# Heme Oxygenase Activity and Some Indices of Antioxidant Protection in Rat Liver and Kidney in Glycerol Model of Rhabdomyolysis

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Activity of heme oxygenase, superoxide dismutase, and catalase, the content of reduced glutathione and total heme in the liver and kidneys, and serum absorption spectrum in the Soret band were studied in rats with glycerol-induced rhabdomyolysis. Glycerol increased the content of heme-containing metabolites in the serum and the total heme content in the liver and kidneys, and decreased the content of reduced glutathione and catalase activity in the examined organs. Superoxide dismutase activity increased in the liver and decreased in the kidneys. Heme oxygenase activity increased in the liver and kidneys 2 and 6 h postinjection, respectively. The effects of heme delivered to the liver and kidneys from the vascular bed on the antioxidant defense and heme oxygenase activity were studied.

**Key Words:** *heme; heme oxygenase; antioxidant protection system; rhabdomyolysis; glycerol*

Accumulation of heme and hemoproteins in the blood is characteristic of some pathological states, *e.g.* intravascular hemolysis and rhabdomyolysis [12]. Rhabdomyolysis characterized by muscle damage and massive release of myoglobin into the blood can be caused by trauma, hyperthermia, and various toxins and venoms [11]. Glycerol model of rhabdomyolysis is a model of heme-induced damage to the kidney [9]. However, rhabdomyolysis also induces damage to hepatocytes [2]. Massive release of heme and hemoproteins into the vascular bed and their accumulation in tissues and organs is the major damaging factor in rhabdomyolysis. Intracellular accumulation of free heme exhibiting prooxidant properties activates free radical oxidation processes and induces oxidative stress.

The role of induction of heme oxygenase-1 (EC 1.14.99.3; HO-1), an inducible isoform of key enzyme of heme degradation and a stress protein, in cell protection from free heme-induced damage is widely discussed. Induction of HO-1 decreases intracellular content of free heme and increases the level of heme oxygenase products (antioxidant bilirubin and vascular tone regulator carbon monoxide) [6].

Here we studied parameters of antioxidant defense, content of total heme, activity of heme oxygenase in the liver and kidneys, and serum content of heme-containing products in rats with glycerol-induced rhabdomyolysis.

## MATERIALS AND METHODS

Experiments were performed on Wistar rats (150-180 g). Glycerol (50% water solution) was injected intramuscularly in a dose of 0.75 ml/100 g ($^1/_2$ dose into each hindlimb) [9]. The rats were decapitated under light ether narcosis 2, 6, 24 h postinjection. The liver was *in situ* perfused with cold physiological saline.

Accumulation of heme-containing products was assessed by the difference in serum absorption spectra (DA/mg protein) in the Soret band (390-450 nm). The content of total heme in liver and kidney homogenates

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was measured spectrophotometrically and expressed in nmol/mg protein. The content of reduced glutathione (GSH) was determined spectrophotometrically by absorption of its complex with alloxan at 305 nm and presented as μmol GSH/g tissue [1]. Activity of superoxide dismutase (SOD) was determined spectrophotometrically by inhibition of reduction of tetrazolium nitroblue into formazan with superoxide radicals formed in the xanthine oxidase reaction and presented in arb. units/min/mg protein. Activity of catalase (CAT) was measured by spectrophotometrically by utilization of $H_2O_2$ [7] and presented in μmol $H_2O_2$/min/mg protein. Activity of heme oxygenase (HO) was determined as described elsewhere [10]. Enzyme activity was measured by the amount of produced bilirubin and presented in nmol bilirubin/min/mg protein.

Protein content was measured by the method of Lowry modified by Miller. The results were analyzed statistically using Mann—Whitney *U* test.

## RESULTS

The content of heme-containing metabolites in rat serum was increased at all terms of the experiment, but the maximum increase was observed during the first few hours after glycerol injection (Fig. 1). Intramuscular injection of glycerol induces destruction of muscles and hemolysis of erythrocytes, which leads to pronounced accumulation of heme and hemoproteins in the blood [9] and promotes their transport to the liver and kidneys [12]. The total heme content in the liver and kidneys increased 2 h after glycerol injection; in the kidney this parameter remained increased 6 h postinjection (Table 1).

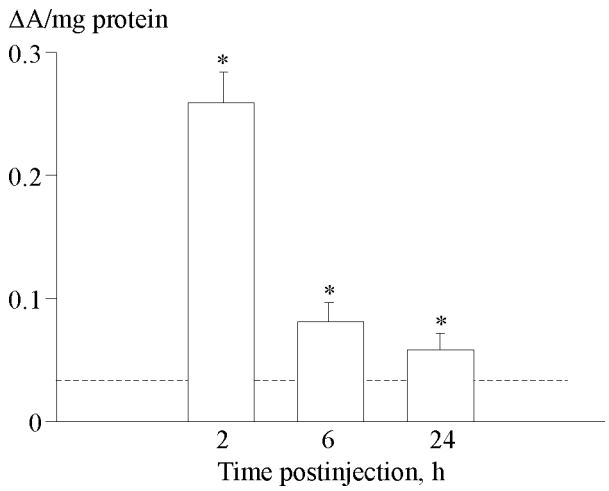


**Fig. 1**. Effect of glycerol on optical density of rat serum in Soret band at various time postinjection (*M*±*m*, *n*=4-6). Dotted line corresponds to the control level. $p<0.05$ *compared to the control.

