UDC 577.23:[616.36-089.873+615.212.035.3] https://doi.org/10.15407/biotech18.02.106

ADENYL NUCLEOTIDES CONTENT IN LIVER MITOCHONDRIA OF RATS WITH ACETAMINOPHEN INTOXICATION AFTER PARTIAL HEPATECTOMY

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

Yuriy Fedkovych Chernivtsi National University, Chernivtsi

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

Received 2025/03/12
Revised 2025/04/11
Accepted 2025/04/30

The work aimed to assess the content of adenyl nucleotides (ATP, ADP, AMP) and Atkinson's adenylate energy charge in the mitochondrial fraction of rat liver with acetaminophen-induced toxic injury following partial hepatectomy.

Materials and Methods. The experiments were carried out on white non-linear rats divided into two groups: I — control rats subjected to resection of 2/3 of the liver tissue, and II — rats that underwent partial hepatectomy following acetaminophen-induced toxic injury. Animals were removed from the experiment at 0 (preoperative period), 24, 48, 72, and 168 hours after partial hepatectomy.

Results. In the mitochondrial fraction of the liver in rats with acetaminophen-induced toxic injury after partial hepatectomy, a decrease in ATP content was observed during the three days of the experiment, accompanied by simultaneous increases in ADP and AMP levels during 72 and 168 hours, respectively, against the background of a decline in the adenylate energy charge with minimal values at 72 h of the regenerative period.

Conclusions. Liver regeneration in rats after partial hepatectomy under conditions of acetaminophen-induced toxic injury is accompanied by a quantitative redistribution of adenyl nucleotides in mitochondria: a maximal increase in AMP content occurs alongside a simultaneous decrease in ATP levels at the terminal stage of organ recovery. Such changes cause the most substantial reduction in Atkinson's adenylate energy charge, which can be regarded as a critical stage in the dysfunction of the energy supply system under these experimental conditions.

Keywords: liver, partial hepatectomy, acetaminophen, adenyl nucleotides.

In ensuring the organism's homeostasis during the progressive development of various pathological conditions of the liver (toxic or traumatic injuries, infectious or metabolic diseases), endowed with a broad functional and metabolic profile, a decisive position belongs to the organ's capacity for compensatory regeneration. The implementation of the regenerative cascade of events, in addition to the typical mechanisms driven by hepatocyte proliferation, may occur through alternative pathways involving hepatic progenitor cells, which are especially important in cases of chronic or significant acute injuries. Reparative regeneration is considered crucial for the restoration of hepatostat and the functional activity of the liver following the hepatotoxic effects of acetaminophen. However, the suppression of regenerative potential noted when threshold doses of hepatotoxins are exceeded leads to the development of liver failure, which in some instances requires transplantation [1, 2]. The liver's unique ability to regenerate creates the possibility of

Citation: Ursatyi, M. S., Kopylchuk, H. P., Nykolaichuk, I. M. (2025). Adenyl nucleotides content in liver mitochondria of rats with acetaminophen intoxication after partial hepatectomy. *Biotechnologia Acta*, 18(2), 106–108. https://doi.org/10.15407/biotech18.02.106

performing partial hepatectomy in cases of tumor formations, parasitic or non-parasitic cysts, abscesses, as well as toxic injuries, including those caused by medicinal xenobiotics [3]. As is known, metabolic reorganization that promotes the regression of liver cell damage is inextricably linked to meeting the increased demands for macronutrient substrates for the endergonic reactions involved in the hepatoregeneration process [4]. The key components of the energy supply system are adenyl nucleotides, whose role lies not only in coupling the processes of energy generation and utilization but also in regulating metabolic (as cofactors and allosteric regulators) and signaling pathways [5, 6]. The work aimed to assess the content of adenyl nucleotides (ATP, ADP, AMP) and Atkinson's adenylate energy charge in the mitochondrial fraction of rat liver with acetaminophen-induced toxic injury following partial hepatectomy.

