Detection of Cerebellum Morphology during Experimental Post-Chemotherapy Cognitive Impairment (PCCI)

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Abstract

Background: Multiple animal studies and clinical investigations have stated are accompanied with Post-Chemotherapy Cognitive Impairment (PCCI), and was proposed several possible underlying mechanisms contributing to PCCI, but exact structural basis is still not well defined.

Aim: Examine the effects of doxorubicin (DOX) on the cerebellum, which would enable us to contribute to the definition of the functional and molecular mechanisms of DOX-induced neurotoxicity.

Methods: The experiment was conducted on four groups of rats, which were administered DOX in different doses, which studied histologically and immunohistochemically investigated.

Results: The study reveals disorders of cerebellum layers, especially Purkinje cell layer, starting from various degrees neuronal damage, dystrophic-degenerative processes, and chromatolysis to total disintegration and reactive astrocytosis, with clear association between DOX effect and cerebellar pathology.

Conclusion: The research database of doxorubicin (DOX) effectsshowed significant changes with a large range of destruction in the cytoarchitecture of the cerebellum. These changes in all neuronal elements can be considered as an essential basis for post-chemotherapy cognitive impairment. (**TCM-GMJ December 2024; 9 (2): P19-P22)**

Keywords: Doxorubicin**,** Chemotherapy-induced cognitive impairment, "Chemo-brain", Cerebellum's Morphology, Purkinje cells, Immunohistochemistry, GFAP.

Introduction

Doxorubicin (DOX) - commonly used in adjuvant chemotherapeutic medicine can cause serious neurotoxicity during and after treatment of cancer patients, but it was generally believed that DOX has a limited capacity to penetrate the blood-brain barrier. Despite this barrier, DOX has been detected in the brain following peripheral administration and can cause severe neurotoxicity. The exact morphological mechanisms underlying chemo-brain, however, remain unclear.

Our study investigates the effects of doxorubicin (DOX) in an acute experiment on the cerebral cortex and cerebellum, aiming to contribute to the understanding of the functional and morphological basis of DOX-induced neurotoxicity.

Chemotherapy is an effective conventional treatment for cancer patients, but is associated with serious short- and long-term neurological side effects, with central (CNS) and peripheral (PNS) nervous system toxicity with wide range of symptoms, among them are: fatigue, emotional instability, anxiety, memory deterioration, difficulty concentrating, the different types of encephalopathies, myelopathy, meningitis, peripheral neuropathies, and numerous cognitive disorders [1-5].

Despite such a variety of manifestations, the most common complication of chemotherapy, is cognitive dysfunction, which was first described 40 years ago as Post-Chemotherapy Cognitive Impairment (PCCI), that occurs during or after treatment, with high frequency [6-9].

The several pathways have been proposed to explain the exact mechanism underlying PCCI, but morphological changes remain unstudied, although almost all authors note a decrease in the volume of white and gray matter of the brain, especially leukoarcosis in white matter and the

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Received November 16, 2024; accepted December 05 , 2024.
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formation of single and multiple foci of gliosis in cortex, sharp decrease in proliferation and atrophy in the subventricular zone, hippocampal dentate gyrus, decreased blood flow velocity and metabolic disorders in the basal ganglia, and frontal gyrus and cerebellum [10-13].

In our experiment we used Doxorubicin (DOX) as it is an anthracycline cytostatic, known since the 1960s, commonly used in adjuvant chemotherapy for various solid and hematogenous tumors, because DOX has a direct effect on the cell membrane, suppresses DNA replication by inhibiting the corresponding enzyme- due to interaction with topoisomerase II [14-19].

The efficacy of doxorubicin against brain tumors is limited due to its poor penetration across the blood-brain barrier (BBB). Despite this, DOX has been detected in the brain following peripheral administration and can cause severe neurotoxicity, so it is believed that DOX causes the production of anti-inflammatory cytokines, cytokines cross the BBB and cause astrogliosis, which can cause PCCI [20-22]. In the literature have been described neuronal dysfunction and death, decreased neurogenesis, and dendritic reduction [23-25].

However, the exact morphological mechanisms and the target structure of DOX action on the brain, remain unclear.

