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## SUPEROXIDE DISMUTASE ACTIVITY IN LIVER CELLS OF RATS UNDER CONDITIONS OF TOXIC DOSES OF ACETAMINOPHEN ADMINISTRATION AND PARTIAL HEPATECTOMY

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*The aim of this work was to investigate the activities of Mn- and Cu,Zn-dependent superoxide dismutase in the mitochondrial and cytosolic fractions of liver cells in rats under conditions of toxic doses of acetaminophen administration and partial hepatectomy. Partial hepatectomy (a surgical procedure for the removal of two-thirds of the liver mass), used to study liver regeneration processes under laboratory conditions, was performed following the method of Mitchell and Willenbring (2008) exclusively under sterile conditions to minimize the risk of infections. The mitochondrial and cytosolic fractions of rat liver cells were obtained using the method of differential preparative centrifugation. The activity of Mn- and Cu,Zn-dependent superoxide dismutase in the mitochondrial and cytosolic fractions of the liver was assessed based on the ability of superoxide dismutase to inhibit adrenaline autooxidation. It was established that in the liver of control rats after partial hepatectomy, activation of Mn- and Cu,Zn-dependent superoxide dismutase occurs only during the early stages (24 h and 48 h) of the regeneration process, likely aimed at increasing the level of superoxide anion radicals. In animals with toxic injury, the course of reparative regeneration is characterized by a decrease in Mn-superoxide dismutase activity in mitochondria against the background of Cu,Zn-SOD activation in the cytosol throughout the entire experimental period, which is likely reflects the implementation of compensatory antioxidant defense mechanisms of hepatocytes in response to their depletion in mitochondria.*

*Keywords: Cu,Zn-superoxide dismutase, Mn-superoxide dismutase, mitochondria, liver, acetaminophen, partial hepatectomy, regeneration*

**Introduction.** Liver regeneration is a highly organized process of tissue restoration, which is the most important reaction of this organ to injury. Overall, the process of liver regeneration comprises three phases: the initiation stage, the proliferation phase, and the termination phase (Asnaashari et al., 2021). Understanding the mechanisms of liver regeneration is an important point in the context of the impact of xenobiotics of various origins, particularly medicinal ones, on this homeostatic organ. One example of such compounds is acetaminophen (N-acetyl-para-aminophenol; APAP), the most widespread antipyretic and analgesic drug. (Kotulkar et al., 2023).

Liver regeneration is accompanied by the activation of prooxidant processes, particularly the formation of reactive oxygen species (ROS), which perform a dual role. On one hand, ROS act as signaling molecules, stimulating regenerative processes such as cell proliferation and the repair of damaged tissues and on the other hand, excessive accumulation of ROS can lead to the development of oxidative stress (Zhang et al., 2023). Superoxide dismutase is a key enzyme of the antioxidant defense system that helps neutralize the superoxide

anion by converting it into the less harmful hydrogen peroxide. There are different isoforms of this enzyme: superoxide dismutase-1 (SOD1, Cu,Zn-SOD, EC 1.15.1.1), which functions in the cytosol, and superoxide dismutase-2 (SOD2, Mn-SOD, EC 1.15.1.1), which plays a critical role in the detoxification of reactive oxygen species in mitochondria (Li et al., 2022).

The aim of the work is to investigate the activity of Mn- and Cu,Zn-dependent superoxide dismutase in the mitochondrial and cytosolic fractions of the liver in rats with paracetamol-induced toxic injury following partial hepatectomy.

**Materials and Methods.** For the study, white non-pedigree rats aged 140–150 days and weighing 150–180 g were used, housed in the experimental-biological vivarium of the Department of Biochemistry and Biotechnology. During the implementation of all necessary procedures on the animals, the requirements of the European Convention adopted in Strasbourg (1986) regarding the protection of vertebrate animals used in scientific research and experiments were clearly observed.

Toxic injury was induced by oral administration of a paracetamol suspension at a dosage of 1250 mg

per kilogram of the animal's body weight, dissolved in a 2% starch gel solution (Kopylchuk et al., 2020). Partial hepatectomy – a surgical procedure for removing two-thirds of the liver mass, used to study liver regeneration processes in laboratory conditions, was performed according to the method of Mitchell and Willenbring (2008) exclusively under sterile conditions to minimize the risk of infections.

