FORENSIC STUDIES

Cite as: Archiv EuroMedica. 2022. 12; 6: e1. DOI 10.35630/2022/12/6.10

Received 4 November 2022;
Published 9 December 2022

DYNAMICS OF MORPHOLOGICAL CHANGES IN CELLS OF THE CEREBELLUM IN ACUTE POISONING WITH CLOZAPINE IN COMBINATION WITH ALCOHOL

***Islam Telipov*[1] ✉, *Dmitriy Sundukov*[1], *Arkadiy Golubev*[1,2]**

[1]*Peoples' Friendship University of Russia, Moscow;*
[2]*V. A. Negovsky Research Institute of General Reanimatology, Federal Research and Clinical Center for Resuscitation and Rehabilitology, Moscow, Russia*

✉ vasy9548@mail.ru

ABSTRACT

According to most researchers, the central mechanism of clozapine poisoning in combination with alcohol is their cholinolytic effect. A life-threatening condition with clozapine occurs as a result of an unintentional overdose of the prescribed drug, suicidal behaviour, or criminal behaviour.

Despite the urgency of the problem of clozapine poisoning in combination with alcohol, the pathogenesis of these conditions and the resulting morphological changes in the brain have not been sufficiently investigated.

In this connection, the purpose of our experimental research is the study of the dynamics of changes in Purkinje cells of the cerebellum in the early period of acute poisoning with clozapine in combination with alcohol.

In sections of the cerebellum of the control group, reversible changes in Purkinje cells (15–20%) predominated in the form of primary irritation and acute swelling. Irreversible damage to Purkinje cells (2-5%).

3 hours after poisoning with clozapine in combination with ethanol, Purkinje cells showed irreversible damage to cerebellar cells with signs of severe changes, neuronophagia, and pericellular edema, with the following ratio: reversible 25-30% and irreversible 60-70%.

24 hours after the combined poisoning with clozapine in combination with alcohol, in percentage terms, the ratio of the changes was the following: reversible 25-30% and irreversible 60-70%.

The study revealed the dynamics of reversible and irreversible changes in Purkinje cells with a predominance of irreversible damage was established, especially after 24 hours from the start of the experiment.

***Keywords**:* clozapine, alcohol, Purkinje cells, poisoning.

INTRODUCTION

Clozapine poisoning is characterized by a severe course and a high probability of death (15-30%). The most pronounced toxic effect of this drug is observed when clozapine and alcohol are taken together [1]. In a forensic medical examination in cases of acute clozapine poisoning, the diagnosis is made by a complex method using clinical and anamnestic, sectional, histological, biochemical data and quantitative

determination of the content of the drug in the biological media of the body.

Clozapine (8-chloro-11-(4-methyl-1-piperazenyl)-5H-dibenzo[b,e] is an international non-proprietary name for the active substance of well-known drugs: Azaleptin and Leponex, alemoxan, iprox. The drug belongs to atypical antipsychotics and is a derivative of 1,4-benzodiazepine [2].

Clozapine has antidopaminergic (D1 D2, D4), antiserotonergic (5HT2A, 5HT1C, 5HT2C, 5HT2D, 5-HT3, 5-HT3, 5-HT6, 5-HT7), antihistamine (H1), antimuscarinic (M1, central and peripheral) anticholinergic and α1, α2-adrenolytic properties [2,9].

This drug has a reinforcing effect of narcotics, hypnotics, sedatives, antihistamines, analgesics, as well as alcohol [2]. Having a weak affinity for D2 receptors, clozapine practically does not cause extrapyramidal disorders at therapeutic doses, while maintaining antipsychotic activity, but if the therapeutic dose of the substance is increased, the risk of developing ESR increases [2, 7-8].

Clozapine has been used to treat acute and chronic forms of schizophrenia, manic states, manic-depressive psychosis, various psychotic conditions, psychomotor agitation in psychoses, aggressiveness, and sleep disorders. Currently, it is accepted in therapy, usually only in the treatment of patients with resistant forms of schizophrenia after an inadequate response to at least two modern antipsychotics [2-4].

The adverse effects of Clozapine include: hyperthermia, neuroleptic and cholinolytic syndromes, agitation, tardive dyskinesia, dizziness, drowsiness, hypo- and hypertension, reflex and sinus tachycardia, dry mouth, hypersalivation, accommodation disorder, urinary incontinence, increased risk of blood vessel thromboembolism, hypertonia, migraines, and various gastroenterological disorders occur, up to the development of atony and obstruction, which is associated with the blockade of 5-HT3 receptors that are present in the central nervous system, platelets, smooth muscle of the blood vessels, the gastrointestinal tract, lungs, and other organs [2, 10]. The literature describes cases of myocarditis, seizures, catatonia, coma, agranulocytosis [14-19].

