Dynamics of glutathione reductase activity in rat liver tissues during cryodestruction of the right atrium

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Abstract. The metabolic processes of the human body are based on multiple redox reactions and oxidative stress occurs when homeostasis is imbalanced. Antioxidant system of the body is represented by such enzymes as catalase, glutathione reductase, superoxidismutase and glutathione peroxidase. Objective: to study the dynamics of glutathione reductase activity in rat liver tissues after cryodestruction of right atrial myocardium to initiate oxidative stress. Materials and methods: 420 male rats were used. The rats were divided into two groups - intact and experimental, 210 animals in each. To initiate oxidative stress, the experimental group rats underwent cryodestruction of the right atrium. The activity of glutathione reductase in the liver tissue was determined by accumulation of oxidized glutathione before the experiment, as well as on 1, 3, 5, 7 and 14 days of the experiment. Conclusions: oxidative stress arising after cryodestruction of the right atrium up to the 7th day of the experiment provokes a decrease in the glutathione reductase activity in the rat liver tissue, but the start of reparative processes helps to restore the disturbed redox equilibrium in the body and normalize the enzyme level.

1 Introduction

Metabolic processes in the human body are based on multiple redox reactions. When homeostasis is imbalanced, oxidative processes and an excess of reactive oxygen species (ROS) prevail in the organism, and this is the trigger mechanism for many diseases, in particular cardiovascular and hepatobiliary systems [1, 2]. The fact is that reactive oxygen species have not only direct toxicity, but are also able to change the signaling pathways of cell, tissue and organ function regulation. For example, when mitochondria are damaged by reactive oxygen species, there are disturbances in the electron transfer of the respiratory chain and this provokes additional production of ROS [3]. Reactive oxygen species also damage vascular endothelium and reduce nitric oxide (II) secretion, which provokes endothelial dysfunction manifested by increased

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vasoconstriction, hypercoagulation and proliferation of muscle cells [4]. Reactive oxygen species destroy cell membranes, which leads to the formation of a large number of free radicals, which in turn damage cardiomyocytes, and as a result myocardial contractile function deteriorates [5, 6]. Free radicals also trigger cardiomyocyte apoptosis and have a direct negative inotropic effect. When free radicals interact with the bilipid layer of cell membranes of cardiomyocytes, lipid radicals, lipid peroxides and lipid hydroperoxide are formed, as a result of which the permeability of their membranes increases. This leads to a significant increase in intracellular calcium content and persistent contraction of myofibrils, and, as a consequence, there is a disturbance of myocardial distensibility and reduction of its contractile function [7, 8].

Antioxidant systems function as a counterbalance to active oxygen forms in the body, but their malfunction leads to destabilization of electron transport chains, and this can provoke a decrease in myocardial contractility. Oxidative stress also reduces the activity of many enzymes and substances, including 2,3-diphosphoglycerate (2,3-DPG), which is localized in erythrocytes and affects their most important function - oxygen transport. A change in the amount of 2,3-DPG changes hemoglobin affinity for oxygen and thus accelerates the dissociation of oxyhemoglobin into hemoglobin and oxygen, while a decrease in 2,3-DPG contributes to a decrease in oxygen tension in the blood [4, 9, 10].

In general, it is worth noting that oxidation products formed under the influence of reactive oxygen species and free radicals in the body are very toxic and their inactivation is provided by the liver.

The body's antioxidant system is represented by such enzymes as catalase, glutathione reductase, superoxidismutase and glutathione peroxidase. They prevent the occurrence and progression of myocardial hypertrophy, cardiomyocyte apoptosis and other processes [11, 12].

The mentioned enzymes also determine the resistance of hepatocytes to the action of free radicals in different zones of hepatic lobules.

The state of antioxidant system and, consequently, the intensity of oxidative stress can be monitored by the activity of antioxidant enzymes and since multiple literature data are quite contradictory this topic does not lose its relevance.

In view of the above, the aim of our work was to study the dynamics of glutathione reductase activity in rat liver tissues after cryodestruction of the right atrial myocardium to initiate oxidative stress.

Objectives of the study: to establish the level of glutathione reductase activity in liver tissues of intact rats and animals with induced oxidative stress in dynamics.

2 Materials and methods

In the study, 420 male rats of eight months of age, weighing 230-250 g, which were kept in the vivarium, were used. The rats were divided into two groups - intact and experimental, 210 animals in each. To initiate oxidative stress, the experimental group rats underwent cryodestruction of the right atrium, which contains mainly secretory cardiomyocytes containing granules with atrial natriuretic factor (ANF). This hormone is a powerful vasodilator; it is involved in the regulation of water-electrolyte metabolism and adipose tissue metabolism.

