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CYTOCHROME P450 ENZYMES ACTIVITY IN RAT LIVER UNDER CONDITIONS OF TOXIC INJURY AND PARTIAL HEPATECTOMY

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The unique liver ability for reparative regeneration plays a decisive role in restoring its homeostatic potential. However, in certain clinical situations, in particular, due to the damage caused by toxicants of a medicinal origin, the regenerative response may be impaired. Uncontrolled use of nonsteroidal anti-inflammatory drug paracetamol (acetaminophen, APAP) is among the leading causes of acute liver failure. The study focuses on evaluating of p-hydroxylase, N-demethylase, N-oxygenase activity of cytochrome P450 (CYP) enzymes as well as the CYP content in the microsomal fraction of the liver of rats subjected to partial hepatectomy following acute acetaminophen-induced toxic injury. White non-linear rats were divided into two groups: with partial hepatectomy (resection of 2/3 of liver tissue) and with partial hepatectomy following oral acetaminophen administration for 2 days at a dose of 1250 mg/kg b. w. Experimental data were obtained at 0 (control), 24, 48, 72 and 168 h after hepatectomy. The regeneration process at the early stages after partial hepatectomy in animals that were not exposed to APAP injury was accompanied by the suppression of aniline p-hydroxylase and dimethylaniline N-demethylase activity, along with a simultaneous decrease in cytochrome P450 content against the background of a compensatory increase of N-oxygenase activity. Liver tissue recovery after partial hepatectomy in animals with APAP injury was characterized by an increase in cytochrome P450 content along with concurrent activation of aromatic hydroxylation, N-dealkylation, and N-oxidation reactions throughout the entire regenerative period. The data obtained indicate the initiating of competing pathways of acetaminophen detoxification and/or toxification at different time intervals during the process of liver reparative regeneration.

K e y w o r d s: cytochrome P450, p-hydroxylation, N-demethylation, N-oxidation, partial hepatectomy, acetaminophen, toxic injury, microsomal fraction, liver.

According to modern concepts, the problem of progressive impairments in liver functional activity under the influence of toxic agents is of particular importance. In restoring the homeostatic potential of liver cells after the damaging effects of hepatotoxic substances, infectious and metabolic diseases, or tissue loss, the unique ability of the organ for reparative regeneration plays a decisive role. The liver's capacity for compensatory regeneration enables segmental resections of hepatobiliary tumors as well as transplantation. However, in certain clinical situations, the regenerative response may be impaired, for instance, due to the presence of hepatopathies (liver cirrhosis) and/or damage caused by toxicants of a medicinal origin (DILI) [1-3].

According to resources such as LiverTox, the Drug-Induced Liver Injury Network (USA), and EudraVigilance (Netherlands), which focus their activities on preventing the development of drug-induced liver injury, the persistence of the trend in DILI occurrence is confirmed due to the increasing risk of uncontrolled use of nonsteroidal anti-inflammatory drugs, particularly paracetamol (acetaminophen, APAP). Acetaminophen-induced injury is among the leading causes of acute liver failure. In certain cases, liver transplantation remains the only treatment option (20% of cases) [4-7].

It should be noted that the mechanisms of a healthy liver's regeneration after resection are described in considerable detail in scientific sources [1, 8]. Nevertheless, there is no information on the

specific features of liver regeneration mechanisms under conditions of partial hepatectomy following toxic injury caused by APAP. As is well known, the implementation of the hepatoxenobiotic effect of APAP is driven by the functioning of multi-stage signaling pathways. With the intake of therapeutic doses, acetaminophen is primarily (approximately 60-90%) metabolized in the liver during phase II biotransformation through sulfate or glucuronide conjugation, followed by the excretion of conjugates (APAP-gluc, APAP-sulfate) by the kidneys. A fairly small proportion (5-15%) of APAP is converted through the functioning of the enzymatic cycle of the heme-thiolate protein superfamily, cytochromes P450 (CYP450), during phase I biotransformation [6, 9]. The formation of the CYP450 complex with substrates primarily occurs due to hydrophobic interactions after the reduction of heme Fe^{3+} and the formation of a high-spin form of the hemoprotein (type I difference spectra) or through the association of substrate amino groups directly with heme iron (type II difference spectra) [10].