Clozapine causes dose-dependent blocking of calcium channels, which leads to cardiac disorders, in the form of non-specific changes in T waves and the ST, QT interval on the ECG and changes in heart rate, without having a direct cardiotoxic. Prolongation of the QT interval above 500 ms. increases the risk of pirouette tachycardia, which leads to death effect [11, 12].

The mechanism for the development of a strong antipsychotic effect is associated with blocking the reuptake of noradrenaline [20] and selective blocking of GABA receptors, while the risk of developing tremor, convulsions, dizziness and nausea may increase [21-22].

By blocking dopamine D2 receptors, clozapine exhibits a central and peripheral antiemetic effect, which is enhanced by anticholinergic, sedative, and antihistamine properties [2].

The sedative effect occurs with the blockade of α-adrenergic receptors. Clinically, this is manifested by inhibition of conditioned reflex activity and motor-defense reflexes, a decrease in spontaneous motor activity, relaxation of skeletal muscles, and a decrease in reactivity to endogenous and exogenous stimuli while maintaining consciousness [23].

Clozapine is well absorbed from the digestive tract. Bioavailability is 50-70%, and the maximum concentration in the blood occurs after 2.5 hours. In the blood, clozapine binds to plasma proteins by 95%, is distributed throughout the body and accumulates mainly in the cells of the brain, heart and liver, lungs, kidneys, and adipose tissues [5,25,26].

A.F. Fartushny presents lethal concentrations of clozapine: blood - 1.3 mg% (13 μg / ml, 13 mg / l), in the liver - 2 mg%, in the kidneys - 2.5 mg% [13].

Clozapine and its metabolites are excreted from the body with urine, bile (more than 30%), the rest is excreted through the gastrointestinal tract [26].

Objective. To reveal the dynamics of changes in Purkinje cells of the cerebellum in the early period of acute poisoning with clozapine in combination with alcohol.

MATERIALS AND METHODS

Experimental studies were carried out on white outbred rats. Under general inhalation anesthesia (sevoflurane 4 vol% with an oxygen flow of 2 l/min in the induction chamber), solutions were administered enterally through a gastric tube in a volume of 10 ml/kg of animal weight.

Of these, 3 groups of animals were distinguished:

I. Control group;

II. Clozapine + alcohol, 3 hours;

III. Clozapine + alcohol, 24 hours.

Group of rats I (control), n=5 - NaCl solution 0.9% was administered - 10 ml/kg;

Group II ("clozapine + alcohol"), n=5 - clozapine was administered at a dose of 150 mg/kg in a solution of 40% ethyl alcohol - 10 ml/kg ml, 3 hours;

Group III ("clozapine + alcohol"), n=5 - clozapine was administered at a dose of 150 mg/kg in a solution of 40% ethyl alcohol - 10 ml/kg ml, 24 hours;

After the administration of these drugs, the animal remained in the vivarium for 3 hours and 24 hours, without food, but with free access to water. Repeated anesthesia was performed (sevoflurane 4 vol% with an oxygen flow of 2 l/min in the induction chamber) after 3 hours and 24 hours, followed by euthanasia of the animal.

Pieces of the cerebellum were fixed with 10% formalin and embedded in paraffin. Histological sections were stained with hematoxylin and eosin and according to Nissl, followed by analysis of morphological changes using an Olympys BX 41 microscope. Neuronal damage was assessed according to the classification, including: 1) acute swelling of nerve cells, 2) primary irritation of nerve cells, 3) hydropic changes in neurons, 4) shrinkage of neurons, 5) ischemic changes in nerve cells, 6) karyocytolysis, 7) "shadow cells", 8) neuronophagia, 9) satellitosis [6]. Damage was assessed for at least 100 cells in each observation. Statistica 10.0 (StatSoft, Inc.) and MedCalc 12.5.0.0 (MedCalcSoftwarebvba) programs were used for statistical analysis. Mean values were represented by the median with an interquartile interval. Intergroup differences in indicators were assessed using the Mann-Whitney U-test and were considered statistically significant at $p<0.05$. The experimental study was carried out in accordance with the requirements of 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 [24].

