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ACTIVITY OF THE THIOREDOXIN SYSTEM IN THE LIVER OF RATS UNDER CONDITIONS OF PARTIAL HEPATECTOMY AFTER TOXIC INJURY

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The work is dedicated to evaluating the activity of thioredoxin, thioredoxin reductase, and selenocysteine β-lyase in the cytosolic fraction of rat liver under conditions of partial hepatectomy after toxic injury by acetaminophen. The experiments were carried out on white non-linear rats, which were divided into two groups by the method of randomization: control animals, which received partial hepatectomy according to the Mitchell and Willenbring method (C/PH), and rats, which had partial resection of 2/3 of the liver tissue following acute toxic injury by acetaminophen through prior two-day administration at a dose of 1250 mg/kg of animal body weight (TI/PH). The study was performed at 0 hours (control), 24 hours (initiation phase), 48 hours (proliferative phase), 72 hours (termination phase), and 168 hours (distant period) after partial hepatectomy. The performance of partial hepatectomy after acetaminophen-induced injury modeling (TI/PH) is accompanied by a statistically significant decrease in thioredoxin activity in liver cells at the initial stages of regeneration (24 h and 48 h) compared to control values (0 h). The established changes occur against the background of suppression of selenium-dependent thioredoxin reductase and selenocysteine β-lyase activities during the initiation period (24 h), active cell proliferation (48 h), and termination (72 h). Depletion of the functional reserves of the thiol-dependent thioredoxin redox system and the suppression of selenide production efficiency, due to the demonstrated impairment in the conversion of organic selenium forms involving selenocysteine β-lyase in animals with acetaminophen-induced injury following partial hepatectomy (TI/PH), can be considered one of the factors reducing the regenerative potential of the liver under conditions of toxic injury.

Keywords: thioredoxin, thioredoxin reductase, selenocysteine β-lyase, partial hepatectomy, acetaminophen, toxic injury, liver

Introduction. The problem of developing xenobiotic-associated dysfunctional disorders and pathological changes of the liver is becoming increasingly relevant. Liver damage of various etiologies, particularly that caused by the adverse effects of hepatotoxins, is accompanied by the activation of compensatory and adaptive mechanisms, an increase in hepatocyte proliferative activity with gradual remodeling of damaged tissue, and the restoration of homeostatic balance. Drug-induced liver injuries (DILI) are considered one of the leading stimuli of excessive hepatocyte loss and suppression of the liver's regenerative potential. According to the DILIN and LiverTox systems, paracetamol (acetaminophen, APAP) injury remains the predictor of DILI development and, consequently, acute liver failure in 50% of situations. It should be noted that the only treatment method for the terminal stages of acute liver failure caused by toxic APAP injury is transplantation, the realization of which is made possible by the liver cells' ability for reparative regeneration (Di-Iacovo et al., 2023; Hora et al., 2023; Liao et al., 2023).

A particular importance in ensuring the compensatory regenerative activity of the liver is attributed to the thiol-dependent thioredoxin system, whose critical components are thioredoxin (EC 1.8.4.10; Trx) and selenium-dependent thioredoxin reductase (EC 1.6.4.5; TrxR). It is known that the thioredoxin system is involved in the preservation of intracellular redox homeostasis and antioxidant protection of cells; however, its role in regeneration is highlighted by its participation in DNA replication, regulation of proliferation, and modulation of apoptotic signaling pathways (Hasan et al., 2022; Liu, 2023). Therefore, the aim of the work was to evaluate the activity of thioredoxin, thioredoxin reductase, and selenocysteine β-lyase in the cytosolic fraction of rat liver under conditions of partial hepatectomy following toxic injury by acetaminophen.

Materials and Methods. The experiments were conducted on white non-linear rats, which were divided into two groups by the method of randomization: control animals that received partial hepatectomy (C/PH) and rats that had partial liver resection following acute toxic injury by acetaminophen (TI/PH). Partial hepatectomy by resection of 2/3 (70%) of the liver tissue was performed in the morning hours under the conditions of general anesthesia according to the method of Mitchell and Willenbring, which involves the

sequential ligation and resection of the left lateral and medial lobes of the liver. The study was performed at 0 hours (control), 24 hours (initiation phase), 48 hours (proliferative phase), 72 hours (termination phase), and 168 hours (distant period) after partial hepatectomy.

Thioredoxin activity was assessed using the turbidimetric method, which is based on recording the rate of insulin disulfide reduction at λ=650 nm over 40 min on a CARY 60 spectrophotometer (USA). Thioredoxin reductase activity was determined by the rate of NADPH-dependent reduction of 5,5'-dithiobis-(2-nitrobenzoic acid) at λ=412 nm. (Arnér et al., 2001) The activity of selenocysteine-β-lyase was evaluated by determining the amount of selenide (HSe^-) formed in the reaction with lead acetate at λ=400 nm over 15 min (Esaki et al., 1982).