The experimental rats were separated into groups: Group I (control) – intact rats that underwent partial hepatectomy (C/PH); Group II – rats with partial hepatectomy and acetaminophen-induced toxic injury (TI/PH). The animals were euthanized by cervical dislocation under light inhalation anesthesia at 24, 48, 72, and 168 hours of the regenerative process.

The mitochondrial and cytosolic fractions of rat liver cells were obtained by differential preparative centrifugation method. The activity of Mn- and Cu,Zn-dependent superoxide dismutase in the mitochondrial and cytosolic fractions of the liver was assessed by the ability of superoxide dismutase to inhibit adrenaline auto-oxidation (Jiao et al., 2023).

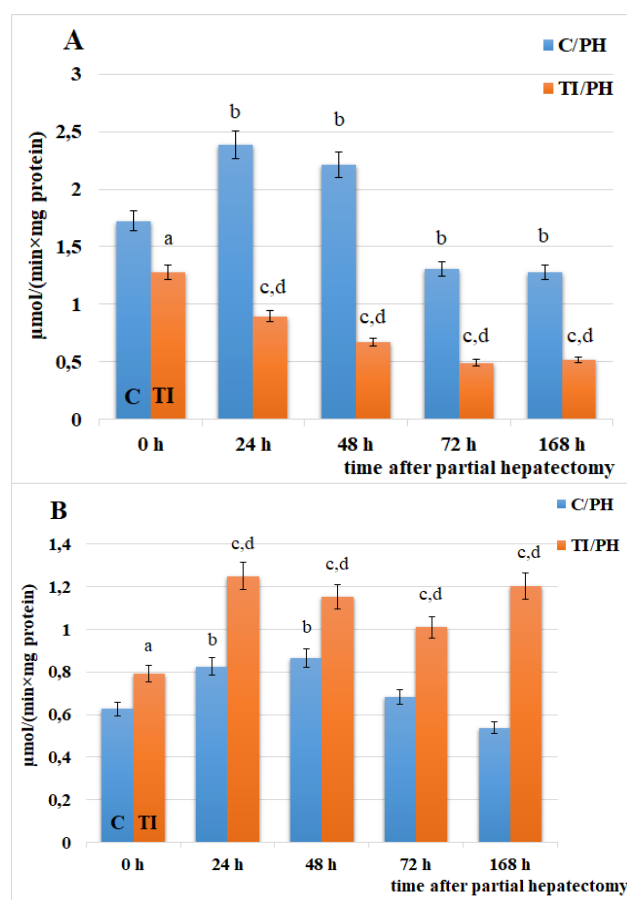
**Results and Discussion.** We have established that in the C/PH group of rats, the increase in Mn-dependent superoxide dismutase activity occurs only at the initial stages of regeneration (24 h) and during the period of active cell proliferation (48 h) compared to the control values at 0 hours (Fig. 1, A).

In return, in the animals of the TI/PH group, the activity of Mn-dependent superoxide dismutase in the mitochondrial fraction of the liver decreases throughout the entire organ recovery period compared to the 0-hour values of the TI group. As shown in Fig. 1, the activity of this enzyme is already reduced in the group of rats in which toxic injury was modeled (TI group) compared to the control at 0 h. The lowest values of Mn-SOD activity were recorded at the final stages of the regeneration process (72 and 168 h) (Fig. 1, A).

It should be noted that MnSOD is an enzyme that is quite hypersensitive to nitration, accounting for up to 20% of the total nitration of proteins under conditions of oxidative stress (Demicheli et al., 2016). Considering that under these experimental conditions, previous studies have shown hyperproduction of nitric oxide in the mitochondria of rat liver, the formation of the highly reactive metabolite peroxynitrite becomes possible, which may cause to local inactivation of Mn-SOD through nitration of critical tyrosine residues.

At the same time, we established that in the cytosolic fraction of liver cells in the control rats (group C/PH), an analogous trend in the changes in the activity of Cu,Zn-superoxide dismutase is observed, which increases only during the 24-hour

and 48-hour periods after partial hepatectomy (Fig. 1, B). This indicates an active response of the antioxidant resources of hepatocytes in response to the intensified formation of superoxide under these conditions. At the same time, in the group of rats with toxic injury, which underwent partial hepatectomy, an increase in superoxide dismutase activity was observed. Such changes were registered by us throughout the entire experimental period, with the highest values at the initial stages (24 h, 48 h) and also at the distant term (168 h) of the regenerative process (Fig. 1, B), which may indicate the activation of antioxidant mechanisms as a result of the damage caused by acetaminophen.



