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ASSESSMENT OF THE DNA DAMAGE LEVEL IN PERIPHERAL BLOOD LEUKOCYTES OF MICE TREATED ORALLY WITH RAPITALAM IN ACUTE AND THERAPEUTIC DOSES

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Abstract
Rapitalam is a drug that is a modulator of the mglur4 receptor - a kind of metabotropic glutamate receptors. Into BelSU Clinical and Preclinical Studies Centre there was performed experimental research, which carried out the identification and quantitative assessment of alkali-labile sites and DNA strand breaks in leukocytes of peripheral blood of male mice treated with Rapitalam. The method is based on the assessment of the integrity of DNA in leukocytes of the whole blood of animals. Rapitalam was administered to animals orally according to 2 schemes: a single acute dose (413 mg/kg, which corresponds to 1/5 LD_{50} dose) and once daily in a therapeutic dose (3 mg/kg) for 4 days. For analysis we used peripheral blood of mice. As an indicator of DNA damage there was used the value of %TDNA. The results of this study established that the level of DNA damage of blood leukocytes (%TDNA) in the groups with acute dose of Rapitalam statistically significantly different from those values in control group of animals, indicating the presence of DNA-damaging activity of Rapitalam in the acute dose. Analysis of the level of DNA damage of blood leukocytes (%TDNA) in groups of animals treated with therapeutic dose of Rapitalam showed significant differences between animals treated with solvent (dimethyl sulfoxide) and animals treated with Rapitalam dissolved in DMSO. Conspicuous is the fact that there is observed a significant reduction in DNA damage in the therapeutic dose of Rapitalam as compared to the acute dose and the group receiving only the solvent. This suggests that Rapitalam in a therapeutic dose can influence on the processes of intracellular metabolism and acts as a protector.
Key words: Rapitalam, Parkinson's disease, metabotropic glutamate receptors, mGluR4 receptor modulators, DNA strand breaks in leukocytes, alkali-labile sites

Introduction

Rapitalam is a mGluR4 receptor modulator. Mglur4 is a kind of metabotropic glutamate receptors. This group of receptors, as the name implies, doesn't "open" for the current of ions through the membrane of the neuron after activation and exerts its effect indirectly by intracellular signal molecules – second messengers. Metabotropic receptors are divided into three groups depending on their mechanism of action, homology of structure and list of selective ligands (1 – mGluR1, mGluR5; 2 – mGluR2, mGluR3; 3 – mGluR4, mGluR6, mGluR7, mGluR8). Groups of receptors differ in their mechanism of action. Receptors of the first group are associated with Gq-protein. Other groups of glutamate receptors, second and third, make with Gi-protein. It means that the activation of these receptors blocks the function of adenilate cyclase, which in the active state converts ATP into cAMP. Consequently, the work of cAMP protein kinase stops and a phosphorylation pathway that modify the homeostasis of calcium doesn't start [1, 7].

Based on the foregoing, we can understand the differences in the effects of these receptors: activation receptors of 1 group leads to increasing of the activity of NMDA and AMPA receptors is increased (i.e. by increasing synaptic density), but also susceptibility to excitotoxicity increases; activation receptors of 2 and 3 groups, on the contrary, leads to decrease the activity and density of ionotropic receptors and decreases the likelihood of excitotoxicity [8].

Thus glutamate receptors play a huge role in the regulation of functioning and development of the nervous system. For example, glutamate plays a role in neuronal death in hypoxia – in such circumstances, the glutamate transporter is not capable of reuptake of the neurotransmitter into the cell. Therefore, in condition of massive death of neuronal cells, the amount of the released glutamate is growing exponentially, causing exitotoxic excitation in still living neurons and leading to its death.

Glutamatergic system of the brain is one of the most widely specialized signaling systems in our

brain and nervous system, and its role is difficult to overestimate [9].

The aim of this study is the identification and quantitative assessment of alkali-labile sites and DNA strand breaks in leukocytes of peripheral blood of male mice treated orally with Rapitalam.

Materials and methods

The method is based on evaluation of DNA integrity in whole blood leukocytes of animals and humans.

The experiment was performed on small laboratory rodents (males of mice), with an average weight of 35-40 g and 2-4 months of age. The animals were kept in accordance with the applicable Sanitary rules on the device, equipment and maintenance of experimental biological clinics in BelSU Clinical and Preclinical Studies Centre, on a standard diet, with 12-hour light mode, in conditions of free access to water and food. Obtained from the nursery animals were distributed in randomized groups of 6 individuals. As a negative control there was used animals that were injected solvent. The exposure time, the conditions of the keeping of the negative control animals and animals receiving the test substances were identical [2].

The test pharmaceutical substance of Rapitalam was dissolved in dimethyl sulfoxide to final concentration of solvent 5%. Acute dose was 413 mg/kg, which corresponds to 1/5 of LD_{50} dose (according to studies acute toxicity the LD_{50} of the test drug was 2066 mg/kg). The therapeutic dose was 3 mg/kg. All solutions and suspensions were prepared immediately before use. Rapitalam was administered to animals orally in two ways, either a single acute dose or once a day therapeutic dose for 4 days. The substance was administered orally.