A characteristic manifestation of APAP hepatotoxicity processes is the impairment of its detoxification due to the saturation of glucuronidation/sulfation pathways. As a result, there is an enhanced production of the reactive N-acetyl-p-benzoquinoneimine (NAPQI) via N-oxidation, in contrast to the formation of 3-hydroxy-acetaminophen (3-OH-APAP) via hydroxylation mediated by CYP450. The metabolic bioactivation of acetaminophen and the formation of NAPQI are primarily carried out by the CYP1A2, CYP2E1, and CYP3A4 isoforms against the background of the depletion of glutathione reserves. Accordingly, the extensive generation of the reactive acetaminophen metabolite by cytochrome P450 determines the efficiency of the functioning of the entire xenobiotic metabolism system and is critical in the occurrence of APAP-induced liver injury [6, 9, 11].

From a biochemical point of view, the establishment of markers of the functional state of the liver detoxification system under conditions of partial hepatectomy following toxic injuries is crucial for the appropriate therapy of a range of pathological conditions associated with acute drug-induced liver failure. Considering the above, the aim of the study was to evaluate the activity of cytochrome P450 enzyme systems in the liver of rats under conditions of partial hepatectomy following acetaminophen-induced toxic injury.

Materials and Methods

Animals, experimental design and procedures. The experimental studies were performed on white non-linear rats of reproductive age (140-150 days) weighing 130-160 g. The animals were placed in the vivarium of the Educational and Scientific Institute of Biology, Chemistry, and Bioresources at Yuriy Fedkovych Chernivtsi National University under standard conditions of temperature range (20–24°C), humidity, and lighting (12/12 hours). Throughout the study, the rats were kept on a complete diet with free access to water in plastic cages with sand bedding.

All manipulations with the animals were performed in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the Law of Ukraine On the Protection of Animals from Cruelty (as amended by Law No. 5456-VI of October 16, 2012). Compliance with the ethical principles of the work was certified by the Committee on Bioethics of Scientific Research at Yuriy Fedkovych Chernivtsi National University (Protocol No. 1, dated April 4, 2024).

Partial hepatectomy, involving the resection of 2/3 of the liver tissue, was carried out in the morning hours under anesthesia with pre-sterilized instruments, following the method of Mitchell and Willenbring [12]. This surgical technique involves sequential ligation and aseptic resection of the left lateral and median liver lobes. Narcotic sleep was initiated with thiopental sodium at a dose of 7 mg/kg. Sterile surgical silk “IGAR” (Ukraine) was used for liver tissue ligation. The resected liver parts subsequently served as controls for comparison with the regenerating liver (0 hours). After partial hepatectomy, internal and external sutures were applied using sterile surgical material “Kapron B” (Ukraine). Before each surgical intervention, quartz treatment (UV disinfection) of the room was performed. Modeling of acute toxic injury was performed by oral administration of acetaminophen (LD_{50} = 2402 mg/kg) using the gastric intubation method at the calculated rate of 1250 mg/kg for 2 days in the form of a suspension in a 2% starch gel solution [13].

The animals were randomly divided into two groups: Group I – rats that underwent partial hepatectomy (C/PH); Group II – rats with acetaminophen-induced injury that underwent partial liver tissue resection (TI/PH). The experimental animals were removed from the experiment with adherence

to the requirements of current ethical standards. Further experimental studies were conducted at 0 (control), 24, 48, 72 and 168 hours after partial hepatectomy. In each of the three replicates (*n*) of each group of experimental rats, 25 animals were used. The mortality rate of control animals after partial hepatectomy (C/PH) was 13%, while in rats with acetaminophen-induced injury after partial hepatectomy (TI/PH), this indicator was 40%.

The microsomal fraction of the rat liver homogenate was obtained by differential centrifugation (Heraeus Biofuge Stratos, USA) [14]. The protein content in the microsome suspension was determined using the Bradford method.

The determination of *p*-hydroxylase activity of cytochrome P450 was carried out on the basis of the rate of NADPH-dependent *p*-hydroxylation of aniline, established by the amount of *p*-aminophenol formed. The absorption of the experimental samples was measured at λ = 630 nm using a CARY 60 spectrophotometer (USA). The determination of N-demethylase activity of cytochrome P450 consists in the assessment of the rate of NADPH-dependent demethylation of dimethylaniline by the amount of formaldehyde formed at λ = 412 nm [15]. The determination of N-oxygenase activity of cytochrome P450 was performed by evaluating the rate of dimethylaniline oxidation based on the amount of the colored compound N-nitrosodimethylaniline at λ = 420 nm [16].