Statistical analysis was performed with the use of GraphPad Prism 8.0.1 program. The obtained results were analyzed by the method of two-way ANOVA and expressed as mean ± SEM. Differences between groups were considered statistically significant at $p<0.05$.

Results and Discussion. The results of the experiments demonstrate a statistically significant increase in thioredoxin activity in the cytosolic fraction of the liver in animals of the C/PH group within 72 h after conducting partial hepatectomy compared to the control (0 h). It should be noted that the maximally pronounced increase in thioredoxin activity (by 50 % compared to 0 h) in this group of animals is registered during the period of active cell proliferation (48 h). At the same time, under the conditions of conducting partial hepatectomy after acetaminophen-induced toxic injury (TI/PH), a completely opposite trend of changes in Trx activity is observed. Specifically, in the liver cells of animals in the TI/PH group, a significant decrease in thioredoxin activity is tracked during the initiation phase (24 h) and active cell proliferation phase (48 h), by 55–58 % compared to the control values (0 h) (Fig. 1, A).

It is known that the activation of the cascade of regenerative events in response to damaging stimuli, particularly tissue loss after partial hepatectomy, is accompanied by the release of pro-inflammatory cytokines and growth factors (TNF-α, IL-6, and HGF), which promote cell cycle progression and induce DNA replication (S phase) (Court et al., 2002; Di-Iacovo et al., 2023; Hora et al., 2023). A critically important stage in DNA synthesis is considered the de novo formation of 2'-deoxyribonucleotides (dNDP) from the corresponding ribonucleotides (NDP), catalyzed by ribonucleotide reductase (EC 1.17.4.1; RNR). Interestingly, the reaction cycle of RNR envisions the oxidation of catalytically active sulfhydryl groups of cysteines in the enzyme's active site (E(SH)2) to disulfides (E(S-S)) with substrate reduction. The regeneration of ribonucleotide reductase is carried out with the participation of reduced thioredoxin, a dithiol coenzyme endowed with functional activity, through the conversion of two cysteine residues in the –Cys–Gly–Pro–Cys– sequence of the Trx-SH2 molecule into a cysteine disulfide (Bhagavan et al., 2011; Sengupta et al., 2019). Given the above, the increase in thioredoxin activity observed in the animals of the C/PH group during the period of the active course of synthetic processes that we identified likely reflects the accumulation of Trx-SH2 and occurs in response to the increased demand of actively proliferating liver cells for RNR activity restoration and DNA synthesis following the conducting of partial hepatectomy. In turn, the decrease in thioredoxin activity in the animals of the TI/PH group, which may indicate the depletion of reserves of functionally active Trx-SH2, probably leads to the suppression of ribonucleotide reductase activity, subsequent disruption of DNA replication, and cell cycle arrest.

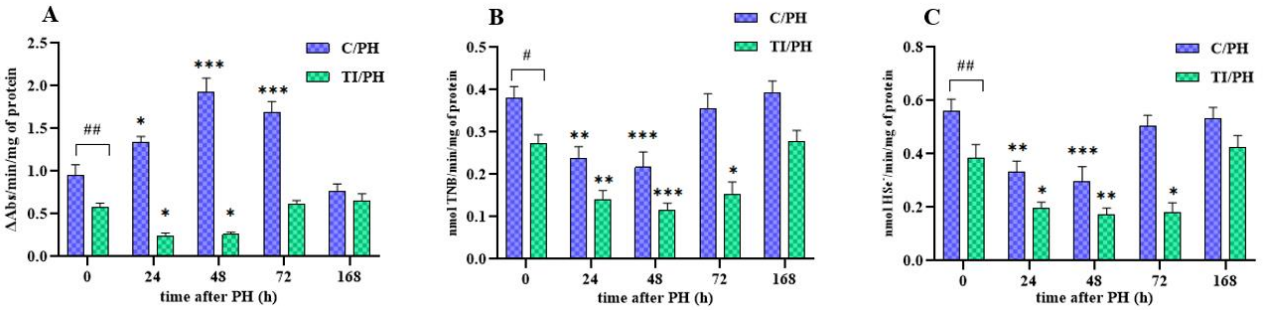

Table 1.

Fig. 1. Activity of thioredoxin (A), thioredoxin reductase (B), and selenocysteine β-lyase (C) in the cytosolic fraction of rat liver under conditions of partial hepatectomy following acetaminophen toxic injury.

****, **, *** – levels of significance of differences compared to the control (0 h), P<0.05; P<0.01; P<0.001, respectively. #, ## – levels of significance of differences compared to the C/PH group (0 h), P<0.05; P<0.01, respectively.***

In addition to its ability to maintain the redox-dependent thiol-disulfide state of proteins, including RNR, thioredoxin possesses antioxidant activity, which is determined not only by its capacity to function as a scavenger of reactive oxygen species (ROS) but also by its ability to restore the catalytic activity of peroxiredoxins and glutathione peroxidases (Hasan et al., 2022; Liu, 2023). Accordingly, the decrease in Trx activity in animals of the TI/PH group during the initiation period and active cell proliferation, against the background of the decrease in reduced glutathione levels throughout the entire regeneration period (168 h), as shown in our previous studies, is likely to result in the suppression of the antioxidant potential of liver cells and disruption in the course of regenerative events under conditions of toxic injury.

