RESEARCH ARTICLE

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Nitrozine Stress and Neurological Disorders in Experimental Alcohol Intoxication and Their Pharmacological Correction by Neuropeptides

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Abstracts: The article aims to provide a comparative estimation of Cerebrocurin®, Cortexin® and Cerebrolisin® neuroprotective effect on the outcome of experimental chronic alcoholism in rats. 50 Wistar rats were subjected to transient, experimental chronic alcoholism and were randomly assigned to 5 groups (n=10 each): (1) Intact, (2) Control, (3) Cerebricurin®, (4) Cortexin®, (5) Cerebrolisin®. Investigated preparations were administered during 14 days parenteraly after 30-days of violent alcoholization. Functional deficits were quantified by daily neurological examinations (Garcia et al., 1995); rats' behavior was quantified in the test of Passive Avoidance Conditioned Response (PACR). Nitrotirosine values were measured after treatment. Against the background of chronic alcohol intoxication in rats, we have elevated indicators, nitrozile proteins in plasma and brain reflecting the activation processes of nitrozine stress in each groups. We have conducted correlation between the level of nitrotirozine in rat brain and the manifestations of neurological deficit in scores on McGrow in the control group at the end of the experiment. The most active drug was Cerebrocurin®, which demonstrated a significant reduction of nitrotirozine in plasma and especially in the brain of the rats relative vs vehicle-treated controls and normalized neurological status. This is an experimental justification for inclusion of Cerebrocurin® in the traditional model of treatment of chronic alcoholism.

Key Words: neurons, alcoholization, nitrozine stress, neuroprotection.

INTRODUCTION

Oxidative and nitrozine stress develops in various brain disorders - acute infringement of cerebral blood flow in the form of ischemic and haemorrhagic stroke, in cases of cranial trauma with dyscirculatory encephalopathy, toxic encephalopathy with different etiologie [1, 2].

We know that the onset of events which leads to death of neurons may be caused by cytokines, hormones, reactive oxygen forms (ROF), nitric oxide derivatives (NO), oxidized thiols, products of oxidative modification of proteins and nucleic acids [3 - 5].

Due to a number of reasons, the brain is highly sensitive to nitrozine stress. NO and its derivatives play an important role in the damage of proteine and nucleic acids by activating poly-(ADR-ribose)-polymerase (PARP) which can activate apoptosis [6 - 8].

NO-radicals, peroxynitrites, that attack protein molecules, forming o-tyrosine, 6-nitrotryptofan, nitrotirozine 3, 3-chlortirozine, 2-oxohistidine and various carbonile derivatives, play an important role in the oxidative degradation of proteins [9 - 12].

N-, S-nitrozine destruction of protein's fragments of membranes of neurocytes impairs the sensitivity and specificity of receptors, generation, formation and conduction of nerve impulse and breaks synaptic transmission. These changes lead to a breach of the secretory, incretory, transport function of neurons as well as reduce cognitive and memory body functions [13 - 17].

We have received the data about an increase in NO-syntaze activity in the brain of rats with experimental alcoholism in our previous work. But now, available literatures contain little data regarding the pathogenic role of nitrozine stress in the mechanism of alcohol brain damage that determines the relevance of the chosen research theme, but present results are fragmentary and controversial [17-20].

Also, we have obtained data about neuroprotective properties of drugs with peptide structure (Cerebrocurin®, Cortexin® and Cerebrolisin®) on metabolic processes in nerve cells and the interaction of brain neuromediator systems.

Therefore, it is necessary to assess the impact of these drugs on the parameters of nitrozine stress and neurological deficiency in animals with experimental alcoholic intoxication.

METHODS

The experiment was performed on 50 white male Wistar rats, m 250-300 gr, bred in the Arboretum of the Institute of Pharmacology and Toxicology of Medical Science Academy of Ukraine. Animals were cared for before and at all stages of the experiment in compliance with applicable institutional guidelines and regulations of the bioethics committee of Ukraine. All experimental procedures were carried out according to "Regulations on the use of animals in biomedical research" [21, 22].

