remained unchanged throughout the test and served as the spatial cues. The training and testing sessions consisted of 4 swim trials from different starting points. The starting points for a trial were marked on the edge of the pool as north (N), south (S), east (E), and west (W). In each trial, mice were given a maximum time of 60 s to find the invisible platform. If the mouse failed to find the platform in 60 s, it was gently guided to the platform and the escape latency score was count as 60 s. After landing or being placed on the platform, rats remained there for 30 s before the next trial.

Before nickel exposure, the mice were trained to find the escape platform in six training sessions conducted twice/day for three days. On day 4, after the baseline testing session was completed, the animals were treated with one of the doses and subjected to the testing sessions at different time points (1, 3, 12, 24, and 48 h after treatment). Thus, each mouse was tested at five time points after exposure. 4 trials were used at each time point. The average time required to locate the platform in 4 trials was defined as the escape latency.

The open field test was conducted using the corresponding module of the TM-Vision system, which comprised a 50 cm × 50 cm × 30 cm black box, a computer-controlled camera, an adjustable light and a surrounding curtain. The illuminance of arena was measured by an illuminance meter (1332 A, TES, Taiwan, China) and kept to 100 Lx throughout the test. The baseline testing trials of open field test started at 7:00 A.M., and then the animals were treated with nickel during 9:00–9:20 A.M. In each trial, the mouse was placed at the center of the arena, and the moving activity was monitored for 3 min. The thermal maps were conducted with the moving orbit by the control software. The total distance traveled was calculated.

2.3. Nickel concentration assay

At 3 or 24 h post-treatment, the brains of the mice were removed, and the cerebral cortex of the right hemisphere was isolated on a cold plate. Both the cerebral cortex and the left hemisphere were weighed and heated on a thermal plate at 200 °C for 2 h, followed by subsequent incineration in a muffle furnace at 450 °C for 4 h. The ashes were dissolved and resuspended in 0.1 M nitric acid. The nickel concentrations were analyzed using a furnace atomic absorption spectrometer (iCE 3500, Thermo Scientific, USA) according to the manufacturer's recommended protocol (SOLAAR, V11.02, Thermo Scientific, USA). The certified reference material of nickel was purchased from the National Certified Reference Center of China (GBW08618). The total nickel was normalized to the tissue weights. The ratios of the nickel amounts in the cerebral cortex to that in the brain were calculated as the percentages of deposited nickel in the cerebral cortex (% cortex).

2.4. Sample preparation

A new herd of mice were treated with nickel for biochemical experiments. Their cerebral cortexes were appropriately prepared for analysis in the mitochondrial activity kept (MTAK) and non-kept (MTANK) assays. In the MTAK procedure, dissected cerebral cortices were homogenized using a TissueLyser (Qiagen, USA) with 1 ml of hypotonic buffer (120 mM KCl, 20 mM HEPES, 2 mM $MgCl_2$, and 1 mM EGTA, pH 7.4) and centrifuged at 600 × *g* for 10 min. The