The content of cytochrome P450 was determined by the method of Omura and Sato, which involves spectrophotometric measurement of the difference in the absorption spectra of the oxidized and reduced forms of cytochrome P450. In order to form the reduced CYP–CO complex, the experimental samples were bubbled with CO for 1 min [14]. Carbon monoxide was obtained by adding concentrated CH_2O_2 to concentrated H_2SO_4. To purify carbon monoxide from residual oxygen, the gas was passed through a pyrogallol solution. To reduce cytochrome P450, $Na_2S_2O_6$ was added to the experimental samples. The absorption measurements of the experimental samples were performed at λ = 450 nm (absorption maximum) and λ =490 nm (absorption minimum), taking into account the molar extinction coefficient of cytochrome P450 of 91 $mmol/l^{-1} \cdot cm^{-1.}$

The determination of the inactivation rate of cytochrome P450, which involves the conversion of cytochrome P450 into the inactive form (cytochrome P420), was performed by recording the differential absorption spectra of reduced carboxy-complexes of hemoproteins and was calculated based on the difference in absorption values at λ = 420 nm and λ = 450 nm ($\Delta A_{420-450}$), taking into account the protein content in mg [14].

Statistical analysis of the experimental results was performed in the GraphPad Prism 8.0.1 program (GraphPad Software, San Diego, California, USA) using two-way analysis of variance (ANOVA) with Tukey's post hoc test. The relationship between the indicators was assessed using the Pearson correlation coefficient. Differences between groups were considered statistically significant at $P < 0.05$. Data were expressed as mean ± SEM.

Results and Discussion

The efficiency of biotransformation of xenobiotics and endogenous metabolites is directly related to the cytochrome P450-containing oxygenase system of the endoplasmic reticulum. CYP enzymes catalyze the monooxygenation of a wide range of substrates, primarily through hydroxylation, epoxidation, N-, O-, or S-dealkylation, and N- and S-oxidation [14, 17].

The results of the studies showed that the early stages of liver regeneration after partial hepatectomy in control rats (C/PH) were accompanied by a decrease in microsomal aniline *p*-hydroxylase activity of cytochrome P450 in the period of 24 h by 65% ($P < 0.05$) and in the period of 48 h by 47% ($P < 0.01$) compared to the control values at 0 h (Fig. 1, *A*). During the assessment of oxidative N-dealkylation reactions, a decrease in the level of N-demethylase activity in the microsomal fraction of rat liver cells in the C/PH group by 42% ($P < 0.05$) compared to the control was recorded only in the period of 24 h after tissue resection of this organ (Fig. 1, *B*).

It is known that the liver's unique ability to regenerate is a complexly regulated system that is triggered and controlled by a number of pro-inflammatory mediators with cross-interactions between biochemical pathways. The release of pro-inflammatory cytokines and growth factors (IL-6, IL-1β, TNF-α, and HGF) during the activation of the regenerative cascade in response to acute injury after partial hepatectomy is primarily focused on eliminating damaged cells [1, 18, 19].

In recent years, during studies of the metabolic response of the body to inflammation using animal models, a decrease in the expression of CYP enzymes in liver tissues has been noted [20]. There-

fore, the results we obtained regarding the decrease in p-hydroxylase and N-demethylase activities of CYP450 in the group of control rats after partial hepatectomy are consistent with the studies conducted by Fujino et al., where a reduction in CYP mRNA levels was demonstrated during the initiation of inflammatory stimuli at the early stages of liver parenchymal recovery [21].

At the same time, the established suppression of *p*-hydroxylation and N-demethylation reactions of cytochrome P450 at the early stages of regeneration can be considered a stress-induced response to changes caused by surgical liver tissue resection or a consequence of the regenerative reaction. Theoretical evidence indicates that during the implementation of a set of regenerative reactions in the remaining liver tissue, specific metabolic changes develop and cease with the restoration of the hepatostat. Metabolic reorganization that arises in response to acute liver injury is an important physiological determinant of proper regeneration [22]. In view of the above, it can be assumed that after partial hepatectomy in control animals (C/PH), the prioritization of functional support for hepatocyte proliferation is compensated by the suppression of specialized functions, particularly the activity of the detoxification system.

During the 24-hour period of remodeling of damaged liver tissue, against the background of a decrease in the intensity of hydroxylation and N-dealkylation reactions of cytochrome P450 in the microsomal fraction of the rat liver in the C/PH group, we recorded an increase in dimethylaniline N-oxygenase activity of the cytochrome P450 system, which exceeds the control value at 0 h by 52% *(P* < 0.05) (Fig. 2). Although a significant number of aliphatic, alicyclic, aromatic, and mixed substrates of cytochrome P450 are metabolized through oxidative N-dealkylation involving the carbon atom of the alkyl group that is bonded to the nitrogen atom (α-carbon atom), a portion of primary, secondary, and most commonly tertiary amines undergo metabolic N-oxidation via nitrogen atoms, resulting in the formation of N-oxide products. N-oxidative biotransformation of functional groups in nitrogen-containing compounds may be accompanied by both metabolic toxification and detoxification of the parent amines through the production of highly polar metabolites [23-25]. This suggests that the increase in N-oxygenase activity of the cytochrome P450 system under these experimental conditions is likely occurring in response to an elevated need for the conversion of endogenous amine-substituted compounds. One

