Phosphorylation pathway

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molecules

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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].
RESEARCH RESULT


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 LD50 dose (according to studies acute toxicity the LD50 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 37°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 NaCl, 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-1/5-1N (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.

| 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 | 12.7±1.3 | 1 | 150 | 24.5±6.5 |
| 2 | 150 | 17.7±6.8 | 2 | 150 | 27.3±3.5 |
| 3 | 150 | 21.3±5.4 | 3 | 150 | 23.6±4.0 |
| 4 | 150 | 19.5±2.5 | 4 | 150 | 24.5±5.3 |
| 5 | 150 | 15.8±3.9 | 5 | 150 | 22.0±9.1 |
| 6 | 150 | 11.8±8.5 | 6 | 150 | 17.3±3.8 |

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

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Acute dose, 1/5 of LD50</th>
<th>Therapeutic dose, 3 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%TDNA</td>
<td>p</td>
</tr>
<tr>
<td>Rapitalam</td>
<td>23.25 ± 3.35</td>
<td>0.008</td>
</tr>
<tr>
<td>DMSO, 5%</td>
<td>16.51 ± 3.75</td>
<td></td>
</tr>
</tbody>
</table>

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

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).

**References**


