INTRODUCTION

The pathology of the cardiovascular system is the major health and social problem, because it takes the first place in the structure of morbidity and mortality [1-2]. The prevalence and incidence of cardiovascular diseases have been attracting considerable attention in recent decades. This is partly due to the fact that myocardial fibrosis is the major consequence of the most nosological units of cardiovascular diseases. The special attention is focused on the research of diagnostic markers of degradation and reparation of myocardial tissue [3-5], which would reflect the dynamic changes in the myocardium and were predictors of prognosis the diffuse cardiosclerosis [2-3]. The purpose of this investigation was to determine the changes of the content of protein-bound oxyproline in blood as a diagnostic marker of metabolic activity of collagen at the experimental diffuse ischemic necrotic cardiosclerosis in the rats of different resistance to hypoxia.

We believe that early pathogenic therapy of myocardial fibrosis should be taken into consideration as a solution to this issue as at the recent study we have demonstrated that the use of trimetazidine as inducer of endogenous cardio-protection in the development of diffuse ischemic necrotic cardiosclerosis is manifested via decreased manifestations...
of oxidative and nitrooxidative stress, optimization of immune and cytokine response, stabilization of humoral immune responsiveness [6].

**THE AIM**

The current work carried out to study the effects of TM on the improvement of metabolism of connective tissue elements in myocardium, indicating inhibition of cardiосclerotic process.

**MATERIALS AND METHODS**

**ANIMALS AND TREATMENT**

Experiments were done on 192 male albino rats (190–250 g) (Ternopil State Medical University vivarium, Ukraine). All animals received care in compliance with the “Guide for the Care and Use of Laboratory Animals” (National Institute of Health Publication № 85-23, revised 1985). The studies were carried out according to the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the local animal protection committee.

The experimental animals were divided into 3 groups according to their different resistance to hypoxia using the method of hypobaric hypoxia [6] [Berezovskyi, 1975; Markova, 1998]. Each group was divided into four equal subgroups: control group, diffuse ischemic necrotic cardiосclerosis group (2 times repeated injections of epinephrine hydrotartrate (0.5 mg/kg body weight) and calcium gluconate (5 mg/kg body weight), control group introduced with trimetazidine dihydrochloride (10 mg/kg body weight) and calcium gluconate (5 mg/kg body weight) treated with trimetazidine dihydrochloride treated with trimetazidine dihydrochloride group (2 times repeated injections of epinephrine hydrotartrate (0.5 mg/kg body weight) and calcium gluconate (5 mg/kg body weight) group introduced with trimetazidine dihydrochloride (10 mg/kg body weight) for all period of observation (n=8 each group).

**MEASUREMENT OF PROTEIN-BOUND OXYPROLINE IN BLOOD SERUM**

Concentration of protein-bound oxyproline in homogenate of myocardium was determined biochemically [7] at 7, 14 and 30 days after the modelling pathology.

**HISTOPATHOLOGY STUDY**

A portion of the tissue from the ventricles of myocardium was taken at 30 days after the modelling pathology, then fixed in 10 % neutral-buffered formalin solution for 5 days, embedded in paraffin, and sectioned. Histological examination of Masson trichrome staining of myocardium was performed [8].

**STATISTICAL ANALYSIS**

Statistical analysis was carried out by OriginPro Program. Results are expressed as mean±standard deviation. The results were statistically analyzed using non-parametric indexes in the Excel software (Microsoft, USA) and STATISTICA 10.0 (StatSoft, USA). The reliability of the differences in values between independent quantitative values was determined with a normal distribution according to the Mann–Whitney U criterion [9]. Values p<0.05 are considered as statistically significant.

**RESULTS**

Before the modeling DINC results determine the protein-bound oxyproline concentration in homogenate of myocardium of rats with low resistance to hypoxia was 17.8 % (p<0.05) higher than in serum of rats with middle resistance to hypoxia (Table I), while the high resistant to hypoxia animals’ oxyproline concentration in homogenate of myocardium was 21.9 % lower (p<0.05) than in homogenate of myocardium of rats with middle resistance to hypoxia. In the simulation DINC the protein-bound oxyproline concentration in homogenate of myocardium of rats gradually increases at all groups.