Free heme is a prooxidant, and its accumulation in cells changes the anti-/prooxidant status due to its direct involvement in redox reactions and due to the increase in $Fe^{2+}$ content [4]. Heme accumulation in the liver and kidneys decreased GSH content in these organs. In the liver this parameter decreased by 47% 2 h after injection of glycerol and remained low for 4 h (Table 1). In the kidneys the content of GSH also decreased 2 h postinjection, although this decrease was less pronounced (Table 1). The observed decrease in GSH content in the examined organs seems to result from its direct interaction with heme [3]. The more pronounced and prolonged decrease in GSH content in the liver can be related to transport of GSH from the liver to other organs, in particular, to the kidneys.

**TABLE 1**. Content of Total Heme, Some Indices of Antioxidant Protection, and Heme Oxygenase Activity in Rat Liver at Various Time After Glycerol Injection (*M*±*m*, *n*=4-6)

| Index | | Control | Time postinjection, h | | |
|---|---|---|---|---|---|
| | | | 2 | 6 | 24 |
| Total heme content | liver | 0.080±0.007 | 0.167±0.021* | 0.086±0.012 | 0.072±0.009 |
| | kidneys | 0.177±0.014 | 0.375±0.048* | 0.357±0.069* | 0.145±0.034 |
| GSH content | liver | 5.40±0.27 | 3.47±0.38* | 3.11±0.81* | 5.91±0.93 |
| | kidneys | 3.26±0.13 | 2.50±0.15* | 3.60±0.25 | 3.75±0.32 |
| SOD activity | liver | 1558.0±44.7 | 1496.0±44.8 | 1829.0±86.7* | 1725±137 |
| | kidneys | 2015±86.5 | 1386±122* | 1409±129* | 1742±119 |
| CAT activity | liver | 258.0±12.7 | 244.0±14.9 | 263.0±8.2 | 187.0±12.9* |
| | kidneys | 150.00±1.07 | 125±12 | 147.00±6.77 | 118.00±8.19* |
| Heme oxygenase activity | liver | 0.043±0.005 | 0.069±0.008* | 0.079±0.009* | 0.088±0.009* |
| | kidneys | 0.043±0.005 | 0.049±0.006 | 0.087±0.007* | 0.074±0.002* |

**Note.** $p<0.05$ *compared to the control.

Injection of glycerol changed activity of antioxidant enzymes SOD and CAT in the examined organs. In the liver, SOD activity increased 6 h postinjection, while CAT activity dropped 1 day postinjection (Table 1). In the kidney, SOD activity was below the control 2 and 6 h postinjection, while CAT activity was low 1 day postinjection (Table 1). The absence of induction of antioxidant enzymes in the kidneys in response to accumulation of free heme was reported previously [8].

The increase in SOD activity against the background of low CAT activity in the liver, on the one hand, and inhibition of antioxidant enzymes in the kidneys, on the other hand, can result in accumulation of active oxygen species, *e.g.* $H_2O_2$ [4]. Accumulation of $H_2O_2$ and $Fe^{2+}$ in cells (the latter is characteristic of rhabdomyolysis [4]) results in the formation of a highly reactive hydroxyl radical in the Fenton reaction, which can damage cell structures.

Induction of HO-1 plays an important role in cell protection from free heme-induced oxidative damage to hepatic and renal cells. In our study, heme oxygenase activity in the liver increased 1.6-fold 2 h postinjection and in the kidney 1.7-fold 6 h postinjection. This activity remained increased 1 day postinjection (Table 1). Both constitutive (HO-2) and inducible (HO-1) isoforms of heme oxygenase are present in the liver and kidneys. The observed activation of heme oxygenase reflects induction of HO-1, because HO-2 is not induced under these conditions [6]. It is known that heme stimulates HO-1 synthesis via activation of some transcriptional factors with participation of active oxygen species [6].

Being a lipophilic molecule, heme can easily cross the cell membranes and accumulate in hepatic and renal cells, when its blood content increased. A receptor-mediated transport of heme in a complex with hemopexin is characteristic of hepatocytes [12]. This specific transport of heme into hepatocytes can explain earlier induction of HO in the liver. It was demonstrated that binding of heme-hemopexin complex to hepatocyte plasmalemma is sufficient for activation of signal pathways leading to induction of HO-1 [5].

Induction of heme oxygenase reduces cell content of free heme, which acts as a prooxidant, and stimulates synthesis of bioactive agents bilirubin and CO. The latter plays an important role in the maintenance of normal vascular tone in the liver and kidneys under conditions of accumulation of heme and hemoproteins [6,8,9].

The earlier induction of HO in the liver contributes to more early normalization of total heme level (6 h postinjection), while in the kidneys this index returns to normal only 1 day after glycerol injection.

Thus, in glycerol model of rhabdomyolysis, accumulation of heme-containing metabolites in the blood is accompanied by an increase in total heme content in the liver and kidneys. These changes modulate parameters of the antioxidant defense system and lead to induction of heme oxygenase. Induction of heme oxygenase and normalization of total heme content in the liver occur earlier than in the kidneys, which probably reflects different mechanisms of heme transport from the blood into these organs.

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