UDC 599.323.452:615.035.3]:577.15 https://doi.org/10.15407/biotech17.02.084

AGE-RELATED CHARACTERISTICS OF GLUTATHIONE-DEPENDENT ENZYMES FUNCTIONING IN RATS UNDER CONDITIONS OF TOXIC INJURY BY ACETAMINOPHEN

M.S. URSATYI, H.P. KOPYLCHUK, I.M. NYKOLAICHUK

Yuriy Fedkovych Chernivtsi National University, Ukraine

E-mail: m.ursatyi@chnu.edu.ua

Received 2024/03/09
Revised 2024/04/17
Accepted 2024/04/30

Currently, the problem with impaired functional potential of homeostatic organs, primarily the liver, is becoming increasingly relevant. One of the leading factors contributing to liver metabolism dysfunction is the hepatotoxic effect from xenobiotics of medicinal origin, particularly acetaminophen (paracetamol, APAP). This analgesic and antipyretic medication is widely used as symptomatic therapy for people of various ages, including infants [1]. The uncontrolled use of paracetamol, despite its low therapeutic index, is a quite common phenomenon during outbreaks of infectious and inflammatory diseases (COVID-19) and during military operations in Ukraine. The primary event in the development of hepatotoxic reactions induced by the toxic effects of acetaminophen is the accumulation in hepatocytes of highly reactive N-acetyl-p-benzoquinone imine (NAPQI), which is detoxified through direct interaction with reduced glutathione (GSH) or in the glutathione-S-transferase (GST) reaction [1, 2]. Therefore, the realization of the xenobiotic effect by APAP is closely associated with the characteristics of the functioning of the glutathione-dependent enzymatic system.

The *aim* of the work was to evaluate the enzymatic activities of the glutathione system in liver cells of rats from different age groups under conditions of toxic injury caused by acetaminophen.

Methods. In the experimental study, 84 white non-breed specific rats of two age categories were used: young (138–150 days) and mature age (348–360 days) with weights ranging from 130–160 grams and 210–250 grams, respectively. Young and mature animals were divided into two groups: rats that were on a complete semi-synthetic diet AIN-93 for 28 days (C) and rats with acute acetaminophen-induced injury who had previously consumed the complete diet AIN-93 for 28 days (TI). Acute toxic injury with acetaminophen was induced by administering it *per os* at a dose of 1250 mg/kg of animal weight on days 29 and 30 of the experiment [3]. The animals were removed from the experiment on day 29 (C) and day 31 (TI). The principle of the method for determining glutathione-S-transferase activity was based on the accumulation of glutathione conjugates with 1-chloro-2,4-dinitrobenzene at $\lambda = 340$ nm. The activity of Se-dependent and Se-independent glutathione peroxidases was assessed by non-enzymatic oxidation of glutathione by hydrogen peroxide or cumene hydroperoxide, respectively, at $\lambda = 260$ nm. Glutathione reductase activity was determined by the rate of NADPH oxidation at $\lambda = 340$ nm over 2 minutes with 30-second intervals. Statistical analysis was conducted using GraphPad Prism 8.0.1. Data were analyzed using a two-way analysis of variance (ANOVA) with Tukey's a posteriori test.

Results and Discussion. The conducted studies demonstrated a statistically significant decrease in glutathione-S-transferase activity in the cytosolic fraction of liver cells of both young and mature rats under conditions of acetaminophen-induced injury. In young animals, GST activity reduced by 29%, and in mature animals, it reduced by 36% compared to the control, and by 34% compared to the TI young age animal group (Figure, *A*). The results demonstrated a dependence of the intensity of reduction in GST activity on the age factor, which was experimentally confirmed by the decrease in GSH content in liver cells of both age groups under conditions of injury with high doses of APAP [4].

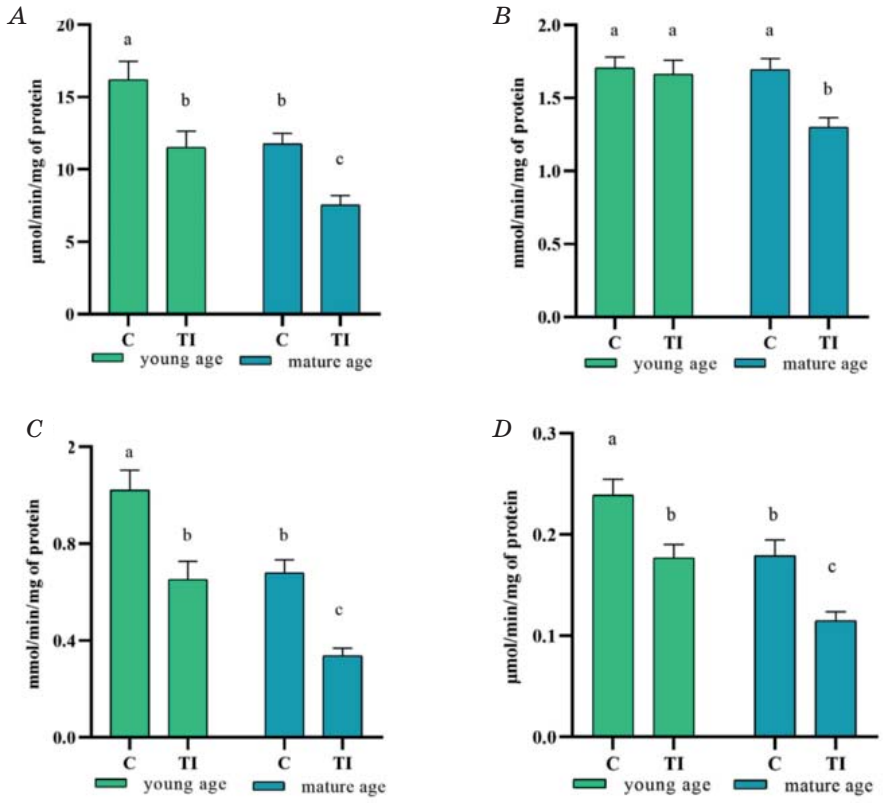

Fig. **Glutathione-S-transferase (*A*), non-Se-glutathione peroxidase (*B*), Se-glutathione peroxidase (*C*) and glutathione reductase (*D*) activities in the cytosolic fraction of liver cells in rats from different age groups under conditions of acetaminophen-induced injury:**
a, b, c — values marked with these letter indices are statistically likely to differ ($P < 0.05$, $P < 0.01$).