Methods

The experiment was carried out on mature male rats of line Wistar(m =170-200gr); A control group (healthy animals) and 4 experimental groups were allocated, each experimental group consisting of rats (n=20) that were administered DOX (doxorubicin hydrochloride – 2.5 mg/kg/ml)according to the following scheme:

Control group – intact animals, standard conventional keeping regime in accordance with the internal protocols of the vivarium (GALS regulation 2023).

Present study was conducted in accordance with ethical principles for research of Ethics committee and Board of medical sciences at Tbilisi State University based on Helsinki - ethical principles declaration for medical research (2013).

I group – DOX one intraabdominal injection 5mg/kg - animals removed from the experiment on the 7th day after injection.

II group – DOX one intraabdominal injection 15mg/kg - animals removed from the experiment on the 7th day after injection.

III group – DOX three times intraperitoneal injection of 15 mg/kg, according to the following scheme: injections every third day - three injections in total - the animals removed from the experiment on the 10th day after the start of administration.

IV group - DOX five-time intraperitoneal injection of 15 mg/kg, according to the following scheme: injections every third day - 5 injections in total - the animals removed from the experiment on the 15th day after the start of administration.

For histological analysis was used hematoxylin and eosin stainingafter fixation in 10% buffered formalin. After selection, the material was prepared for immunohistochemical study – that was

also carried out in all experimental groups to detect the expression of glial fibrillary acidic protein (GFAP) in astrocytes.

Immunostaining with anti-GFAP (incubation time,30 min; clone GA5; dilution 1:400; cat. no. PA0026; Leica Biosystems Newcastle Ltd.), was performed with the manual staining method using the Novolink DAB Polymer Detection system (incubation time, 20 min; cat no. RE7260-CE; Leica Biosystems Newcastle Ltd.) in accordance with the manufacturer's recommendations. Endogenous peroxidase activity was neutralized using the Peroxidase Block reagent [3-4 % (v/v) hydrogen peroxide; Novolink DAB Polymer Detection system; cat. no. RE7260-CE; Leica Biosystems Newcastle Ltd]. To reduce non-specific binding of primary antibody Novocastra Protein Block reagent was used (0.4% casein in phosphate-buffered saline, with stabilizers, surfactant and 0.2% Bronidox L as a preservative; Novolink DAB Polymer Detection system). As the secondary antibody, rabbit anti-mouse IgG (<10 μg/ml) in 10% (v/v) animal serum in Tris-buffered saline/ 0.1% ProClin™ 950 was used (Novolink DAB Polymer Detection system). All reactions for immunohistochemistry were performed at room temperature.

Results

Our experiment was focused on studying the cerebellum, we focused on structural changes in three components of nervous tissue: neurons, astrocytes and blood vessels.

Comparing the obtained data, one can reliably note a progressive decrease in the number of intact neurons, starting from changes of the size and intensity of neuronal expression, pleomorphism of neurons, which will then continue with practically homogenization of neurons nuclei, a sharp disorganization, swelling and fusion of neuronsespecially, in Purkinje cell layer, and ending with the loss of their dendrites, which is the most remarkable feature of complete degeneration, because of diminished contacts with radial glia, especially, as the dose and frequency of administration of DOX increases (**Fig. 1. a, b**).

Regional and, in the last two experimental groups, more pronounced glial hyperplasia, reactive gliosis corresponding to ischemia/hypoxia in the granule cell layer and the Purkinje cell layer, with loss of sharpnessthe border between the granular and Purkinje cell layers, also with a sharp decrease in the size of neurons, an increase in the number of astrocytes, and vacuolization of the substrate were also observed.

Astrocytes, with their polyfunctional ability and complex biochemical properties, are one of the important damage links in Post-Chemotherapy Cognitive Impairment (PCCI). The typical reaction of astrocytes in response to CNS damage - reactive astrogliosis, which seems to be a partially specific protective reaction aimed at restoring tissue homeostasis. When gliosis is maintained, it can become maladaptive, act against some regenerative reactions and thus limit the recovery of function [26, 27].

As a result of toxic effects, the expression of glial fibril-

lary acidic protein (GFAP) reveals both a change in the number of astrocytes (in the form of reactive gliosis) and intracellular compensatory reactions in the form of disorders of number and form the cells [28].