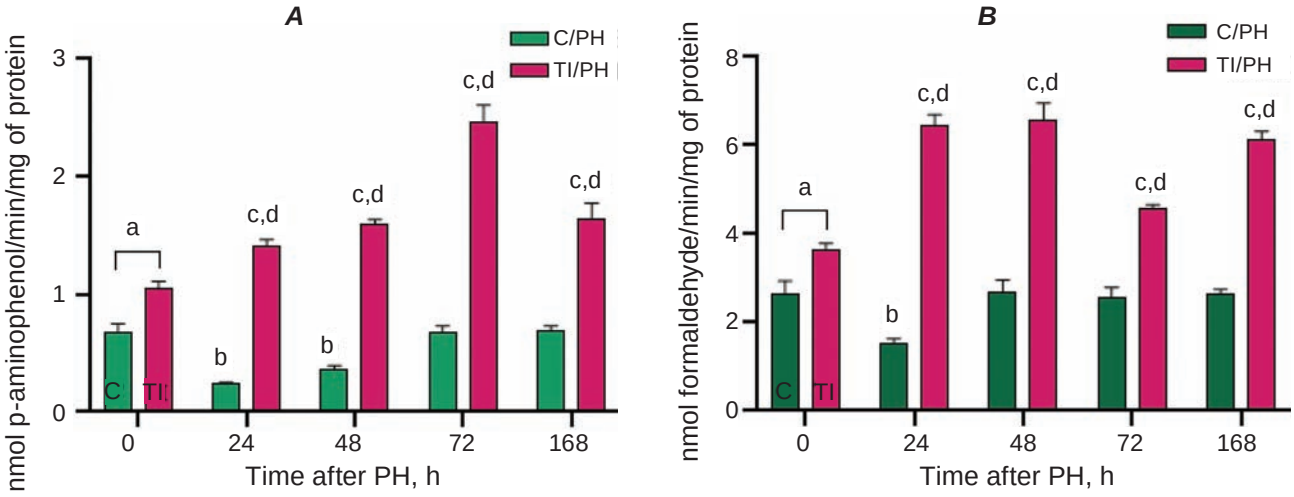

*Fig. 1. p-Hydroxylase activity (**A**) and N-demethylase activity (**B**) of cytochrome P450 in the microsomal fraction of the livers of rats under conditions of partial hepatectomy following acetaminophen-induced toxic injury. C/PH – control animals that underwent partial hepatectomy; TI/PH – rats that underwent partial liver resection after acute acetaminophen-induced toxic injury. a, b, c – 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 compared to the C group at 0 h; c – statistically significant difference of the TI/PH groups compared to the TI group at 0 h; d – statistically significant difference of the TI/PH groups compared to the C/PH groups*

such substrate, considering the structural features of the pyrrole ring, may be bilirubin. As shown by several independent studies, cytochrome P450, along with UDP-glucuronosyltransferase 1A1 (UGT1A1), is considered an alternative enzyme for bilirubin degradation [26, 27]. The CYP1A2 isoform is directly involved in the biotransformation of aromatic and heterocyclic amines. The induction of CYP1A2 promotes the maintenance of homeostasis in cells by enhancing the metabolic clearance of substrates, which may lead to a reduction in blood plasma bilirubin levels and the excretion of hydroxylated metabolites in bile [27-29]. Taking into account the presented information, it can be assumed that the intensification of N-oxidation reactions of the microsomal cytochrome P450 system at the early stages of liver regeneration in control rats after partial hepatectomy is the result of enhanced bilirubin degradation aimed at reducing the risk of developing endogenous intoxication in the remote period.

Thus, the redistribution of marker activities of cytochrome P450 in liver microsomes of control animals after partial hepatectomy occurs through a decrease in *p*-hydroxylase and N-demethylase activities of cytochrome P450 during the 24-hour and 48-hour periods. Instead, a compensatory increase in microsomal N-oxygenase activity of cytochrome P450 in the C/PH group animals is observed only during the 24-hour period. At the final stages of liver parenchyma recovery, the levels of the studied activities return to control values.

Despite its favorable pharmacological properties, the use of acetaminophen may impair hepatobiliary function, leading to dose-dependent or idiosyncratic liver toxicity. This side effect is often asymptomatic and reversible. However, in 20% of cases with acetaminophen consumption, severe symptomatic cytolytic/cholestatic injury develops, requiring liver transplantation or resulting in fatal outcomes. Therefore, even after discontinuation of therapy, acetaminophen may remain at therapeutic levels in the body due to bioaccumulation [4-7, 9, 30].