For analysis we used a peripheral blood of the mice obtained by incising the tip of the tail. The blood aliquots sampling (10 µl) of each animal was performed not later than 24 hours after the completion of treatment. Peripheral blood was sampled in tubes containing phosphate buffer (136.7 mM NaCl, 2.7 mM KCl, 8.1 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.2-7.4) and 1 mM EDTA, shook by vortex to prevent clotting and immediately used for preparation of agarous slides [4, 5].

Aliquots of diluted blood were mixed with an equal volume of 1% low-melting agarous (“Sigma Chem. Co.”, USA) at a temperature of 37º C and applied to the prepared agarous layer. After hardening of the agarous, containing the cells, on top there was applied a new layer of 0.5 % low-melting agarous. The slides were placed in lysing solution (2.5 mol/l NaC1, 0.1 mol/l EDTA, 0.01 mol/l Tris-HCL, pH 10, 1% Triton X-100) at 4-6°C for 1 h. Then the slides were transferred for 20 min in alkaline solution (0.3 mol/l NaON, 0.001 mol/l EDTA, pH >13), transferred to the electrophoresis chamber SE-1/S-1N (LLC “Helicon”, Russia) and subjected to electrophoresis in a fresh portion of the alkaline solution (250 ml) for 20 min at 4-6°C (voltage 27 V, current 260-270 mA, the strength of the electric field 2 V/cm).

After electrophoresis, the slides were washed with distilled water and stained for 1 h in a solution containing 2.0 µg/ml of ethydium bromide. The preparations were analyzed using a fluorescence microscope "LUMAM I-3" ("LOMO", Saint-Petersburg, Russia). Image capture was performed with a digital camera "Nikon CoolPix 995" (Japan) with the subsequent transfer them to the computer. The processing of the photomicrographs was performed using specialized software, where there were implemented the algorithms of calculation of standard parameters of "comet" [2]. For each experimental point there was taken for 6 mice and prepared 3 slides of whole blood from each animal and photographed at 50 "comets" slide [3], that is, for each microslide there were analyzed no less than 150 DNA comets with no overdubs of tails. The analysis of parameters of DNA comets was performed with the stored digital images. As an indicator of DNA damage there was used the value of %TDNA - % DNA in the tail of the comet. Statistical analysis was performed using student's t-test ($p < 0.05$). The middle values presented as M ± SD.

Research results

Data on parameters of DNA damage for each mouse, established after administration of Rapitalam and/or 5% DMSO, are shown in tables 1 and 2. Table 3 shows average values of DNA damage in groups while taking the drug and/or solvent for this drug.

Table 1.

The level of DNA damage in peripheral blood cells of animals after administration of acute dose of DMSO and/or Rapitalam (M ± SD).

DMSO			Rapitalam		
Number of the animal	Number of analyzed cells	%TDNA	Number of the animal	Number of analyzed cells	%TDNA
1	150	12.77±1.3	7	150	24.5±6.5
2	150	17.75±8.4	8	150	27.37±3.5
3	150	21.34±5.4	9	150	23.63±4.0
4	150	19.5±2.5	10	150	24.51±5.3
5	150	15.89±3.9	11	150	22.07±9.1
6	150	11.81±8.5	12	150	17.39±3.8

Note: no significant differences between the values of %TDNA for DMSO and Rapitalam from individual animals.

Table 2.

The level of DNA damage in peripheral blood cells of animals after administration of the therapeutic dose of DMSO and/or Rapitalam (M ± SD).

DMSO			Rapitalam		
Number of the animal	Number of analyzed cells	%TDNA	Number of the animal	Number of analyzed cells	%TDNA
1	150	10.69±2.4	7	150	2.06±1.6*
2	150	16.16±5.8	8	150	3.37±1.0
3	150	14.04±5.1	9	150	3.99±0.3
4	150	16.34±4.4	10	150	1.22±0.6*
5	150	26.62±5.4	11	150	1.00±0.3*
6	150	16.78±5.5	12	150	1.56±0.5*

Comment: * - significantly different from the value for DMSO ($p < 0.05$).

Table 3.

The influence of Rapitalam on the level of DNA damage in peripheral blood leukocytes of the mice (M ± SD).

Parameters	Acute dose, 1/5 of LD_{50}		Therapeutic dose, 3 mg/kg	
	%TDNA	p	%TDNA	p
Rapitalam	23.25 ± 3.35	0.008	2.2 ±1.22 *	0.0009
DMSO, 5%	16.51 ± 3.75		16.77 ± 5.3	

Note: differences between the values of %TDNA for DMSO and Rapitalam as in the acute dose and the therapeutic dose are significant.

* significantly different from the value for acute dose of Rapitalam ($p < 0.05$).

Conspicuous is the fact that there is observed a significant reduction in DNA damage in the therapeutic dose of Rapitalam as compared to the acute dose and the group receiving only the solvent. This suggests that Rapitalam in a therapeutic dose can influence on the processes of intracellular metabolism and acts as a protector.

Conclusions

1. Analysis of the level of DNA damage of blood leukocytes (%TDNA) in the groups with acute dose of Rapitalam (Table 3) showed significant differences between animals treated with the solvent from mice treated with dissolved in DMSO Rapitalam ($p = 0.008$). It indicates the presence of DNA-damaging activity of Rapitalam in the acute dose.
2. Analysis of the level of DNA damage of blood leukocytes (%TDNA) in groups with therapeutic dose of Rapitalam showed significant differences between animals treated with the solvent and animals treated with dissolved in DMSO Rapitalam ($p = 0.0009$) (see Table. 3).

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