Methods. Experiments were conducted on white non-linear rats of reproductive age (140–150 days) weighing 130–150 g. The modeling of acute toxic injury induced by acetaminophen was carried out through its intragastric administration using a gastric probe at a dose of 1.25 g/kg for two days in a 2% starch suspension [7]. A partial hepatectomy was performed according to the Mitchell and Willenbring method [8]. The study model involved dividing animals into two groups: control rats that underwent partial resection of 2/3 of liver tissue (C/PH) and rats that underwent partial hepatectomy after acetaminophen-induced injury (TI/PH). Animals were removed from the experiment at 0 (preoperative period), 24, 48, 72, and 168 hours after partial hepatectomy. Quantification of ATP, ADP, and AMP content in the liver mitochondrial fraction was accomplished by thin-layer chromatography using ALUGRAM Xtra SIL G/UV254 plates (Macherey-Nagel, Germany) [9]. The adenylate energy charge (AEC) was calculated according to the formula by Atkinson: AEC = (ATP + 0.5ADP) / (ATP + ADP + AMP). Statistical analysis was carried out using two-way analysis of variance (Two-way ANOVA) with Tukey's post hoc test in GraphPad Prism version 8.0.1. The results are presented as mean ±SEM; statistical significance was established at $P < 0.05$.

Results and Discussion. Studies have shown that in the mitochondrial fraction of the liver from control rats after partial hepatectomy (C/PH), ATP content decreased during the 48-hour regeneration period (24 h — by 29%; 48 h — by 24%, $P < 0,05$) compared to preoperative values at 0 h (intact control) (Fig. *A*). Instead, ADP and AMP levels in the liver mitochondria of animals in this group exceeded the 0 h values throughout the 48 hours (ADP: 24 h — by 40%, $P < 0,01$; 48 h — by 54%, $P < 0,001$; AMP: 24 h — by 48%, $P < 0,05$; 48 h — by 53%, $P < 0,01$) (Fig. *B* and *C*). Therefore, the multidirectional changes in ATP, ADP, and AMP content established in our study lead to a decrease in AEC only at the initial stages of liver regeneration in animals of the C/PH group (from 0.69±0.01 to 0.51±0.02). Analysis of scientific publications indicates no dysfunction of respiratory and phosphorylation activity in mitochondria following liver tissue resection [10, 11]. Accordingly, the obtained results can be considered adaptive changes associated with enhanced ATP release from mitochondria to meet the energy demands of the processes of structural component synthesis of liver cells. This is clearly reflected in the catalytic activity of DNA replication enzymes that utilize energy from ATP hydrolysis and of protein synthesis enzymes, particularly at the stage of aminoacyl-tRNA formation following partial hepatectomy. In addition, it is known that with an increase in the energy needs of cells (metabolic challenge), there is a compensatory increase in the concentration of ADP [12]. One of the reasons for the increased ADP content in mitochondria may be the intensification of ATP transfer from the mitochondrial matrix to the intermembrane space and, consequently, into the cytosol, which occurs when ADP is simultaneously transported in the opposite direction, mediated by the ADP/ATP antiporter.

Regarding the animals of the TI/PH group, a decrease in ATP content in liver mitochondria occurred during the 72 h of the regeneration process compared to the preoperative values in rats of the toxic injury group at 0 h (24 h — by 34%, $P < 0,05$; 48 h — by 31%, $P < 0,05$; 72 h — by 44%, $P < 0,01$) (Fig. *A*). Such changes were observed against the background of an increase in ADP levels during the 72 hours of liver parenchyma recovery, with a maximum at 48 h (by 60%, $P < 0,001$) and an elevation in AMP levels throughout the entire experimental period (168 h), with maximum values at 72 h (by 54%, $P < 0,001$) (Fig. *B* and *C*). The deepening of disturbances in the adenyl nucleotide system, compared to rats of the C/PH group, is confirmed by the most pronounced decrease in AEC at 72 h of liver regeneration (from 0.61±0.02 to 0.36±0.02). As is known, the toxic metabolite of acetaminophen, N-acetyl-p-benzoquinoneimine, forms adducts with the α-subunit of ATP synthase, with a decrease in its activity [13]. Therefore, the registered changes likely indicate suppression of the resynthesis processes of the main macroregion compound against the background of the