Cryoablation of the rat right atrial myocardium was performed using Cryoinay KI-401 cryoapplicator №4 with a tip diameter of 4 mm, with an exposure time of 10 seconds. (Cryoinai® has: Roszdravnadzor Registration Certificate N° FSR 2009 / 04738.) The animals were operated under ether anesthesia on spontaneous breathing with 2 provisor sutures with access, in the region of the 3-4 intercostal space, to the atriia of the rats. An incision of 7 mm allowed free introduction of the cryoapplicator without exposing the
surrounding tissues to cold, thereby not traumatizing them. Cryoablation was performed with applicator No. 4 with a working surface diameter of 4 mm with exposure of 10 seconds. After cryoablation with the applicator, the thoracic cavity was sutured hermetically, removing air from it with a syringe.

Nitrogen cryodestructor with 4 mm applicator caused formation of icing zone 4-5 mm in diameter and 0.2 mm deep (200 μm), leading to myocardial necrosis in this area. Exposure of myocardium to cryodestructor causes tissue destruction, provokes inflammation, and as a consequence, increases oxidative processes in the body, in addition releasing atrial natriuretic factor from destroyed granules. Which, in our opinion, should be reflected in the dynamics of activity and concentrations of lipid peroxidation system enzymes - antioxidants in the body tissues, in particular glutathione reductase.

The activity of glutathione reductase in liver tissues was determined by the accumulation of oxidized glutathione before the experiment, as well as on days 1, 3, 5, 7 and 14 of the experiment. The animals were decapitated on the indicated days of the experiment, 30 animals in each group [13, 14].

The conclusion of the Bioethics Committee of the "Reaviz Medical University" № 167 from 18 September 2019 was received for the experiment.

Nonparametric statistical analysis was used to analyze the data obtained, which did not correspond to the normal distribution, in order to identify differences in the activity of glutathione reductase in intact animals and rats of the experimental group.

3 Results of the study

Fig. 1 shows the dynamics of glutathione reductase activity in animal liver tissues during the experiment.

![Graph showing dynamics of glutathione reductase activity](image)

**Table:**

| Experiment Day | Glutathione Reductase Activity, nmol/min/1 mg protein |
|---------------|------------------------------------------------------|
| 1 group       | 1 group | 2 group |
| 0             | 103.25  | 103.65  |
| 1             | 103.15  | 99.6    |
| 3             | 103.1   | 91.45   |
| 5             | 103.17  | 85.6    |
| 7             | 103.45  | 80.9    |
| 14            | 103.3   | 89.15   |

Fig. 1. Dynamics of glutathione reductase activity in normal rat liver and right atrium cryodestruction.

In the course of the experiment it was found that cryodestruction of the right atrial myocardium as a result of tissue integrity failure stimulates oxidative processes in the body and provokes a decrease in the activity of glutathione reductase as an antioxidant enzyme in the liver tissues up to 7 days of the experiment, and then, against the background of the start of repair processes the enzyme activity tends to the physiological norm.
An array of the obtained values of glutathione reductase activity in the liver tissues of the experimental group of rats is shown in Table 1.

### Table 1. Dynamics of glutathione reductase activity in tissues of experimental rats.

| Days | N  | M    | Me   | Min  | Max  | 25 Per | 75 Per | 10 Per | 90 Per |
|------|----|------|------|------|------|--------|--------|--------|--------|
| 0 day| 30 | 103,42| 103,65| 99,20| 107,60| 102,30 | 104,70 | 100,40 | 105,40 |
| 1 day| 30 | 98,05 | 99,60 | 92,80| 102,10| 95,10  | 100,90 | 94,40  | 101,55 |
| 3 day| 30 | 91,11 | 91,45 | 86,80| 94,80 | 89,10  | 93,10  | 87,85  | 93,60  |
| 5 day| 30 | 83,62 | 85,60 | 74,40| 88,90 | 79,90  | 87,00  | 76,05  | 87,60  |
| 7 day| 30 | 80,68 | 80,90 | 75,50| 84,80 | 79,00  | 82,60  | 76,75  | 83,95  |
| 14 day| 30 | 90,47 | 91,15 | 86,80| 96,20 | 87,90  | 93,70  | 87,50  | 95,20  |

According to the data presented in the table we can conclude that atrial cryodestruction provokes intensification of oxidative processes in the body and reduction of glutathione peroxidase activity.