<table>
<thead>
<tr>
<th>Resistance of animals to hypoxia</th>
<th>Control (n=8)</th>
<th>The stages of observation DINC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7 days (n=8)</td>
</tr>
<tr>
<td>Low</td>
<td>49.5±0.59</td>
<td>57.45±1.78</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Middle</td>
<td>42.07±1.10</td>
<td>47.92±0.62</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>High</td>
<td>34.52±0.92</td>
<td>38.53±0.55</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

Notes: p<0.05 – significantly different from middle resistant animals to hypoxia at all times of observation; *p<0.05 – significantly different from control at all times of observation.
Under the influence of metabolic therapy by trimetazidine changes of protein-bound oxyproline concentrations in homogenate of myocardium of animals with low resistance to hypoxia were less pronounced (Table II), but significantly the concentration of this metabolite collagen within 7 days after modeling pathology was reduced by 11.0 % (p<0.05) (Figure 1) than in the group of untreated animals at this stage of observation. By stage 14 days DINC protein-bound oxyproline concentration in homogenate of myocardium of rats with low resistance to hypoxia was 25.3 % lower (p<0.001) than in untreated animals, and during 30 days of observation DINC – by 33.9 % (p<0.001) lower than in untreated animals with low resistance to hypoxia in a similar stage of cardiosclerotic process development without correction.

In the serum of rats with middle resistance to hypoxia the protein-bound oxyproline concentration after 7 days DINC and trimetazidine correction was lower by 8.3 % (p<0.05) (Fig. 1) than in the group of untreated animals at this stage of observation. In the next stage of observation, 14 days DINC, protein-bound oxyproline concentration in homogenate of myocardium was 18.6 % lower (p<0.001) than in untreated animals, and during 30 days of observation DINC – by 28.2 % (p<0.001) lower than in untreated rats.

There was no significantly difference between treated and untreated animals with high resistance to hypoxia at the stage of observation 7 days DINC. After 14 days of modeling pathology index was lower by 11.6 % (p<0.05) than in the group of untreated animals at this stage of observation.

Notes: p<0.05 – significantly different from middle resistant animals to hypoxia at all times of observation; *p<0.05 – significantly different from control at all times of observation.

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**Table II.** Influence of trimetazidine on protein-bound oxyproline concentration in homogenate of myocardium at the experimental diffuse ischemic necrotic cardiosclerosis (DINC) due to innate resistance of rats to hypoxia

<table>
<thead>
<tr>
<th>Resistance of animals to hypoxia</th>
<th>Control TM (n=8)</th>
<th>The stages of observation DINC+TM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days (n=8)</td>
<td>14 days (n=8)</td>
</tr>
<tr>
<td><strong>Low</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>51,15±1,36</td>
<td>59,09±1,85</td>
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<tr>
<td></td>
<td>p&lt;0,05</td>
<td>p&lt;0,001</td>
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<td></td>
<td>p&lt;0,001</td>
<td>p&lt;0,001</td>
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<tr>
<td></td>
<td>69,32±1,86</td>
<td></td>
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<tr>
<td><strong>Middle</strong></td>
<td>43,97±1,34</td>
<td>40,58±1,83</td>
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<tr>
<td></td>
<td></td>
<td>p&lt;0,05</td>
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<tr>
<td></td>
<td>52,72±3,15</td>
<td></td>
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<tr>
<td><strong>High</strong></td>
<td>34,90±0,96</td>
<td>36,33±0,91</td>
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<tr>
<td></td>
<td>p&lt;0,05</td>
<td>p&lt;0,001</td>
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<tr>
<td></td>
<td>37,72±1,52</td>
<td>p&lt;0,001</td>
</tr>
<tr>
<td></td>
<td>34,90±0,96</td>
<td>p&lt;0,05</td>
</tr>
</tbody>
</table>

Notes: indicators of control groups expressed in 100 %; * – significantly different from control at all times of observation, p<0.05; # – significantly different from untreated rats at all times of observation, p<0.05.