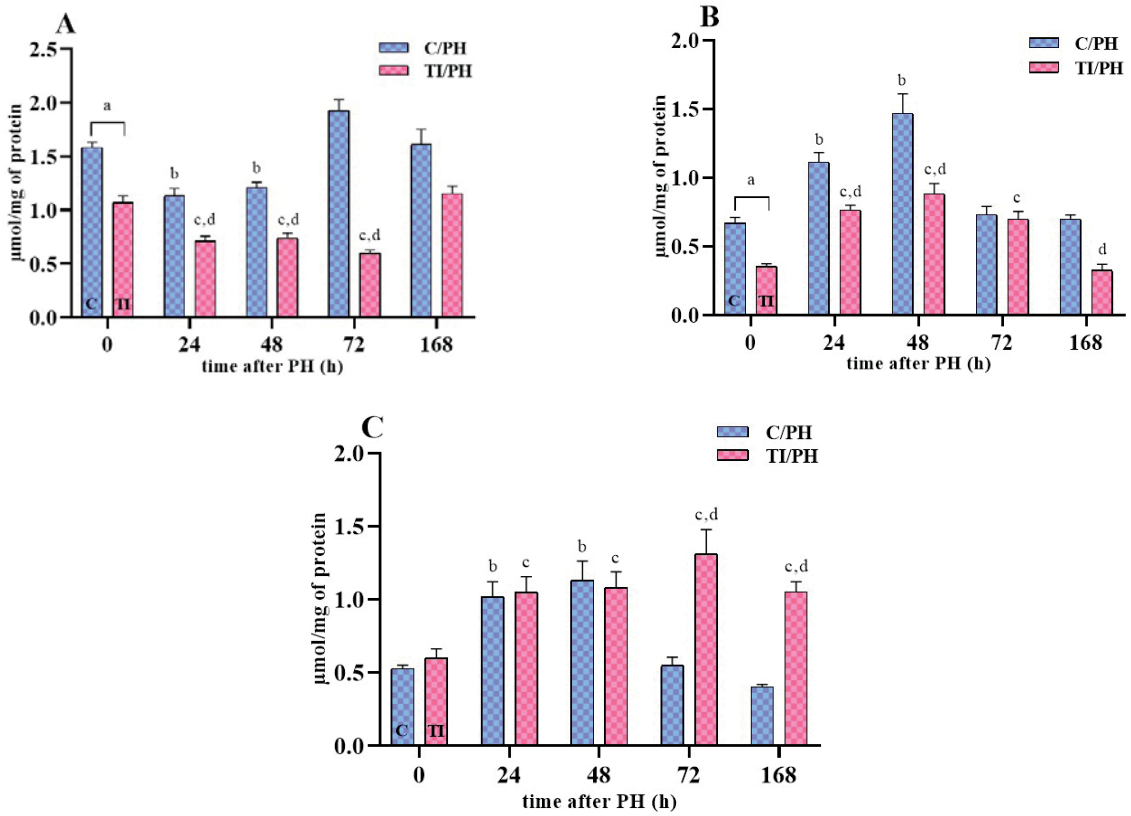

Figure. **ATP (*A*), ADP (*B*), and AMP (*C*) content in the mitochondrial fraction of the liver of rats with acetaminophen-induced toxic injury after partial hepatectomy:**
a, b, c, d — values indicated by these letter indices differ statistically significantly; *a* — statistically significant difference between the TI group and the control group (C) at 0 h; *b* — statistically significant difference of the C/PH groups at each time point compared to the C group at 0 h; *c* — statistically significant difference of the TI/PH groups at each time point compared to the TI group at 0 h; *d* — statistically significant difference between the TI/PH and C/PH groups at the corresponding time point (e.g., 24 h vs 24 h, etc.)

dominance of ATP and ADP dephosphorylation under conditions of increased energy demands caused by partial hepatectomy following acetaminophen-induced injury.

Conclusions. Thus, liver regeneration in rats after partial hepatectomy under conditions of acetaminophen-induced toxic injury is accompanied by a quantitative redistribution of adenyl nucleotides in mitochondria: a maximal increase in AMP content occurs alongside a simultaneous decrease in ATP levels at the terminal stage (72 h) of organ recovery. Such changes cause the most substantial reduction in Atkinson's adenylate energy charge, which can be regarded as a critical stage in the dysfunction of the energy supply system under these experimental conditions. The results obtained may be helpful in creating an algorithm of corrective schemes for restoring liver energy resources in the postoperative period against the background of drug-induced toxic injuries, which is essential under functional load during regeneration.