Consequently, it could be assumed that the results we obtained are probably related to the enhanced use of intracellular GSH reserves for the inactivation of NAPQI. It is known that the consumption of supratherapeutic doses of APAP leads to the exhaustion of sulfation/glutathione pathways, accompanied by a predominance of xenobiotic metabolism via N-oxidation by the cytochrome P450 system, resulting in the formation of a toxic metabolite [5].

In addition to glutathione transferases, GSH functions as a cosubstrate for both non-Se- and Se-glutathione peroxidase (non-Se-GPx, Se-GPx). Significant differences in the form of reduced non-Se-GPx activity in the liver cells were observed only in mature animals of the TI group (by 24% compared to control) (Figure, *B*). Meanwhile, Se-GPx activity decreased in both mature and young rats under conditions of receiving excessive APAP doses, with more pronounced changes in mature age animals (by 50% compared to control; by 48% compared to young rats in the TI group) (Figure, *C*). The consequences of these changes might be impairments in the reduction of hydrogen peroxide and lipid peroxides. The preservation of non-Se-GPx activity at control level, against the backdrop of a decrease in Se-GPx activity in young animals, was likely linked to the activation of a compensatory reaction to counteract the development of oxidative stress.

Maintenance of the intracellular glutathione pool in its reduced state, besides *de novo* synthesis, occured through the regeneration of its oxidized form in an NADPH-dependent glutathione reductase reaction (GR) [6]. As it is shown in the Figure, *D*, reduction in GR activity under conditions of APAP-induced injury occured in the liver of rats of both age groups. More significant reduction in GR activity was observed in animals aged 360 days (by 39% compared to the control and young rats of the TI group) (Figure, *D*), which might be associated with disturbances in the supply of the NADPH cofactor due to the demonstrated decrease in glucose-6-phosphate dehydrogenase activity.

Conclusions. Therefore, the influence of the age component can be considered as one of the critically important factors in suppressing the functional activity of the glutathione-dependent enzymatic system, manifested by decreased activity of GST, non-Se-GPx, Se-GPx and GR under

conditions of toxic injury caused by the medicinal xenobiotic acetaminophen. More pronounced decrease in glutathione-dependent enzymes under conditions of acetaminophen intoxication was observed in animals aged 360 days (mature age).

Key words: glutathione transferase, glutathione peroxidase, glutathione reductase, liver, acetaminophen, different age groups.

Authors' contribution

M.S. Ursatyi (reporter) — executing the experimental part of the work, creating graphical materials, analyzing data, and writing theses.

H.P. Kopylchuk — planning the research, establishing animal model, summarizing the results and formulating conclusions.

I.M. Nykolaichuk — analyzing data and results, writing theses.

Funding source. The work was carried out within the framework of the departmental theme "Biochemical mechanisms of integral regulation of nutrient-metabolic status of living organisms and complex biotechnological approaches to their correction" (2021–2025, State registration number 0120U105710).

REFERENCES

1. *Chidiac A. S., Buckley N. A., Noghrehchi F., Cairns, R.* Paracetamol (acetaminophen) overdose and hepatotoxicity: mechanism, treatment, prevention measures, and estimates of burden of disease. *Expert Opinion on Drug Metabolism & Toxicology*. 2023, 19 (5):297–317. https://doi.org/10.1080/17425255.2023.2223959
2. *Mazaleuskaya L. L., Sangkuhl K., Thorn C. F., FitzGerald G. A., Altman R. B., Klein T. E.* PharmGKB summary: pathways of acetaminophen metabolism at the therapeutic versus toxic doses. *Pharmacogenet Genomics*. 2015, 25 (8):416–426. https://doi.org/10.1097/FPC.0000000000000150
3. Preclinical studies of drugs: guidelines. In: *Stefanov O.V.*, editor. Kyiv: *Avicenna*. 2001, 344–345.
4. *Kopylchuk H., Nykolaichuk I., Ursatyi M.* The redox potential of glutathione in the livers of rats of different ages under conditions of toxic injury by acetaminophen. Youth Science for Peace and Development: coll. of materials of the International scientific and practical conference (November 8–10, Chernivtsi). Chernivtsi: *Yuriy Fedkovych Chernivtsi National University*. 2023, 65–68.
5. *Kopylchuk H., Nykolaichuk I., Ursatyi M.* Effect of dietary protein deficiency on the activity of cytochrome P450 enzyme systems in the liver of rats of reproductive age under acetaminophen-induced injury. *Acta Scientific Gastrointestinal Disorders.* 2022, 5 (4):39–48. https://doi.org/10.31080/ASGIS.2022.05.0402
6. *Vašková J., Kočan L., Vaško L., Perjési P.* Glutathione-related enzymes and proteins: A review. *Molecules*. 2023, 28 (3):1447. https://doi.org/10.3390/molecules28031447