Chronic alcohol intoxication was achieved by daily introgastral introduction during the first 10 days – 15% solution of ethanol in doses of 4g/kg, next 10 days – 15% solution of ethanol in doses of 6g/kg and in another 10 days rats get 25% ethanol solution in doses of 3g/kg (R. Mirzoyan, 2001). At 30 days we stopped alcoholization and conducted experimental drug therapy and continued surveillance within 14 days.

The 50 rats used in these experiments were randomly divided into five groups (n=10) (four experimental groups and one control group):

- **Group 1:** received ethanol within 30 days and Cerebrocurin® from day 31 to 44 in dose 0.06 mg / kg [23].
- **Group 2:** received ethanol within 30 days and Cerebrolizin® from day 31 to 44 in doses of 4 mg / kg [23].
- **Group 3:** received ethanol within 30 days and Cortexin® from day 31 to 44 in the dose 0.5 mg / kg [23].
- **Group 4**: received ethanol within 30 days (control).
- **Group 5:** intact (instead ethanol received saline solution).

Every day each rat`s neurological status was assess according to the scale of stroke-index McGrow (up to 3 points - the mild degree, from 3 to 7 points - the moderate level, 7 points or more - severe degree).

Nitrozine protein quantitation was conducted using ELISA-set NITROTIROSINE (NK501 set number, series 4513K19). ELISA-set NITROTIROSINE is a solid phase immunosorbent enzyme set that operates on the principle of sandwich.

Nitrotirozine concentration in the samples, measurements of which is parallel with the standards which can be determined by a standard curve.

Statistical processing of results was conducted by methods of mathematical statistics with application software packages «biostatistics for Windows, version 4.03» and« Microsoft Excel 2002». Distribution normality was tested using Kolmogorov-Smirnov test. Subject to compliance normality distribution reliability obtained different values that are compared, evaluated using the Student t-criterion (or the equivalent nonparametric Mann-Whitney). The reliability of the differences of relative quantities were estimated using criteria $\chi 2$. Relationship between quantitative parameters was assessed using criteria Spearmen (R) and through regression analysis. For all analysis, confidence level differences between the comparative figures are believed to be < 0, 05.

RESULTS

Cerebricurin®

Neuropeptide of new generation, received from embryos of large horned livestock. Cerebricurin®contains free amino acids, neuropeptides and low-molecular products of controllable proteolysis low-molecular fibers and peptides of embryos of large horned livestock.

As it is known, the embryo at an early stage of ontogenesis contains the greatest concentration of regulative neuropeptides which at appropriate technological processing lay the basis of Cerebricurin®. It is not excluded, that in initial suspension of a preparation neuroblast stam cells are obtained.

Regulative neuropeptides, making the basis of the preparation, assist in remyelinisation, glial proliferation and regeneration of new neurons.

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Table 1: Indicators of nitrotirozine in plasma of rats

Group of rats (n=10)	Nitrotirozine in plasma, nml/g proteins
Intact	6.91±1.52
Control	21.61±3.33
Cerebrolisin®	12.97±2.28*
Cortexin®	9.88±1.77*
Cerebrocurin®	7.03±1.81*

*P<0.05 vs vehicle-treated controls

Table 2: Indicators of nitrotirozine in the brain of rats

Group of rats (n=10)	Nitrotirozine in the brain, nml/g proteins
Intact	17.23±3.05
Control	144.27±28.56
Cerebrolisin®	110.55±19.90*
Cortexin®	87.71±23.46*
Cerebrocurin®	25.26±2.63*

*P<0.05 vs vehicle-treated controls

Table 3: Evaluation of Neurological Deficits of Animals with Experimental Chronic Alcoholism from 1 to 44 Days According to the Scale of C.P.McGrow, Apartments in Points (1-30 days - Alcoholization, 31-44 days - Healing stage)

Group of rats (n=10)	1 day	14 day	31 day	36 day	44 day
Intact	0	0.1±0.1*	0	0	0.1±0.1*
Control	0	3.4±0.47*	5.75±0.5*	5.35±0.72*	2.05±0.41*
Cerebrolisin®	0	4.25±0.49*	4.15±0.6*	1.85±0.37*	0.35±0.12*
Cortexin®	0	4.4±0.53*	4.6±0.42*	1.65±0.28*	0.3±0.15*
Cerebrocurin®	0	4.6±0.3*	4.1±0.3*	0.3±0.15*	0.15±0.1*