Authors' contribution

MSU — experimental work, data analysis, and interpretation, manuscript writing; HPK — experiment planning, result generalization, critical editing of the manuscript; IMN — experiment planning, result analysis, manuscript writing.

Funding source

The work was conducted 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. Kiseleva, Y. V., Antonyan, S. Z., Zharikova, T. S., Tupikin, K. A., Kalinin, D. V., Zharikov, Y. O. (2021). Molecular pathways of liver regeneration: A comprehensive review. *World journal of hepatology*, 13 (3), 270–290. https://doi.org/10.4254/wjh.v13.i3.270
2. Bhushan, B., Apte, U. (2019). Liver Regeneration after Acetaminophen Hepatotoxicity: Mechanisms and Therapeutic Opportunities. *Am. J. Pathol.*, 189 (4), 719–729. https://doi.org/10.1016/j.ajpath.2018.12.006
3. Sun, R., Zhao, H., Huang, S., Zhang, R., Lu, Z., Li, S., Wang, G., Aa, J., Xie, Y. (2021). Prediction of Liver Weight Recovery by an Integrated Metabolomics and Machine Learning Approach After 2/3 Partial Hepatectomy. *Front. Pharmacol.*, (12), 760474. https://doi.org/10.3389/fphar.2021.760474
4. Huang, J, Rudnick, D. A. (2014). Elucidating the metabolic regulation of liver regeneration. *Am. J. Pathol.*, 184 (2). 309–321. https://doi.org/10.1016/j.ajpath.2013.04.034
5. De la Fuente, I. M., Cortés, J. M., Valero, E., Desroches, M., Rodrigues, S., Malaina, I., Martínez, L. (2014). On the dynamics of the adenylate energy system: homeorhesis vs homeostasis. *PloS One*, 9(10), e108676. https://doi.org/10.1371/journal.pone.0108676
6. Camici, M., Allegrini, S., Tozzi, M. G.(2018). Interplay between adenylate metabolizing enzymes and AMP-activated protein kinase. *FEBS J.*, 285 (18), 3337–3352. https://doi.org/10.1111/febs.14508
7. Kopylchuk, H. P., Nykolaichuk, I. M., Ursatyi, M. S. (2024). Age-dependence of biochemical manifestations of hepatotoxicinjury in rat exposed to xenobiotics of various genesis. *Biological systems*, 16(1), 3–13. https://doi.org/10.31861/biosystems2024.01.003
8. Kopylchuk, H., Voloshchuk, O. (2024). Adenine nucleotide content and activity of AMP catabolism enzymes in the kidney of rats fed on diets with different protein and sucrose content. *Studia Biologica*, 18(3), 57–68. https://doi.org/10.30970/sbi.1803.783
9. Mitchell, C., Willenbring, H. (2008). A reproducible and well-tolerated method for 2/3 partial hepatectomy in mice. *Nature protocols*, 3(7), 1167–1170. https://doi.org/10.1038/nprot.2008.80
10. Crumm, S., Cofan, M., Juskeviciute, E., Hoek, J. B.(2008). Adenine nucleotide changes in the remnant liver: An early signal for regeneration after partial hepatectomy. *Hepatology*, 48(3), 898–908. https://doi.org/10.1002/hep.22421
11. Kataoka, M., Tanaka, A., Ikai, I., Yamaoka, Y., Ozawa, K., Chance, B. (1993). Kinetic analysis of ATP-synthetase and ATPase in regenerating rabbit liver. *Eur. Surg. Res.*, 25(2), 91–97. https://doi.org/10.1159/000129262
12. Wilson, D. F. (2017). Oxidative phosphorylation: regulation and role in cellular and tissue metabolism. *J. Physiol.*, 595 (23), 7023–7038. https://doi.org/10.1113/JP273839
13. Ramachandran, A., Jaeschke, H. (2019). Acetaminophen hepatotoxicity: A mitochondrial perspective. *Adv. Pharmacol.*, 85. 195–219. https://doi.org/10.1016/bs.apha.2019.01.007