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CLOZAPINE AND CLOZAPINE-ETHANOL POISONING AS A CAUSE OF HISTOMORPHOLOGICAL CHANGES IN THE CEREBELLUM

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ABSTRACT — Clozapine is an atypical neuroleptic with a narrow therapeutic index which is widely used in clinical practice. Due to its psychotropic effect the drug is often misused. Experimental studies were performed on 25 outbreed mail rats. The animals were divided into 5 groups (the controls and four experimental groups). The controls included 5 intact rats. The experimental groups 1 and 2 were treated with clozapine at a dose 150 mg/kg. The experimental groups 3 and 4 received clozapine at the same dose and ethanol (2 ml/kg 70% ethanol). Severe damage to the Purkinje cells of the cerebellum was confirmed by an increase in the number of neurons that register signs of both reversible and irreversible damage. Purkinje cell alteration has increased by 24 hours from the beginning of the experiment.

KEYWORDS — clozapine, ethanol, poisoning, cerebellum, histomorphological changes.

INTRODUCTION

Poisoning is one of the most important issues of forensic medicine and is among the first three main reasons of violent death [1].

Clozapine poisoning is quite common in forensic medical practice. This substance is an atypical neuroleptic. It has a pronounced sedative and antipsychotic effect. Clozapine is widely used in clinical practice to treat acute and chronic forms of schizophrenia, psychosis, manic conditions, bipolar disorders and others. It is considered not to provoke extrapyramidal reactions. Clozapine has been shown almost not to have an effect on the level of prolactin in the blood [2].

According to Russian scientists, about 1 million patients undergo clozapine therapy every year in more than 60 countries of the world [3], which determines the high risk of poisoning with this drug. The therapeutic threshold of clozapine is comparatively narrow. Its single therapeutic dose is 50–200 mg, the highest daily dose is 900 mg, whereas a fatal clozapine dose for an adult is about 2 g [4, 5]. In addition to accidental clozapine poisonings, there is a large number of criminal poisoning with this substance. There is also a large number of combined clozapine-ethanol poisonings [6] .

Clozapine is metabolized in the liver. Its main metabolites are desmethylclozapine (norclozapine) and clozapine-N-oxide. According to the literature, clozapine and its metabolites are excreted by the kidneys [7]. The main target organ in case of such poisonings is the brain.

The objectives of the study

was to estimate histomorphological changes in the cerebellum in acute clozapine and combined clozapine-ethanol poisonings 3 and 24 hours after the intoxication in the experiments on laboratory rats.

MATERIAL AND METHODS

Experimental studies were performed on 25 outbreed mail rats. The animals were divided into 5 groups (the controls and four experimental groups). The controls included 5 intact rats. The experimental groups 1 and 2 were treated with clozapine at a dose 150 mg/kg. The experimental groups 3 and 4 were treated with clozapine at the same dose and ethanol (2 ml/kg 70% ethanol). Groups 1 and 3 were euthanized 3 hour after the administration of the drug; groups 2 and 4 were euthanized 24 hours after the administration of the drugs.

Keeping animals and working with them were carried out in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes.

The samples were fixed in 10% neutral formalin and immersed into paraffin. Histological sections were processed according to the standard method and stained with hematoxylin and eosin and Nissl-staining. The sections were examined by light microscopy using Nikon Eclipse E-400 microscope with a video system based on the Watec 221S camera (Japan) at 400× magnification.

Neuron damage was assessed according to a classification including: 1) acute swelling 2) primary irritation 3) hydrophic changes 4) fat dystrophy 5) calcification 6) shrinking 7) severe changes 8) ischemic change 9) karyocytolysis 10) *shadow cells* 11) neuronophagy 12) satellite disease 13) pigmented dystrophy [8].

RESULTS AND DISCUSSION

In the group of controls reversible changes in cerebellar Purkinje cells primary irritation, acute swelling were found (15–20%). The share of irreversible damage to the Purkinje cells of the cerebellum (*shadow cells*, shrinking of neurons, etc.) was about 2–5%.

