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Original Article

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 previouslyreported 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.

INTRODUCTION

Despite the discovery of new methods for diagnosing myocardial infarction (MI), the assessment of the level of a biomarker remains advantageous compared with other diagnostic techniques. First, biomarkers can help clinicians efficiently formulate a differential diagnosis. Second, because biomarker levels often correlate with the severity of a disease, they can be used to guide therapy. Third, some biomarkers provide prognostic value [1]. And last but not least, the cost of assessment (via biochemical analyses) of biomarkers is much lower than the cost of procedures with similar predictive capabilities.

Irreversible myocardial necrosis is the hallmark of acute myocardial infarction. Myocardial injury leads to the release of specific cytosolic substances that can be used as markers of injury [1]. These markers includecreatine phosphokinase (CK-MB), aspartate amino transferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), and others [2]. The activity of these enzymes increases dramatically in blood serum after the onset of ischemia, which is the indicator of the extent and depth of tissue damage [3].

Earlier works have reported on the effectiveness of the L17 compound of the group of substituted 5R1,6R,2,3,4-thiadiasine-2-amines for treating experimental myocardial infarction, conditioned by the immune-modifying action of the compound. These studies assert that a reduction in neutrophilous infiltration and activation of lympho/monocytic infiltration occurs in the nidus of inflammation [4, 5].

The purpose of this study was to evaluate the action of the L17 compound on the extent of injury and the possible recurrence of experimental MI by the dynamic assessment of transaminase activity in blood and myocardial homogenate (tissue).

LDH, AST, and CPK activity was measured, because the basal levels of these enzymes in the myocardium are higher than that in other tissues and that is why an increase in activity of these enzymes is a very sensitive and specific marker of acute heart damage, especially MI [6].

MATERIALS AND METHODS

Test compound

The test compound L-17 from the group of 5-phenyl substituted-6H-1,3,4-thiadiazine-2-amines studied in these experiments has been proven to have a biological effect [4, 5, 7-9].

Animal preparation

The experiment was carried out on healthy, sexually mature, nonlinear albino male rats. The animals used in the tests were quarantined in the vivarium of the Institute of Immunology and Physiology of the Ural Division of Russian Academy of Science (Yekaterinburg, Russia). The animals had no symptoms of any disease. All animals were housed under equivalent conditions and were fed according to the customary schedule. All experimental procedures performed on the animals were approved by the Institute of Animal Care and Use Committee at the Institute of Immunology and Physiology of the Ural Division of RAS (# S-FL17-2014-25) and were performed in accordance with the principles formulated by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, France, 18.03.1986), APS's Guiding Principles in the Care and Use of Vertebrate Animals in Research and Training, and in accordance with the Laboratory Practice Regulations of RF (Ministry of Public Health Order no. 267 from 19.06.2003) [10].

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 decapitated. 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 repeatedly injected 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 and during the process of MI development was investigated on days 1, 5, and 7 of the experiment.

Tissue enzyme activity of LDH and CK-MB was evaluated at days 1, 7, and 14. For this evaluation, a 10% myocardial homogenate was prepared. 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 tissue samples into a cup containing ice. Then, the homogenate was centrifuged for 5 minutes at 3000 rpm. Subsequently, the 10% homogenate was diluted 20 times.

The activity of the enzymes was investigated in the resulting solution (reagent kits LDH B 23.01. and CK-NAC-03/13 "Vital Development Corporation", Saint-Petersburg, Russia).

Statistical analysis

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.

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 (18.7±3.31 U/l), and LDG 1-2 (262.3±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).

Results of the biochemical investigation of LDH activity are presented in tables 1-2.

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

Time	Intact animals, (Group A), n=5 M±m	Experimental infarction without treatment (Group C), n=10 M±m	Experimental infarction with L17 compound administration (Group D), n=15 M±m
total LDH activity,U/g (units per grams)			
1st day	5,10±0,31	3,86±0,45*	3,83±0,10*
7th day		4,37±0,29	3,73±0,37*#
14th day		4,08±0,54	4,22±0,24
LDH 1-2 isoenzyme activity			
1st day	5,06±0,31 (99,4 %)	3,45±0,11* (89,4 %)	3,81±0,36* (99,5 %)
7th day		3,76±0,11* (86,0 %)	3,31±0,28* (88,7 %)
14th day		3,92±0,17* (96,1 %)	3,46±0,26* (82,0 %)

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

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 3.83±0.10 U/g protein in group D vs. 5.10±0.31 U/g protein 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.

