INFLUENCE OF A BIOLOGICALLY ACTIVE COMPOUND FROM SUBSTITUTED THIADIAZINES ON TRANSAMINASE ACTIVITY IN MYOCARDIAL HOMOGENATE IN EXPERIMENTAL MYOCARDIAL INFARCTION

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ABSTRACT

Objective: Earlier works have reported on the effectiveness of the compounds of the group of substituted 5R1, 6R2, 3,4-thiadiasine-2-amines for treating experimental myocardial infarction, conditioned by the immune-modifying action of the compound. The purpose of this study was to evaluate the action of the L17 compound of the group of substituted 5R1, 6R2, 3,4-thiadiasine-2-amines on the extent of injury and the possible recurrence of experimental myocardial infarction by the dynamic assessment of transaminase activity in blood and myocardial homogenate (tissue).

Methods: Modelling of myocardial infarction in rats was performed in accordance with the author's modification of the standard ligation model. Tissue enzyme activity of LDH and CK-MB was evaluated at days 1, 7, and 14.

Results: According to the results, the decrease in LDH 1-2 activity in tissue (after experimental myocardial infarction) corresponded to the increase in enzyme activity in blood on the first day of the experiment. However, on the seventh day of the experiment, the decrease of LDH 1-2 activities in the tissue of animals treated with L17 compound corresponded with the decrease of LDH activity in blood, while in non-treated animals the relation between the enzyme levels in blood and tissue was typical for the onset of MI.

Conclusions: The evaluation of enzyme levels in myocardial tissue confirms previously-reported data that the administration of a thiadiazine compounds prevents the recurrence and decreases the size of experimental myocardial infarction.

Keywords: Enzymes, L-17 compound, Myocardial infarction, Thiadiazines, Tissue enzymes.
All animals undergoing surgery received a similar level of care and attention. Surgery was performed with the use of aseptic techniques. Instruments were sterile. For separate experiments, the procedure was as follows. After an overnight fast, the rats were anesthetized with ether and the experimental MI was performed. At the end, all rats were anesthetized with ether and capitated. Diazepam (2.5 mg/kg) was used to lessen the dosage of general anesthetic and to produce a smoother induction and recovery [11, 12].

Modelling of MI in rats was performed in accordance with the author’s modification of the standard ligation model [RF Patent No 2407062 of 20/12/2010] [4]. After surgery, every rat was maintained in a separate, labelled cage. The methods have been previously reported [4, 5].

**Experimental protocol**

The animals were divided into four groups: Group A included 5 intact animals with an average body weight of 220 g each; Group B (sham-operated) included 10 animals (average body weight 216 g each) that underwent a thoracotomy (only plasma enzyme levels were detected [4, 5]); Group C (MI-group) included 10 animals (average body weight 232 g each) that underwent experimental MI, but no preparation was administered; and Group D included 15 animals (average body weight 210 g each) that had undergone the experimental MI and received intraperitoneal injection of the L-17 compound, dosed at 40 mg/kg an hour after surgery. Later, a 40-mg/kg dose of L-17 was repeated as often as once every 24 hours.

**Laboratory testing**

For biochemical analysis, 3 ml of blood was obtained via a heart puncture for subsequent centrifugation and serum separation. Serum activity of CK-MB, ALT, AST, LDH (isoenzymes 1 and 2) before puncture for subsequent centrifugation and serum separation. The weighed portion of myocardial tissue with physiological saline (1.0 ml of physiological saline per 100 mg of tissue) was homogenized for 1 minute by placing a glass containing physiological saline per 100 mg of tissue in a separate labelled cage. The methods have been previously reported [4, 5].

**Table 1: LDH activity in rats with myocardial infarction in the myocardial tissue homogenate (based on milligrams of protein tissue homogenate)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Intact animals, (Group A), n=5 M±m</th>
<th>Experimental infarction without treatment (Group C), n=10 M±m</th>
<th>Experimental infarction with L17 compound administration (Group D), n=15 M±m</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st day</td>
<td>5.10±0.31</td>
<td>3.86±0,45*</td>
<td>3.83±0.10*</td>
</tr>
<tr>
<td>7th day</td>
<td>4.37±0,29</td>
<td>4.09±0.54</td>
<td>4.22±0.24</td>
</tr>
<tr>
<td>14th day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH 1-2</td>
<td>5.06±0.31 (99.4 %)</td>
<td>3.45±0,11* (89.4 %)</td>
<td>3.81±0.36* (99.5 %)</td>
</tr>
<tr>
<td>1st day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7th day</td>
<td>3.76±0,11* (86.6 %)</td>
<td>3.31±0.28* (88.7 %)</td>
<td></td>
</tr>
<tr>
<td>14th day</td>
<td>3.92±0,17* (96.1 %)</td>
<td>3.46±0.26* (82.0 %)</td>
<td></td>
</tr>
<tr>
<td>Note:</td>
<td>* statistical reliability with group A was significant (p&lt;0.05); # statistical reliability between Groups C and D was significant (p&lt;0.05).</td>
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</tr>
</tbody>
</table>