RESULTS AND DISCUSSION

In the study of sections of the cerebellum, reversible and irreversible changes were found. Reversible include: acute swelling, primary irritation. The following were classified as irreversible: hydropic changes, pigmental degeneration, pigmental atrophy of nerve cells, adipose degeneration, calcification, wrinkling, ischemic and severe changes, karyocytolysis accompanied by neuronophagia, satelliteitis.

In sections of the cerebellum of the control group, reversible changes in Purkinje cells (15–20%) predominated in the form of primary irritation and acute swelling. Irreversible damage to Purkinje cells (2-5%).

3 hours after poisoning with clozapine in combination with ethanol, Purkinje cells showed irreversible damage to cerebellar cells with signs of severe changes, neuronophagia, and pericellular edema. In the cells, the nuclei and nucleoli were displaced to the periphery, they became hyperchromic, deformed, in some of them the nucleolus was not stained, "shadow" cells appeared, there was an absence of cells in long areas, of which 37-55% are reversible, 40-60% are irreversible.

24 hours after the combined poisoning with clozapine in combination with alcohol, pronounced severe changes sharply prevailed in the cells of the cerebellum, massive neuronophagia occurred, the absence of neurons in almost the entire field of view, pronounced pericellular and perivascular edema, and in percentage terms were: reversible 25-30% and irreversible 60-70%.

The results of the study are presented in table No. 1.

Table 1. Damage rates in percent (%) of Purkinje cells in different experimental groups, Me (LQ; HQ)

Damage type	I	II	III
	Control	Clozapine + alcohol 3 hours	Clozapine + alcohol 24 hours
Reversible damage, %	17.5 (15.9-17.7)	47.4*^ (42.3-52.1)	27.2*^#& (24.3-29.2)
Irreversible damage, %	3.6 (2.8-4.1)	36.7* (33.2-39.1)	60.5*^# (57.1-63.5)

*Note: * - p<0.05 when compared with control; ^ - when compared with clozapine 3 hours; # - p<0.05 when compared with clozapine with alcohol 3 hours; & - when compared with clozapine 24 hours*

CONCLUSIONS

Histomorphological study of the cerebellum of laboratory animals revealed the dynamics of reversible and irreversible changes in Purkinje cells in clozapine poisoning in combination with alcohol.

The progression of these changes over time with a predominance of irreversible damage was established, especially after 24 hours from the start of the experiment, when their number increased to 60-70%.

Thus, the conducted study allows us to clarify not only the immediate cause of death, but also to speak about the elapsed time from the moment of taking the drug to the onset of death.

REFERENCES:

1. Sljundin D. G., Livanov A. S., Anuchin V. V. [i dr.] Kriminal'nye otravlenija klozapinom - Anesteziologija i reanimatologija. – 2007. – N 4. – P. 61-64. PMID: 17929492
2. Mashkovskij M. D. Lekarstvennye sredstva 16-e izdanie. M. Novaja volna; 2019: 73-74. ISBN 978-5-7864-0218-7
3. A. S. Babkina, A. M. Golubev, D.V. Sundukov, A. R. Bashirova, M. A. Golubev. Klozapin: mehanizmy toksichnosti i pobochnyh jeffektov. GENERAL REANIMATOLOGY, 2018, 14: 35-45. DOI: 10.15360/1813-9779-2018-2-35-45
4. Tochilov V.A., Kushnir O.N. Klozapin – preparat vybora dlja lechenija bol'nyh s ostrymi psihozami. Social'naja i klin. psihiatrija. 2011; 21 (2): 37-42. ISSN: 0869-4893
5. Jellenhorn, M. Dzh. Medicinskaja toksikologija: diagnostika i lechenie otravlenij u cheloveka: V 2 tomah / Per. s angl. G. K. Faizovoj. — Medicina, 2003. — Vol 1: 1048 p. ISBN 5-225-03320-2
6. Ermohin P.N. Gistopatologija central'noj nervnoj sistemy (pod red. A.P.Avcyna). M. Medicina. 1969.
7. Elliott E.S., Marken P.A., Ruchter V.L. Clozapine-associated extrapyramidal reaction // Ann. Pharmacother. 2000. -N 34(5). - P. 615-618. DOI: 10.1345/aph.19213
8. Clozapine versus typical neuroleptic medication for schizophrenia (Cochrane Review). / K. Wahlbeck, M. Cheine, M.A. Essali et. al. Oxford, 2000.- 126 p. DOI: 10.1002/14651858.CD000059
9. Clozapine dosages and plasma drug concentrations / W.H. Chang, S.K. Lin, H. Y. Lane et al. J. formos. Med. Assoc. -1997. N 96(8). - P. 599-605. PMID: 9290269
10. Hagg S., Spigset O., Soderstrom T.G. Association of venous thromboembolism and clozapine. Lancet Lond Engl. 2000;355(9210):1155–1156. DOI: 10.1016/S0140-6736(00)02066-3
11. Freudenrich O., Weiner R.D., McEvoy J.P. Clozapine-induced electroencephalogram changes as a function of clozapine serum levels // Biol. Psychiatry. 1997. -N42.-P. 132-137. DOI: 10.1016/S0006-3223(96)00298-3
12. Abdelmawla N, Mitchell AJ. Sudden cardiac death and antipsychotics. Part 1. Risk factors and mechanisms. Adv Psychiatr Treat. 2006;12:35–44. DOI: https://doi.org/10.1192/apt.12.1.35
13. Fartushnyj, A. F. Smertel'nye dozy i koncentracii nekotoryh lekarstvennyh veshhestv v biologicheskih ob#ektah / A. F. Fartushnyj // Sudebno-medicinskaja jekspertiza. — 1999. — T. 42, № 5. — S. 16–19.
14. Bellissima B.L.,Tingle M.D., Cicovic A.,Alawami M., Kenedi C. A systematic review of clozapine-induced myocarditis. Int. J. Cardiol. 2018; 259: 122-129. DOI: 10.1016/j.ijcard.2017.12.102
15. Savvatis K., Mohiddin S.A. Clozapine-induced myocarditis: “Psychoanalysing” drug-induced myocarditis. Int. J. Cardiol. 2018; 259: 130-131. DOI: 10.1016/j.ijcard.2018.02.071
16. Datta T., Solomon A.J. Clozapine-induced myocfrditis. Oxf. Med. Case Reports. 2018; 1: omx080. DOI: 10.1093/omcr/omx080
17. Andrew M Williams, Susie H Park. Seizure associated with clozapine: incidence, etiology, and management. CNS Drugs. 2015 Feb;29(2):101-11. DOI: 10.1007/s40263-014-0222-y
18. Bibily J., McCollum B., de Leon. Catatonia Secondary tu Sudden Clozapine Withdrawal: A Case with Three Repeated Episodes a Literature Rview. Case Rep. Psychiatre. 2017; 240: 27-31. DOI: 10.1155/2017/2402731
19. Reddy S.M., Khairkar P.H., Jajoo U. 70 hours of coma by clozapine intoxication. J. Neuropsychiatry Clin. Neurosci. 2013; 25(4): E22-23. DOI: 10.1176/appi.neuropsych.12090221
20. Owen, J.A. Clozapine concentrations and clinical response in schizophrenic patients / J.A. Owen, N.J.

Delva, J.S. Lawson. // Am. J. Psychiatry. - 1992.-Vol. 149.-P. 1120-1121. DOI: 10.1176/ajp.149.8.1120

21. Simpson, G.M. Clozapine plasma levels and convulsions / G.M. Simpson, T.A. Cooper. // Am. J. Psychiatry. 1978. - Vol. 135. - P. 99-100. DOI: 10.1176/ajp.135.1.99
22. Devinsky D., Honigfeld G., Patin J. Clozapine-related seizures // Neurology. -1991.-N41.-P. 369-371. DOI: https://doi.org/10.1212/WNL.41.3.369
23. Kervin R.W. Clozapine: back to future for shizopbrenia research // Lancet. - 1995 Apr 29; 345(8957):1063-4. DOI: 10.1016/s0140-6736(95)90811-0
24. 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. Sankt-Peterburg: Rus-LASA NP «Ob'edinenie specialistov po rabote s laboratornymi zhivotnymi» Rabochaja gruppa po perevodam i izdaniju tematicheskoj literatury; 2012: 48.
25. Ackenheil, M. Clozapine pharmacokinetic investigations and biological effects in man / M. Ackenheil. // Psychopharmacology. - 1989. - Vol. 99.-P. 32-37. DOI: 10.1007/BF00442556
26. Govard D.M. General pharmacology of clozapine // Br. J. Psychiatry Suupl. -1992.-N 17.-P. 5-11. PMID: 1358127

back