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Original Research Article

Rat spinal ganglia in assessment of protective action of antioxidants: A morphological study

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ARTICLE INFO

Article history:
Received 30 January 2017
Received in revised form
7 November 2017
Accepted 21 November 2017
Available online xxx

Keywords:
Mercury chloride
Antioxidants
Neurons
Satellite cells

ABSTRACT

Background and objective: Mercury pollution is one of the most pressing environmental problems. Therefore, the impact of mercury on human body, the nervous system in particular, remains topical. The aim of the study was to identify the morphological characteristics of neurons and neuroglia in spinal ganglia of rats receiving antioxidants in the presence of small doses of mercury (II) chloride.

Materials and methods: A total of 100 white Wistar rats were divided into 5 series (10 groups), with 10 animals in each group. The first series comprised intact animals receiving saline solution instead of drugs administered in other series (control). In the second series 10 injections of mercury (II) chloride were performed (group of short-term neurointoxication) and 50 injections (group of long-term neurointoxication). In the third to the fifth series, the short- and long-term neurointoxication was followed by 10 daily injection of the drugs: unithiolum, thiotriazolinum and mildronate respectively. Spinal ganglia were obtained two weeks after the completion of drugs administration and studied microscopically and ultramicroscopically.

Results: Administration of thiotriazolinum, unithiolum and mildronate mitigated manifestations of toxic effects of mercury (II) chloride on spinal ganglia. Unithiolum and thiotriazolinum activated synthetic processes, while mildronate had a positive effect on restoration of cells metabolism.

Conclusions: Morphological data show that unithiolum and thiotriazolinum action decreases toxic effects of mercury chloride and are similar. They demonstrate pronounced activation of synthetic processes in sensory neurons and satellite cells of spinal ganglia. Mildronate also restores cell ultrastructure and has more pronounced effect on their energetic processes and interaction between neurons and satellite cells.

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https://doi.org/10.1016/j.medici.2017.11.001

1. Introduction

Mercury pollution is one of the most pressing environmental problems [1–3]. Widespread use of mercury and its compounds as well as devices containing mercury in manufacturing, scientific research, medical, educational and household measuring devices in the absence of effective control system resulted in mercury pollution going far beyond the specific chemical plants and its current almost universal occurrence, including homes, schools, and kindergartens. Therefore, the impact of mercury on human body remains topical. Moreover, mercury being a neurotropic poison makes the effect of its low doses on the nervous system an urgent issue [4]. Low doses of mercury primarily affect the nervous system causing numerous neurological diseases and quite often presenting as other diseases [5,6], hence the relevance of further neuromorphological researches aimed at finding drugs to prevent neurointoxication caused by small doses of mercury.

The aim of the study was to identify the morphological characteristics of neurons and neuroglia in spinal ganglia of rats receiving antioxidants in the presence of small doses of mercury (II) chloride.

2. Materials and methods

The study was conducted on 100 white Wistar rats weighing 160–180 g (at the beginning of the experiment). Experimental animals were divided into 5 series (10 groups) with 10 animals in each group (Table 1). Animals received standard vivarium (animal facility) diet with natural alteration of day and night and had free access to drinking water. All procedures were performed in accordance with the ''Regulations on the animal use in biomedical research'', the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, and the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). The research was approved by Bioethical Committee for Human Subjects or Animal Research in Bogomolets National Medical University, Minutes N 12, December 30, 2015.

The first series comprised intact animals receiving saline solution instead of drugs administered in other series: control of short-term exposure (10 injections) and control of long-term exposure (50 injections). In the second series 10 injections of mercury (II) chloride (group of short-term neurointoxication) and 50 injections (group of long-term neurointoxication) were performed. In the third series 10 injections of unithiolum (2,3-dimerkaptopropansulfonate sodium, Russia) were performed after short-term and long-term neurointoxication. In the fourth series 10 injections of thiotriazolinum (morpholinium-3-methyl-1,2,4-triazolyl-5-thioacetate, Ukraine) [7] were performed after short-term and long-term neurointoxication. In the fifth series 10 injections of mildronate (3-(2,2,2-trymetylhydraziniumyl) propionate, Latvia) [8,9] were performed after short-term and long-term neurointoxication. All substances were injected daily intraperitoneally with insulin syringe: normal saline – 0.5 mL, mercury (II) chloride (0.3 mg/100 g of weight), unithiolum in a dose of 50 mg/kg of weight (5%, in 5 mL ampules), thiotriazolinum – 100 mg/kg (2.5%, in 2 mL ampules), and mildronate 50 mg/kg (in ampules 500 mg in 5 mL).

Samples from experimental animals (spinal ganglia L2–S1 from both sides – 12 ganglia) were obtained two weeks after the completion of injections under thiopental anesthesia (35–40 mg/kg). Perfusion-fixation was made with 4% paraformaldehyde through the left ventricle of the heart [10]. Harvested ganglia were fixed with 2.5% glutaraldehyde and postfixed using 1% osmium tetroxide.

Semi-thin and ultrathin sections were made following the standard technique from small fragments of spinal ganglia on ultratoms LKB-3 and LKB-8800 (Sweden) in longitudinal and transverse projections. The semi-thin sections were stained with toluidine blue, analyzed and photographed, using Olympus BX51 microscope having appropriate software. Ultrathin sections were contrasted by 2% solution of uranyl acetate for 15 minutes and solution of lead citrate for the same time according to Reynolds ES [11]. They were examined and photographed in the electron microscopes PEM 125K and EMV 100B (Ukraine).

Morphometric study was conducted with the image analyzer: microscope Olympus BX51 with digital camera C-4040zoom and personal computer. Metric characteristics were evaluated with software UTHSCSA Image Tool® for Windows® (version 2.00) interactively. The number of gliocytes that were localized around one neuron (direct contact between cells) and percentage of intact myelinated nerve fibers (without visible damages of axon and myelin sheath structure) were expressed as mean and standard error.

Statistical analysis was conducted using Statistica 4.0 (Statistica Inc. USA) and MS Excel programs. Changes between groups were assessed using the parametric Student *t* test after confirming the normality of distribution. Changes with significance level over 95% ($p < 0.05$) were considered reliable.

Table 1 – Experimental design.

2 + 2 weeks – short-term			10 + 2 weeks – long-term		
	2 weeks 10 injections	2 weeks		10 weeks 50 injections	2 weeks
Control	Normal saline	Normal saline	Control	Normal saline	Normal saline
Hg	$HgCl_2$	Normal saline	Hg	$HgCl_2$	Normal saline
Hg + U	$HgCl_2$	Unithiolum	Hg + U	$HgCl_2$	Unithiolum
Hg + Th	$HgCl_2$	Thiotriazolinum	Hg + Th	$HgCl_2$	Thiotriazolinum
Hg + M	$HgCl_2$	Mildronate	Hg + M	$HgCl_2$	Mildronate

Please cite this article in press as: Sokurenko LM, et al. Rat spinal ganglia in assessment of protective action of antioxidants: A morphological study. Medicina (2017), https://doi.org/10.1016/j.medici.2017.11.001