Analysis of total LDH activity in rats with experimental MI (Groups C and D) in the myocardial tissue homogenate (in terms of milligrams of tissue homogenate protein) revealed significantly lower levels of activity compared with that in Group A animals on day 1 of the experiment (3.86±0.45 U/g protein in group C and 3.38±0.10 U/g protein in group D vs. 5.10±0.31 U/g protein in Group A).

Analyses of LDH 1-2 iso enzyme activities showed that the values of LDH1-2 did not differ significantly between Groups C and D but were significantly lower than the values for Group A animals (table 1). Moreover on the seventh day of the experiment, LDH 1-2 activity in the myocardial tissue of Group D animals was significantly lower than not only the values for Group A animals (3.31±0.28 U/g protein in Group D versus 3.73±0.375, 10±0.31 U/g protein in Group A), but also the values for Group C animals (3.31±0.28 U/g protein in group D vs. 4.37±0.29 U/g of protein in B).

Analysis of total LDH activity in rats with experimental MI (Groups C and D) in the myocardial tissue homogenate (in terms of gram wet weight tissue) revealed the absence of any significant differences between the experimental groups. However, analyses of LDH 1-2 iso enzyme activities showed that the values of activity in Group C animals were significantly lower than the values of Group A animals on days 1 and 7.

**RESULTS**

The results of routine biochemical studies of enzyme activity (markers of myocardial infarction) in the serum have been previously reported [4].

According to the results of biochemical analyses, the amino transferring enzyme levels in the serum of sham-operated rats (Group B) reliably exceeded the corresponding indicators of healthy rats in Group A. Enzyme activity returned to basal levels (close to the values of intact animals) on day 7 of the experiment.

Throughout the study, in animals with experimental infarction without treatment (Group C), determined levels of almost all enzymes exceeded that of intact animals as well as the enzyme indicators in sham-operated animals. Only on the fifth day of experimental infarction were the activity indicators AST [17.4±3.0 U/l], ALT [10.7±3.1 U/l], and LDG 1-2 [262.6±22.1 U/l] significantly decreased compared with those on the first day, although these rates remained higher than in Groups A and B [4].

Of interest is the comparison of serum enzymes between Groups C and D. According to the data obtained, throughout the entire experiment, the levels of AST, ALT, and CK-MB in Group D animals were significantly lower than that in the blood of Group C animals. In addition to the biochemical analyses, the evaluation of enzyme activity in myocardial tissue was performed (tables 1-4).

The Student t test and a nonparametric Mann-Whitney test were applied to compare the groups. The Statistica Six Sigma Release 7 computer program was used for mathematical data processing. All the data are expressed as mean±SD. P<0.05 was considered statistically significant.
more informative than other enzymes, because it increases after MI. However, LDH1 isoenzyme present mostly in the heart is considered a biomarker of MI and reacts later to injury than other enzymes do. Total LDH (there are 5 LDH isoenzymes) is known to be the late level of special importance for the diagnosis of MI [6, 13-16].

**Table 2: LDH activity in rats with myocardial infarction in the myocardial tissue homogenate (in terms of gram wet weight tissue)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Intact animals, (Group A), n=5 Mzm</th>
<th>Experimental infarction without treatment (Group C), n=10 Mzm</th>
<th>Experimental infarction with L17 compound administration (Group D), n=15 Mzm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total LDH activity, U/g (units per grams)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st day</td>
<td>331.5±7.8</td>
<td>332.4±39.9</td>
<td>309.2±8.1</td>
</tr>
<tr>
<td>7th day</td>
<td>326.0±24.3</td>
<td>335.7±43.9</td>
<td>348.6±35.6</td>
</tr>
<tr>
<td>14th day</td>
<td>322.2±13.2</td>
<td>322.2±13.2</td>
<td>381.0±20.7</td>
</tr>
<tr>
<td>1st day</td>
<td>329.5±7.9 (99.4%)</td>
<td>297.4±8.7* (89.5%)</td>
<td>308.1±29.3 (99.6%)</td>
</tr>
<tr>
<td>7th day</td>
<td>280.6±8.5* (86.1%)</td>
<td>309.8±26.2 (88.9%)</td>
<td></td>
</tr>
<tr>
<td>14th day</td>
<td>322.2±13.2</td>
<td>322.2±13.2</td>
<td>312.5±22.0 (82.0%)</td>
</tr>
</tbody>
</table>

Note: * statistical reliability with group A was significant (p<0.05).