*P<0.05 vs vehicle-treated controls

The procedures of preparing of Cerebricurin® consist of some stages. Brain tissue is taken from the embryo of an animal, and homogenization weight is diluted with a physiological solution and then preserved before completion of extraction process, and to the solution collected after removal of the formed deposit is added preservative in quantity not less than 0, 5 %. Make sterilization of a solution by filtering and maintain it before formature of lipid layer, and after which the remainins solution preserved before the termination of processes of aggregation at a temperature, not exceeding physiological. Then after branch of the formed particles solution subject to interoperability with immobilized proteolysis enzyme. The mode of interoperability established, proceeding from control test of received means, and the received solution is preserved within 30 days at temperature not above 10°C.

CORTEXIN®

A multicomponent Preparation

Its components are presented by L-amino acids, vitamins and mineral substances.

Peptides of Cortexin® consist of some amino acids: asparaginic acid (446 nm/mg), treonine (212 nm/mg), serine (268 nm/mg), glutamic acid (581 nm/mg), proline(187 nm/mg), glycine (298 nm/mg), alanine(346 nm/mg), valine(240nm/mg), isoleycine (356 nm/mg), tyrosine (109 nm/mg), phenylalanine(162 nm/mg), hystidin (116 nm/mg), lysine(253 nm/mg), arginine and others (202 nm/mg).

A fraction of asparagine acid of about12 % is necessary, and glutamine acids — about 15 % from the general content of amino acids in structure of peptides. Glycine, present in the preparation, at the same time carries out the role of a stabilizer.

Cortexin® contains water-soluble (thiamine — 0.08 mkg/10 of mg, riboflavin— 0.03 mkg/10 of mg, niacin — 0.05 mkg/10 of mg) and fat-soluble vitamins (retinol— 0.011 mkg/10 of mg, an alpha-tocopherol — 0.007 mkg/10 of mg).

At preparation there are mineral substances (Cu — 0.2129 mkg/10 of mg, Fe — 2.26 mkg/10 of mg, Ca — 22.93 mkg/10 of mg, Mg — 8.5 mkg/10 of mg, K — 19.83 mkg/10 of mg, Na — 643.2 mkg/10 of mg, S — 152.65 mkg/10 of mg, P — 91.95 mkg/10 of mg, Zn — 4.73 mkg/10 of mg, Mb — 0.0203 mkg/10 of mg, Co — 0.0044 mkg/10 of mg, Mn — 0.0061 mkg/10 of mg, Se — 0.0745 mkg/10 of mg, Al — 0.3104 mkg/10 of mg, Li — 0.0340 mkg/10 of mg).

The positive effect of Cortexin® is not only as a result of the action of polypeptide components, but also neurochemical activity of macro-and microelements, as well as vitamins (A, E, B1 and PP).

In Cerebrolisin® officially allocate active fraction. It consist of the balanced and stable mixture of amino acids (85 %) and biologically active neuropeptides (15 %), possessing total multifunctional action. However the structure of Cerebrolisin® is more complex.

First, carried out researches have shown, that in cleared Cerebrolisin® there are present more than 100 oligopeptides and motives of fibers with weight basically up to 5800 Da. These are numerous short combinations of amino acids, fragments of peptides, received at tripsinolysis of proteome cortex of pigs` brains.

They serve as a potential trophic product for nerve cells metabolism.

The importance of this research was tracking down the structure of Cerebrolisin® which is vital for the neurochemical activity of the brain`s oligopeptides. Its tripeptides — glutation Glu-Cis-Gly and tiroliberine Glu-His-Pro, as well as motives enkephalin Tyr-Gly-Gly-Phe and collagen Gly-Pro-Hyp.

Is was discovered also that membrane fraction of lipids and maybe effects of Cerebrolisin® connected with increase of plasticity of neurones, can lean in the action not only on membrane fraction of peptides, but also on heterogeneous fraction of neurospecific lipids.

Against a background of chronic alcohol intoxication in rats, we used promoted indicators of nitrozile proteins in plasma and brain reflecting the activation processes of nitrozine stress.