In our study we used evaluation of anti-GFAP expression in the astrocytes.

Reactive gliosis is the result of the death of neurons sensitive to DOX direct action.

Anti-GFAP positive reaction of astrocytes was evident in the cerebellum: with astrocytes hypertrophy, activation of filaments, enhancement of cell proliferation and migration to the injured area, with next development of glial scar (**Fig. 2. a, b**).

Conclusion

Thus, a study of doxorubicin (DOX) effect showed significant dose-dependent changes in all neural elements of cytoarchitecture of cerebellum - disorders of neurons of various degrees of dystrophic-degenerative processes, from chromatolysis to total disintegration and reactive astrocytosis with high diffuse-focal expression of anti-GFAP and formation of glial scar in the area of damage. These changes can be considered as a significant basis of post-chemotherapy cognitive impairment.

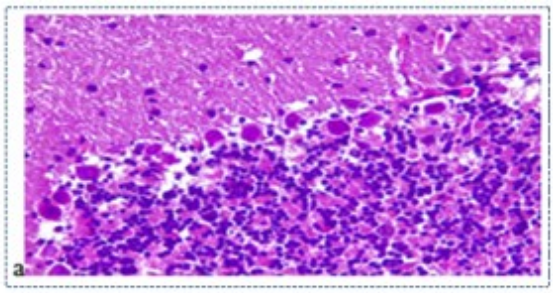
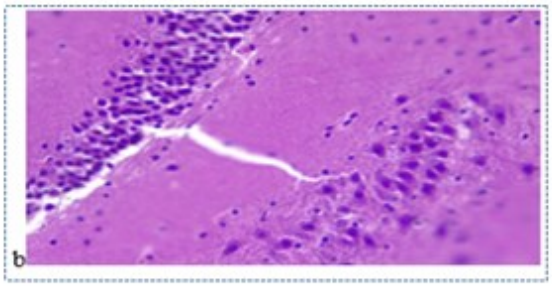


Fig.1. a. II group – cerebellum, H&E, x200. Homogenization of Purkinje cells nuclei, diminished and number of cells, fusion of neurons. Regional hyperplasia of glia in the granular and Purkinje cells layer.



b. III group – cerebellum, H&E, x200. Necrobiosis in granular layer and Purkinje neurons. Involution of Purkinje cells.

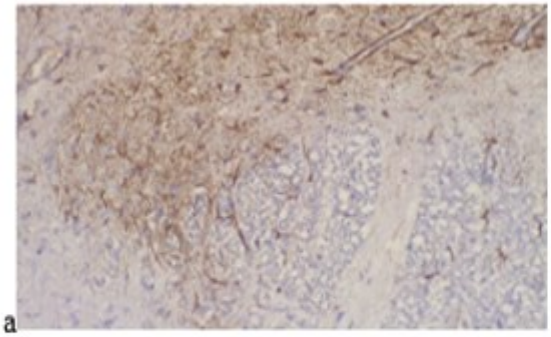
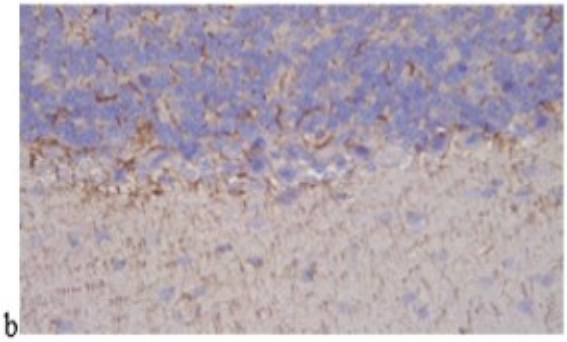


Fig.2. a. III group - cerebellum. IHC, anti-GFAP, x200. Tissue substrate heavily vacuolated, preserved neurons are shrunken, reactive gliosis with positive anti-GFAP reaction.



b. IV group – cerebellum. IHC, anti-GFAP, x400. Disorder of the cerebellum layers borders. Purkinje cells disoriented, different immunohistochemical expression of anti-GFAP.

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