**Table 3: CPK activity in rats with myocardial infarction in the myocardial tissue homogenate (in terms of gram wet weight tissue)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Intact animals, (Group A), n=5 Mzm</th>
<th>Experimental infarction without treatment (Group C), n=10 Mzm</th>
<th>Experimental infarction with L17 compound administration (Group D), n=15 Mzm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total CPK activity, U/g(units per grams)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st day</td>
<td>3.34±0.23</td>
<td>5.33±0.39*</td>
<td>5.10±0.38</td>
</tr>
<tr>
<td>7th day</td>
<td>5.66±0.44*</td>
<td>3.46±0.30#</td>
<td>5.18±0.28</td>
</tr>
<tr>
<td>14th day</td>
<td>5.18±0.33*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: * statistical reliability with group A was significant (p<0.05); # statistical reliability between groups C and D was significant (p<0.05).

According to the study data, total CPK activity (in terms of milligrams of tissue homogenate protein) in Group C animals was significantly higher than the activity of this enzyme in Group A animals. However, because of L17 compound administration (Group D) on day 7 of the experiment, total CPK activity was significantly lower (3.46±0.30 U/g protein) than that in Group C (5.66±0.44 U/g protein) animals and did not differ from the values for intact animals (Group A).

**Table 4: CPK activity in rats with myocardial infarction in the myocardial tissue homogenate (based on milligrams of protein tissue homogenate)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Intact animals, (Group A), n=5 Mzm</th>
<th>Experimental infarction without treatment (Group C), n=10 Mzm</th>
<th>Experimental infarction with L17 compound administration (Group D), n=15 Mzm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDH activity, U/g(units per grams)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st day</td>
<td>215.5±15.7</td>
<td>458.9±31.2*</td>
<td>411.6±28.6</td>
</tr>
<tr>
<td>7th day</td>
<td>421.5±29.1*</td>
<td>323.6±26.9* #</td>
<td></td>
</tr>
<tr>
<td>14th day</td>
<td>425.9±26.2*</td>
<td>467.7±26.8*</td>
<td></td>
</tr>
</tbody>
</table>

Note: * statistical reliability with group A was significant (p<0.05); # statistical reliability between groups C and D was significant (p<0.05).

A similar pattern in the values of CPK activity was recorded during the study analysis in terms of gram wet weight tissue. According to the results, total CPK activity in Group D animals was significantly lower (322.2±13.2 U/g protein) than that in Group C (421.5±29.1 U/g protein) animals.

**DISCUSSION**

All transaminases, including the CPK-MB, are present in skeletal muscles, and even average damage to muscles can increase their levels in the blood to abnormal values [12]. Therefore, of special significance was the comparison of enzyme levels in animals with (Groups C and D) and without myocardial tissue damage.

The most sensitive tests in MI diagnostics are the evaluation of the CPK, AST, and LDH activity in blood serum [3, 6]. For example, AST activity after MI can increase 2 to 20 times, but during angina it usually remains within normal values [13, 14]; the gradual decrease in AST and ALT levels is of special importance for the diagnosis of MI [6, 13-16].

Total LDH (there are 5 LDH isoenzymes) is known to be the late biomarker of MI and reacts later to injury than other enzymes do. However, LDH1 isoenzyme present mostly in the heart is considered more informative than other enzymes, because it increases after MI before other isoenzymes and can increase against the normal values of total LDH [15].

As for the CPK-MB, it is located almost in the myocardium, and for that reason, its increase is a highly specific and sensitive indicator of cardiomyocyte damage [17].

In contrast to the abovementioned enzymes, the increase of ALT levels after MI is not so significant and sharp, but its lower levels in comparison with levels of AST (ALT/AST ratio) are of great diagnostic value because it excludes liver pathology [18, 19].

According to previous data [4, 5], the significant increase in transaminase activity on the first day of the experiment indicated the effectiveness of the experimental MI model used in the study. The increase in enzyme activity on the fifth day of the experiment is also common in acute MI development [6].

The influence of L17 compound administration on tissue enzyme levels

Significant lower CPK activity in Group D animals (on the background of L17 compound administration) was the indirect confirmation of the compound’s action in reducing the size of...
CONFLICTS OF INTEREST
The authors declare no conflict of interest.

ACKNOWLEDGMENT
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REFERENCES