Nitrotirosine value was 21.61±3.33 pg/ng tissue (mean SD) in vehicle-treated controls, 12.97±2.28 pg/ng tissue ($p < 0.05$ compared with vehicle) in animals that received Cerebrolisin®, 9.88±1.77 pg/ng tissue ($p < 0.05$ compared with vehicle) in animals that received Cortexin® and 7.03±1.81pg/ng tissue ($p < 0.05$ compared with vehicle) in animals that received Cerebrocurin® (figure 4).

Nitrotirozine level in plasma of rats (Table 1) in group Cerebrolizin® is below nitrotirozine relative to the control group at 39.98

Nitrotirozine level in the brain of rats (Table 2) in group Cerebrolizin® is lower than nitrotirozine relative to the control group on 23.37%.

We estimated the daily neurological status of rats from the beginning till the end of the experiment and we noted that alcohol intoxication led to persistent neurologic disorders (tremor, totter, violations of physical

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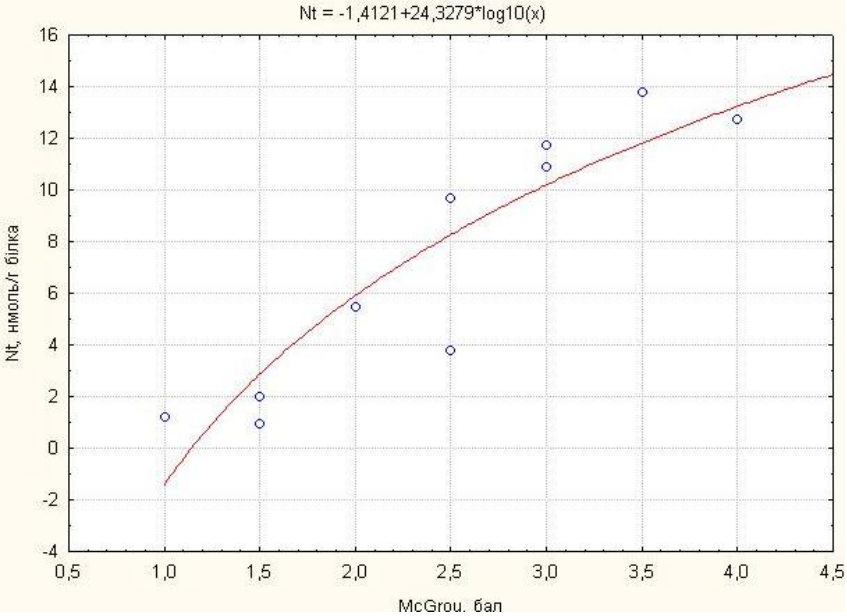

Figure 1: The results of the regression analysis (scattering diagram) interconnection 'communication indicators integrated marker nitrozine stress (nitrotirozine in the brain) expression and neurological deficits (ballroom index on a scale C. P. McGrow)

activity), that persist and after the withdrawed of ethanol. Introducing Cerebrolizin®, Cortexin® led to regression of neurological symptoms within 7 days from 6-7 to 1-2 points on McGrow, from 7 to 14 day rats had 1-2 score on McGrow (Table 3).

In the group Cerebrocurin® nitrotirozine level in plasma decreased by 67.47% relative to reliably control and in the brain of rats reliably below nitrotirozine compared with the control group to 82.49%.

Cerebrocurin® was the most active drug, reducing the manifestations of neurological symptoms in the first 3 days of treatment of 6 – 7 to 1 - 2 points on McGrow. Against the background of the animals receiving Cerebrocurin® significantly reduced manifestations of neurological disorders: tremor, stiff tail, chaotic motions in a cage, ptosis, hyperactivity, convulsive muscle contraction in the first days of admission in contrast to other drugs. Cerebrocurin® also had a significant sedative effect on animals compared with other groups (reduced forms of hyperactivity, aggressiveness).

Mechanism of action and point of impact of Cerebrocurin® is fundamentally different from other drugs of neuropeptid nature, especially Cerebrolizin®. Cerebrocurin® containing peptides that have a program analysis and construction of the CNS. So the result is a qualitative difference between different mechanisms of action. Protective effects of brain tissue of Cerebrocurin® include optimizing its effect on brain energy metabolism and calcium homeostasis, stimulation of intracellular protein synthesis, inhibition the processes of glutamate-calcium cascade and lipid peroxidation. However, the drug has significant neurotrophic effects.