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

Time	Intact animals, (Group A), n=5 M±m	Experimental infarction without treatment (Group C), n=10 M±m	Experimental infarction with L17 compound administration (Group D), n=15 M±m
total LDH activity,U/g (units per grams)			
1st day	331,5±7,8	332,4±39,9	309,2±8,1
7th day		326,0±24,3	348,6±35,6
14th day		335,7±43,9	381,0±20,7
LDH 1-2 isoenzyme activity (% activity)			
1st day	329,5±7,9 (99,4%)	297,4±8,7* (89,5%)	308,1±29,3 (99,6%)
7th day		280,6±8,5* (86,1%)	309,8±26,2 (88,9%)
14th day		322,2±13,2 (96,0%)	312,5±22,0 (82,0%)

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)

Time	Intact animals, (Group A), n=5 M±m	Experimental infarction without treatment (Group C), n=10 M±m	Experimental infarction with L17 compound administration (Group D), n=15 M±m
total CPK activity, U/g(units per grams)			
1st day	3,34±0,23	5,33±0,39*	5,10±0,38*
7th day		5,66±0,44*	3,46±0,30#
14th day		5,18±0,33*	5,18±0,28*

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)

Time	Intact animals, (Group A), n=5 M±m	Experimental infarction without treatment (Group C), n=10 M±m	Experimental infarction with L17 compound administration (Group D), n=15 M±m
LDH activity, U/g (units per grams)			
1st day	215,5±15,7	458,9±31,2*	411,6±28,6*
7th day		421,5±29,1*	323,6±26,9* #
14th day		425,9±26,2*	467,7±26,8*

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 (323.6±26.9 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

experimental MI, because, according to MRI studies, CPK activity strongly correlates with the area of MI [20].

Total LDH activity (based on milligrams of protein tissue homogenate) in experimental groups (Group C and D) did not differ significantly from the values of intact animals. However, the levels of cardio-specific LDH 1-2 significantly declined throughout the study, both in Groups C and D, compared with that in control group (Group A) values.

The principal differences between the experimental groups and thus the action of the L17 compound can be revealed only after the comparison of enzyme activity in blood [4, 5] and tissue. According to the results, the decrease in LDH 1-2 activity in tissue (both in Group C and D) corresponded to the increase in enzyme activity in blood on the first day of the experiment, and was in accordance with data in the literature [3, 21-23].

However, on the seventh day of the experiment, the decrease of LDH 1-2 activity in the tissue of Group D animals corresponded with the decrease of LDH activity in blood (229.7±60.6 U/l), while in group C animals the relation between the enzyme levels in blood (345.5±52.9 U/l) and tissue was typical for the onset of MI [21, 24].

The changes in CPK activity revealed a similar pattern. On the seventh day of the experiment, the decrease in CPK activity almost to the values of intact animals was observed both in the blood (108±16.8 U/l vs 248.1±41.5 in Group C) and the tissue of Group D animals (on the background of L17 compound administration).

The described changes in the dynamics and relationships between LDH and CPK levels in blood and tissue confirmed the occurrence of recurrent MI (as a consequence, the increase in tissue damage volume) in animals in Group C and prevention of its development in animals in Group D.

Thus, the evaluation of enzyme levels in myocardial tissue confirms previously reported data that the administration of a thiadiazine compound prevents the recurrence and decreases the size of experimental MI.

CONCLUSION

According to the previous data, administration of L17 compound strongly induced the activation of the apoptosis process after experimental MI. The activation of the intrinsic apoptosis pathway (CD 95 marker) was observed from the first day of the experiment, and activation of the extrinsic pathway (P53 marker) was found on the seventh day of the experiment [9].

These findings, coupled with data that transaminase (AST) activity strongly correlates with the size of the necrotic area after MI [25-27], allow us to assume that apoptosis activation is one of the mechanisms of L17 action. According to this hypothesis, cardiomyocyte apoptosis activation reduces the number of cells that die by necrosis and as a result decreases enzyme levels.

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Author contributions

O Chupakhin and P Sarapultsev developed the concept. O Chupakhin and L Sidorova provided the L17 compound. P Sarapultsev, I Danilova and M Chereshneva designed the experiment and analysed data. I Gette conducted the biochemical analysis. AP Sarapultsev administered the experiment and wrote the manuscript. All authors discussed the results and implications and commented on the manuscript at all stages.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest

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