**Fig. 1.** Activity of Mn-dependent superoxide dismutase in the mitochondrial fraction (A) and Cu,Zn-dependent superoxide dismutase in the cytosolic fraction (B) of liver cells in rats with toxic injury following partial hepatectomy.

Note: C/PH – control animals that underwent partial hepatectomy; TI/PH – animals with toxic injury that underwent segmental partial liver resection; a – statistically significant difference between the indicators of rats in the TI group and control,  $P \leq 0.05$ ; b – statistically significant difference in the indicators of the C/PH group compared to the values of C at 0 h,  $P \leq 0.05$ ; c – statistically significant difference between the TI/PH groups compared to the indicators of rats in the TI group at 0 h,  $P \leq 0.05$ ; d – statistically significant difference between the TI/PH groups compared to the indicators of the C/PH group,  $P \leq 0.05$ .

In the literature (Chandrasekharan et al., 2021), it is noted that Cu/ZnSOD is excessively expressed under conditions of elevated levels of pro-inflammatory factors IL-1 $\beta$  and TNF- $\alpha$ . This is in reliable agreement with the results of previous studies on the activation of inflammatory processes in the liver of rats with toxic injury following partial hepatectomy, induced by pro-inflammatory cytokines.

**Conclusions.** It has been established that in the liver of control rats, following partial hepatectomy, the activation of Mn- and Cu,Zn-dependent superoxide dismutase occurs only at the early stages (24 h and 48 h) of the regenerative process, which is likely aimed at increasing the level of superoxide anion radical.

In animals with toxic injury, the course of reparative regeneration is characterized by a decrease in the activity of Mn-superoxide dismutase in the mitochondria, against the backdrop of Cu,Zn-SOD activation in the cytosol throughout the entire experimental period. This likely reflects the implementation of compensatory mechanisms of antioxidant defense of hepatocytes in response to their depletion in the mitochondria.

**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 СУПЕРОКСИДДИСМУТАЗИ В КЛІТИНАХ ПЕЧІНКИ ЩУРІВ ЗА УМОВ ВВЕДЕННЯ ТОКСИЧНИХ ДОЗ АЦЕТАМІНОФЕНУ ТА ЧАСТКОВОЇ ГЕПАТЕКТОМІЇ

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Метою даної роботи стало дослідження активностей Mn- і Cu,Zn-залежної супероксиддисмутазу в мітохондріальній та цитозольній фракціях клітин печінки щурів за умов введення токсичних доз ацетамінофену та часткової гепатектомії. Часткову гепатектомію (хірургічну процедуру для видалення 2/3 маси печінки), що використовується для вивчення процесів регенерації печінки в лабораторних умовах, здійснювали за методом Mitchell і Willenbring (2008) виключно в стерильних умовах з метою мінімізації ризиків інфекцій. Мітохондріальну та цитозольну фракцію клітин печінки щурів отримували методом диференційного препаративного центрифугування. Визначення активності Mn- і Cu,Zn-залежної супероксиддисмутазу в

мітохондріальній та цитозольній фракціях печінки оцінювали за здатністю супероксиддисмутази пригнічувати аутоокислення адреналіну. Встановлено, що печінці контрольних щурів після часткової гепатектомії активація Mn- і Cu,Zn-залежної супероксиддисмутази відбувається лише на ранніх етапах (24 год та 48 год) регенераційного процесу, що, ймовірно, спрямовано на підвищення рівня супероксидного-аніон-радикалу. У тварин із токсичним ураженням перебіг репаративної регенерації характеризується зниженням активності Mn-супероксиддисмутази в мітохондріях та тлі активації Cu,Zn-СОД у цитозолі впродовж всього експериментального періоду, що, вірогідно, відображає реалізацію компенсаторних механізмів антиоксидантного захисту гепатоцитів у відповідь на їх виснаження в мітохондріях.

*Ключові слова:* Cu,Zn-супероксиддисмутаза, Mn-супероксиддисмутаза, мітохондрії, печінка, ацетамінофен, часткова гепатектомія, регенерація.

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