Evaluating the dynamics of changes in thioredoxin reductase activity in the cytosolic fraction of the livers of rats in the C/PH group, we observed its significant decrease during the initiation period (24 h) by 37 % and during active cell proliferation (48 h) by 43% compared to the control (0 h). On the other hand, in the liver cells of animals in the TI/PH group, thioredoxin reductase activity remains below the control values not only at 24 h (by 49 %) and 48 h (by 58 %) of the experiment, but also during the termination period (72 h; by 44 %) (Fig. 1, B). Thioredoxin reductase is a selenium-containing flavoprotein that provides NADPH-dependent recirculation of reduced thioredoxin from its oxidized form (Hasan et al., 2022; Liu, 2023). Thus, the observed increase in Trx activity, reflecting the restored state of its reserves, alongside the decrease in TrxR activity and, accordingly, the disruption of oxidized Trx conversion in animals of the C/PH group within 48 hours, may serve as direct evidence of the activation of a compensatory mechanism that contributes to the intensification of Trx synthesis after PH. At the same time, the suppression of TrxR activity in animals of the TI/PH group during the initial stages of regeneration can likely be considered one of the primary causes of the shown Trx dysfunction under these experimental conditions.

The functional activity of thioredoxin reductase is directly determined by the efficiency of selenide (HSe^-) formation and the incorporation of selenocysteine (Sec) into the active site of the selenoenzyme. One of the pathways for producing the Sec precursor, HSe^-, is considered the conversion of organic selenium forms (Sec and SeMet) with the participation of selenocysteine β-lyase (Zhang et al., 2020; Kang et al., 2020). Consequently, the decrease in TrxR activity in rats of both studied groups can be explained by the observed reduction in selenocysteine β-lyase activity (Fig. 1, C), followed by the disruption of the formation of the selenoprotein domain in the active site of TrxR.

Conclusions. Hence, conducting partial hepatectomy following acute toxic injury by acetaminophen is accompanied by a decrease in thioredoxin activity during the initial stages of regeneration (24 h and 48 h), along with simultaneous suppression of thioredoxin reductase and selenocysteine β-lyase activities during 72 h of the experiment.

Conflict of Interest: *The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be interpreted as a potential conflict of interest.*

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АКТИВНІСТЬ СИСТЕМИ ТІОРЕДОКСИНУ В ПЕЧІНЦІ ЩУРІВ ЗА УМОВ ЧАСТКОВОЇ ГЕПАТЕКТОМІЇ ПІСЛЯ ТОКСИЧНОГО УРАЖЕННЯ

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Робота присвячена оцінці активності тіоредоксину, тіоредоксинредуктази та селеноцистеїн-β-ліази в цитозольній фракції печінки щурів за умов часткової гепатектомії після токсичного ураження ацетамінофеном. Експерименти були проведені на білих нелінійних щурах, яких методом рандомізації поділяли на дві групи: контрольні тварини, яким здійснювали часткову гепатектомію згідно методу Mitchell and Willenbring (C/PH) та щури, яким проводили часткову резекцію 2/3 тканини печінки після гострого токсичного ураження ацетамінофеном шляхом попереднього дводенного введення в дозі 1250 мг/кг маси тіла тварини (TI/PH). Дослідження виконували на 0 (контроль), 24 (фаза ініціації), 48 (проліферативна фаза), 72 (фаза термінації) та 168 (віддалений період) години після здійснення часткової гепатектомії. Проведення часткової гепатектомії після моделювання ацетамінофен-індукованого ураження (TI/PH) супроводжується статистично достовірним зниженням активності тіоредоксину у клітинах печінки на початкових етапах регенерації (24 год та 48 год) порівняно із контрольними величинами (0 год). Встановлені зміни відбуваються на тлі пригнічення селенозалежної тіоредоксинредуктазної й селеноцистеїн-β-ліазної активностей впродовж періоду ініціації (24 год), активної проліферації клітин (48 год) та термінації (72 год). Виснаження функціональних резервів тіолозалежної редокс-системи тіоредоксину та пригнічення ефективності продукування селеніду через показане порушення перетворення органічних форм селену за участі селеноцистеїн-β-ліази у тварин з ацетамінофен-індукованим ураженням після часткової гепатектомії (TI/PH) можна розглядати як один із факторів зниження регенеративного потенціалу печінки за умов токсичного ураження.

Ключові слова: тіоредоксин, тіоредоксинредуктаза, селеноцистеїн-β-ліаза, часткова гепатектомія, ацетамінофен, токсичне ураження, печінка

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