Cerebrocurin® has the ability to increase glucose transporter gene expression through blood-brain barrier and also increases its transport to the brain. Cerebrocurin® neurotrophic properties related to the protection of neuronal cytoskeleton by Ca-dependent protease inhibitors, including kalpayin, and increased expression microtubule acidic protein-2. At the same time Cerebrocurin® affinity of BDNF which increases binding to its receptors. Influence of preparation for trk-B-receptors neutrofin shows its effect on the regulation of growth factors. Also Cerebrocurin® regulates the hyperactivity of microglia and reduces production of IL-1α and other proinflammatory cytokines that reflects the influence of the drug to the local manifestations of inflammatory reactions and processes nitrozine and oxidative stress.

Currently Cerebrocurin® is widely used in clinical practice for the above mentioned [24, 25].

We conducted interrelations' between the level nitrotirozine in rat brain and the manifestations of neurological deficit in scores on McGrow in the control group at the end of the experiment.

As the results of regression analysis and the evaluation chart scattering integral relationship between stress marker nitrozine stress and parameter characterizing the degree of neurological deficit (score on a scale ballroom CPMcGrow), approximated by a linear regression model (Fig.).

Equation

Score on a scale ballroom CPMcGrow = 1.43 +0.31 * nitrotirozine.

This allows you to program in the future based on data nitrotirozine concentration and its correlation with the degree of neurological deficit according to the scale C.P.McGrow.

NT = -1.4121 +24.3279 * log10 (x) sets. R = 0.92 F = 47.56 R2 = 0.86 the resulting relationship is highly reliable - R = 0.000125, β = 0.92, Fisher ratio F = 47.56, calculating the standard error 0.219. According to the regression analysis we conclude that the level of values nitrotirozine to 10 nml/g protein value neurological deficit according to the scale of C.P. McGrow does not exceed 3 points (because the value scale dance performance by C. P. McGrow concentrated in the bottom of the chart in the initial part of the regression line). Improving nitrotirozine reflects the exacerbation of oxidative modification of signaling molecule NO, also observed is the progression of worsening neurological deficit.

At the level of values nitrotirozine more than 10 nml/g protein in 100% of the value scale of neurological deficit according C.P.McGrow exceeds 3 points that shows more significant changes in neurological status.

The resulting error of approximation (standard error of approximation is 0.219) shows the adequacy built graph interrelation and the possibility of good prognosis according to the scale dance performance C.P. McGrow and nitrotirozine.

This data shows a statistically significant effect of nitrotirozine on neurological deficit and need correction oxidation – reduction imbalance of NO – in the modulating effect of rational neuroprotection in chronic alcohol intoxication.

The above data indicates that the neuroprotective mechanism of action of investigated drugs (Cerebrocurin®, Cortexin® and Cerebrolizin®) mechanism is an important antioxidant that prevents the development of nitrozine stress. It should be noted Cerebrocurin® drug that approached the level of nitrotirozine in the brain of rats almost intact and demonstratively show the best efficiency among preparations. The second performance was Cortexin® drug, which demonstrated lower nitrotirozine in the brain of 39.20% compared with control. Cerebrolizin® showed nitrotirozine level of 23.37% less than control, have appeared as the least effective antioxidant.

CONCLUSIONS

- We have demonstrated statistically significant interconnection between nitrotirozine level in rat brain and neurological Ballroom Score deficit according to the scale of C. P. McGrow in model of chronic alcohol intoxication in rats. This allows you to program in the future on the basis of concentration of nitrotirozine and their relationship with degree of neurological deficit.
- The above data indicates that the neuroprotective mechanism of action of investigated drugs (Cerebrocurin®, Cortexin® and Cerebrolizin®) mechanism is an important antioxidant that prevents the development nitrozine stress.

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- The most active drug was Cerebrocurin®, which demonstrated a significant reduction nitrotirozine in plasma and especially in brain relative to controls and background parallel normalization of neurological status. This is an experimental justification for inclusion Cerebrocurin® in traditional model of treatment of chronic alcoholism.