3 hours after clozapine administration an increase in the number of Purkinje cells with signs of both reversible (60–70%) and irreversible (30–40%) damage was detected.

24 hours after clozapine poisoning the number of Purkinje cells with signs of reversible(40–50%), irreversible (45–55%) damage increases (fig. 1, 2).

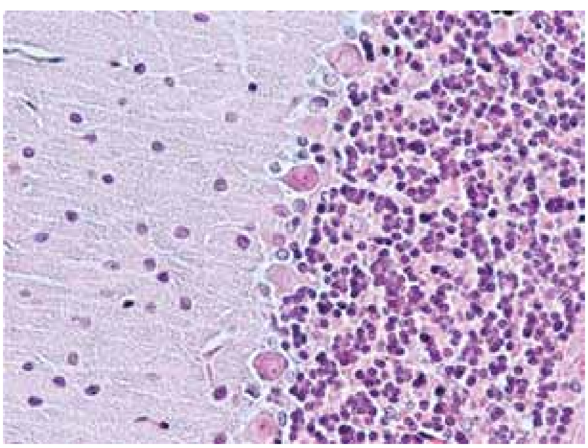

***Fig. 1.** Reversible changes in Purkinje cells of the cerebellum 24 hours after clozapine (150 mg/kg) administration. Hematoxylin-eosin. Magnification 400. Cell swelling, primary irritation*

3 hours after combined clozapine and ethanol administration damage to Purkinje cells is detected (37–55% — reversible, 40–60% — irreversible).

24 hours after combined clozapine and ethanol poisoning 25–30% of the damage was reversible and 60–70% was irreversible. The absence of Purkinje cells in significant areas of their localization was also found. There was a decrease in the intensity of Nissl staining of the substance up to its complete disappearance.

The combination of clozapine and ethanol has a damaging effect on Purkinje cells. Previous studies show the development of dystrophic changes in cortical neurons [9]. Mechanisms of clozapine toxicity are shown in a number of studies [10]. Clozapine causes damage to myocardial and lung cells [1, 11, 12]. In case of combined clozapine and ethanol poisoning the damage to organs is more significant. After administration ethanol is converted to acetaldehyde which damages cell membranes. Ethanol and acetaldehyde inhibit the energy metabolism of cells by damaging the mitochondria. Moreover, they activate lipid peroxidation. In addition, cell damage is aggravated by the activation of lysosomal hydrolytic enzymes. The mechanisms of damaging effect of ethanol include the release of catecholamines. An important component of the pathogenesis of damage caused by ethanol is the effect of catecholamines and violation of hemomicrocirculation [12, 13]. In addition, clozapine damages mitochondria [14], affects the functional activity of astrocytes and microglia [15], and reduces the content of ceramide and sphingomyelin in the liver [16].

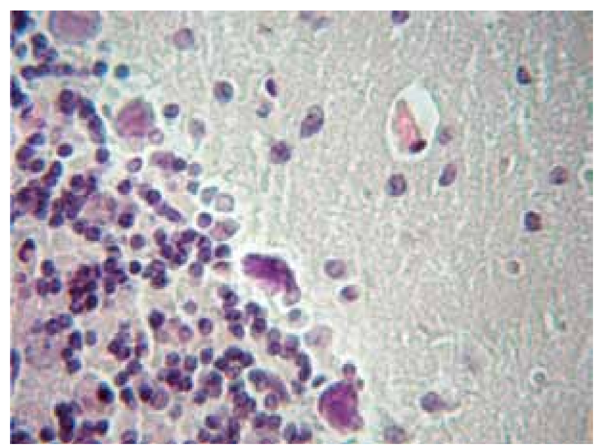

***Fig. 2.** Irreversible changes of Purkinje cells of the cerebellum 24 hours after clozapine (150 mg/kg). Hematoxylin-eosin. Magnification 400 Severe changes, cariocitolysis, "cells shadows"*

CONCLUSION

Clozapine poisoning severely affects the Purkinje cells of the cerebellum, which is confirmed by an increase in the number of neurons that register signs of both reversible and irreversible damage. Purkinje cell alteration increases by 24 hours from the beginning of the experiment.

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