**Fig. 1.** Influence of trimetazidine on protein-bound oxyproline concentration in homogenate of myocardium at the experimental diffuse ischemic necrotic cardiosclerosis (DINC) due to innate resistance of rats to hypoxia.
and after 30 days of observation and correction DINC by metabolic therapy the concentration of protein-bound oxyproline in serum was 28.4 % (p<0.001) lower than in untreated animals with high resistance to hypoxia without correction.

Histological examination of the myocardium during 30 days of DINC showed that the of micropreparations hearts healthy animals, irrespective of the resistance to hypoxia, connective tissue provided very little in the form of thin collagen fibers (Figure 2), whereas in micropreparations of hearts in the development of DINC (Figure 3) revealed the presence of focal cardiosclerosis, perivascular sclerosis hyperelasticity of the inner membrane of vessels, cardiomyocyte hypertrophy, diffuse proliferation of connective tissue. Fibrotic regions in myocardium are rich in collagens and therefore appear in blue upon Masson trichrome staining. In addition, centralized nuclei as well as the shape and the size distribution of the myofibers are visualized and show expressed hypertrophy of cardiomyocytes.

All the above-mentioned symptoms are the highest in the low resistant animals to hypoxia, indicating the intense development of diffuse cardiosclerosis in animals with low resistance to hypoxia and confirm the results obtained in determining the concentration of protein-bound oxyproline serum of rats with different resistance to hypoxia.

**DISCUSSION**

Using the determination of protein-bound oxyproline concentrations in homogenate of myocardium in modeling DINC with and without trimetazidine correction and given that hydroxyproline contained mainly composed of collagen and is the product of its metabolism [4-5], it can be used as a biological marker of the intensity of the synthesis of collagen in tissue infarction can draw the following conclusion: the intensity of the metabolic imbalance of connective elements with diffuse ischemic necrotic cardiosclerosis and trimetazidine correction depends on the resistance of animals to hypoxia. In animals with low resistance to hypoxia manifested maximum effect of trimetazidine correction, however, given the more pronounced changes in the concentration oxyproline them in modeling DINC without correction, this effect was not enough for leveling differences between animals with different resistance to hypoxia. This pattern is similar for the group of animals with middle resistance to hypoxia, but the changes were less pronounced. Animals with high resistance to hypoxia were characterized by lower concentrations oxyproline changes in modeling DINC, so the effect of the use manifested to a lesser extent, but in general, they are characterized by minimal metabolic disorders of connective tissue elements in the development of DINC and correction by trimetazidine [10-16]. The activity of the connective tissue metabolism was studied in experimental diffuse ischemic necrotic cardiosclerosis due to different resistance of the organisms to hypoxia. The investigations were based on the changes of concentration of protein-bound oxyproline in homogenate of myocardium that reflect adequacy metabolic changes of collagen [4-5].

**CONCLUSIONS**

The development of the experimental diffuse ischemic necrotic cardiosclerosis at all times of observation is accompanied by metabolic imbalance in the connective tissue of the heart, and argumented by the increasing of oxyproline level in homogenate of myocardium of animals with different resistance to hypoxia. The intensity of metabolic imbalances in diffuse connective tissue elements is the highest in the low resistant animals to hypoxia. Those results are confirmed by histological examination of the myocardium of rats with different resistance to hypoxia.
resistance to hypoxia. Fibrotic regions in myocardium are rich in collagens. It has been revealed that the most pronounced therapeutic effect of TM is observed in animals with low resistance to hypoxia, slightly less – in animals with medium resistance to hypoxia, and the lowest – in animals with high resistance to hypoxia. This pattern was observed at all stages of observation, but it was most expressed in the early period of cardiosclerotic process, indicating the feasibility of early use of metabolic therapy. It explains the absence of cardioprotective effect of trimetazidine in the later stages of cardiosclerosis, when the myocardial fibrosis is already formed.

REFERENCES

Authors’ contributions:
According to the order of the Authorship.
Conflict of interest:
The Authors declare no conflict of interest.

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