REFERENCES AND NOTES

1. Belenichev I.F., Kolesnik Y.M., Pavlov S.V., Abramov A.V., Buhtiyarova N.V. Mitochondrial dysfunction in cerebral pathology. Neuroprotection by Cerebrocurin® / by international neurological journal. - 2008. - № 4 (20). - p. 23-29.
2. Belenichev I.F., Levitskyy E.L., Pavlov S.V. The role of early response gene c-fos in normal and neurodestruktiv toxic pathology. Features farmakocorection neuropeptid drugs / / Modern problem of toxicology. - 2008. - № 1. - p. 17-27.
3. Belenichev I. F., Chernykh V.A., Kolesnik M.U. Rational neuroprotection / / Donetsk: A.U. Zaslavsky publisher, 2009.-262pp.
4. Levytskyy E.L., Hubskyy Y.I. Freeradicale damaged of nuclear genetic apparate of the cell. / / Ukr. Biochim. Gourn. -1994. -T. 66, № 4. -C. 18-30.
5. Sokolovskyy V.V. / / Questions of medical chemistry. -1998. -T. 6, № 34. -p. 2-11.
6. Dubnina E.E, Morozova M.G., Havrovskaya S.V., Kuzmich E, V., Leonova N.V. / / Biochemistry. - 2002. - T. 67, № 3. - P.413-421.
7. Belenichev I.F., Pavlov S.V., Sokolik E.P., Buhtiyarova N.V., New treatment possibilities of alkohole disease. Cerebrocurin® application prospects. / / International neurology gournal.- 2008. - № 2 (20).
8. Zaidi A., Miachaels ML / / Free Rad. Biol. -1999. -V. 27. -P. 810-821.
9. Dean RT, Hunt JV, Grant AJ et al. / / Free Rad. Biol. Med. - 1991. - V.11, № 12. - P.161-165.
10. Artsukevych A.N., Maltsev A.N., Zynchuk V.V. / / Biochemical aspect of life of biology systems. Gathering. Teach. Labour congress of biochemisters of Belarus. Grodno. - 2000. - P.19-23.
11. Miayata T., Inagi R., Asashi K., Hhorie K. et al. / / FEBS Lett. - 1998. - V. 437, №1-2. - p. 24.
12. Salo P.S., Pacifici R.E., Lin S.W. et al. / / Biochem J. - 1990. - V. 265, № 20. - P.11919-11927.
13. Levine R.L., Garland D., Oliver C.N. / / Meth. Enzymol. - 1990. - V. 186. -p.464-478.
14. Dubnyna E.E., Burmystrov S.O., Chassis D.A., Porotov I.A. / / Questions of medical chemistry. - 1995. - T.41, №1. - P.24-26.
15. Sokolovskyy V.V. Thiodisulfide interaction of blood as indicators the state of nonspecific resistance of organism. SPb, 1996. - 30pp.
16. Packer L., Prilipko L., Christen Y. Free Radicals in the Brain. Aging / / Neurological and Mental Disorders. -Berlin; New York: Springer-Verlag, 1992. - P.21-41.
17. Bongarzone ER, Pasquini JM, Soto EF / / J. Neurosci Res. -1995. - V. 41. - P.213-221.
18. Crune T., Michel P., Sitte N. et al. / / Free Radic. Biol. Med. - 1997. - V. 23. -p.357-360.
19. Ciolino H.P. and Levine R.L. / / Free Rad. Biol. - 1997. - V.22. - P.1277-1282.
20. Pigeolit E., Corbiser P., Houbion A. et al. / / Mech. Ageing and Develop. -1990. - V.51. - p.283-287
21. Kozhemyakin Y., Khromov A.S., Filonenko M.A., Sayfetdinova G.A., Scientific and practical advice on maintenance of laboratory animals and work with them, Kyiv, 2002.
22. Habriev R.U., Recommendations on experimental (preclinical) study of new pharmacological substances, Moscow, 2005.
23. The directory VIDAL"Medications of Russia", Moscow, Russia, 2010.
24. Yena L.M., Kuznetsova S.M., Kuznetsov V.N. and others. Material and experimental clinical trials of preparation 'Cerebrocurin®, Kyiv, 1997. - 115 pp.
25. Serhiyenko A.N. Application of the drug 'Cerebrocurin® in the treatment of degenerative-dystrophic diseases of the retina / News of medicine and pharmacy. - 2001. - № 12(97). - p.8.

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