The obtained numerical data on the dynamics of glutathione reductase activity in the liver tissues of the control and experimental groups did not correspond to the normal distribution and were subjected to nonparametric statistical analysis to establish the reliability of differences in the studied groups (Table 2).

### Table 2. Statistical analysis of the dynamics of glutathione reductase activity in rat liver tissue against the background of oxidative stress induced by right atrial cryodestruction.

| Day  | Groups | Statistical test      | Criterion                        | P value |
|------|--------|-----------------------|----------------------------------|---------|
| 0 day| 1 and 2| Mann - Whitney         | $U = 423,5000$                   | 0,722720|
|      |        |                       | $Z = -0,354826$                  |         |
|      |        | Kolmogorov-Smirnov     | Max Neg Differece $= -0,16667$   | >0,10   |
|      |        |                       | Max Pos Differece $= 0,133333$   |         |
|      |        | Wald-Wolfowitz        | $Z = -1,04165$                   | 0,297570|
|      |        |                       | $Z_{adjstd} = 0,911453$          | 0,362057|
| 1 day| 1 and 2| Mann - Whitney         | $U = 45,50000$                  | 0,000000|
|      |        | Kolmogorov-Smirnov     | Max Neg Differece $= 0,00$       | <0,001  |
|      |        |                       | Max Pos Differece $= 0,866667$   |         |
|      |        | Wald-Wolfowitz        | $Z = -5,46872$                   | 0,000000|
|      |        |                       | $Z_{adjstd} = 5,338512$          | 0,000000|
| 3 day| 1 and 2| Mann - Whitney         | $U = 0,0$                       | 0,000000|
|      |        | Kolmogorov-Smirnov     | Max Neg Differece $= 0,00$       | <0,001  |
|      |        |                       | Max Pos Differece $= 1,000000$   |         |
|      |        | Wald-Wolfowitz        | $Z = -7,55204$                   | 0,000000|
|      |        |                       | $Z_{adjstd} = 7,421834$          | 0,000000|
| 5 day| 1 and 2| Mann - Whitney         | $U = 0,0$                       | 0,000000|
|      |        | Kolmogorov-Smirnov     | Max Neg Differece $= 0,00$       | <0,001  |
|      |        |                       | Max Pos Differece $= 1,000000$   |         |
|      |        | Wald-Wolfowitz        | $Z = -7,55204$                   | 0,000000|
|      |        |                       | $Z_{adjstd} = 7,421834$          | 0,000000|
| 7 day| 1 and 2| Mann - Whitney         | $U = 0,0$                       | 0,000000|
|      |        | Kolmogorov-Smirnov     | Max Neg Differece $= 0,00000$    | <0,001  |
|      |        |                       | Max Pos Differece $= 1,000000$   |         |
|      |        | Wald-Wolfowitz        | $Z = -7,55204$                   | 0,000000|
|      |        |                       | $Z_{adjstd} = 7,421834$          | 0,000000|
| 14 day| 1 and 2| Mann - Whitney        | $U = 0,0$                       | 0,000000|
|       |        | Kolmogorov-Smirnov     | Max Neg Differece $= 0,00000$    | <0,001  |
|       |        |                       | Max Pos Differece $= 1,000000$   |         |
|       |        | Wald-Wolfowitz        | $Z = -7,55204$                   | 0,000000|
|       |        |                       | $Z_{adjstd} = 7,421834$          | 0,000000|
The tabulated data allow us to conclude that the activity of glutathione reductase in the liver tissues of the control and experimental groups differed significantly from the first day of the experiment.

Cryodestruction of right atrium leads to damage of secretory cardiomyocytes and is accompanied by inflammatory process and release of atrial natriuretic factor into surrounding tissues, which provokes myocardial ischemia [15]. Reduced oxygen supply to the area of myocardial ischemia contributes to further decrease of functional activity of antioxidant system with activation of radical oxygen forms production processes [16, 17]. All these events induce systemic inflammatory response and are the cause or an important link in the pathogenesis of many serious pathologies. The findings are consistent with the results of other studies reported in the specialized literature [18, 19].

4 Conclusions

Oxidative stress arising from cryodestruction of the right atrium up to 7 days of the experiment provokes a decrease in glutathione reductase activity in rat liver tissues, but the launch of reparative processes helps to restore the disturbed redox equilibrium in the body and normalize the enzyme level.

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