At the same time, liver regeneration in rats with acetaminophen-induced injury after segmental organ resection (TI/PH) was accompanied by completely opposite changes in marker activities of the cytochrome P450 system compared to the experimental control group (C/PH). As the liver parenchyma recovered in rats of the TI/PH group, an increase in aniline *p*-hydroxylase, dimethylani-

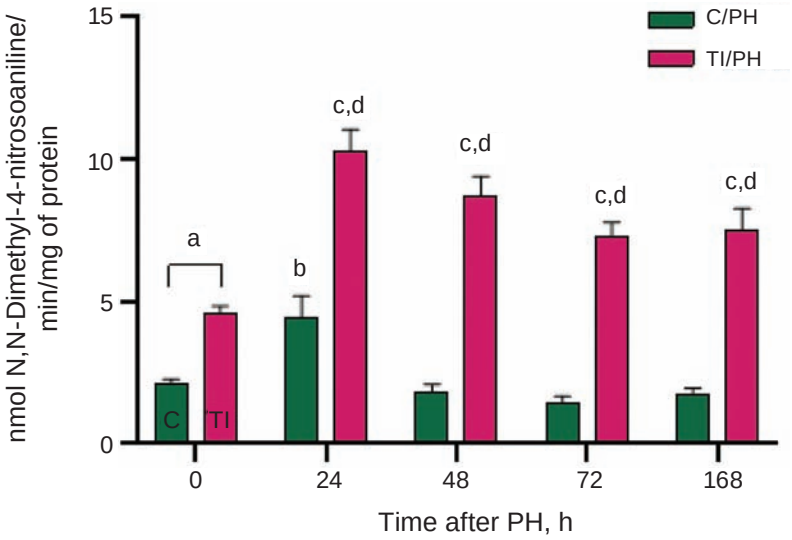

Fig. 2. N-oxygenase activity of cytochrome P450 in the microsomal fraction of the livers of rats under conditions of partial hepatectomy following acetaminophen-induced toxic injury. C/PH – control animals that underwent partial hepatectomy; TI/PH – rats that underwent partial liver resection after acute acetaminophen-induced toxic injury. a, b, c – 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 compared to the C group at 0 h; c – statistically significant difference of the TI/PH groups compared to the TI group at 0 h; d – statistically significant difference of the TI/PH groups compared to the C/PH groups

line N-demethylase and N-oxygenase activities of cytochrome P450 in the microsomal fraction was recorded compared to the values of TI animals at 0 h. It is important to note that in the early stages of regeneration (24 and 48 h) in the livers of animals with acetaminophen-induced injury, N-dealkylation (by 43–45%, $P < 0.001$) and N-oxidation (by 47-56%, $P < 0.001$) reactions predominate, while at the 72-hour time point p-hydroxylation reactions are maximally activated (by 57%; $P < 0.001$) with the involvement of cytochrome P450 (Fig. 1, 2).

It is known that cytochrome P450-dependent enzymes catalyze the monooxygenation of substrates predominantly through an identical sequence of reactions. Hydroxylation and N-dealkylation are two of the most important types of CYP metabolic reactions occurring through different mechanisms. The mechanism of the hydroxylation reaction is a hydrogen abstraction/oxygen rebound mechanism, known as the Groves rebound mechanism. Whereas in CYP-mediated N-dealkylation, there are two alternative mechanisms: one is hydrogen atom transfer, and the other is single electron transfer. The reaction barriers for hydrogen atom transfer in N-dealkylation are significantly lower than those for hydroxylation, which may explain why the predictability of N-dealkylation is higher than that of hydroxylation [31, 32]. Perhaps this is why during the 24-hour and 48-hour periods in liver microsomes of rats with acetaminophen-induced injury after partial hepatectomy, an increase in N-demethylase activity occurs (Fig. 1, *B*). After all, each stage of the catalytic cycle of CYP450 provides a unique spectral signature that reflects changes in the oxidation states or spin state of Fe^{3+} in the heme, deformation of the porphyrin ring, or alteration of dioxygen fragments [31]. The formation of type I complexes occurs when nonpolar substrates (such as dimethylaniline) interact with the low-spin form of cytochrome P450. The spectral changes that occur are associated with the transition of the Fe3+ atom in the heme from the initial six-coordinated low-spin position to a five-coordinated high-spin position, bound to the substrate, and the type I binding site is located in the hydrophobic region of the CYP apoenzyme [10, 14, 33].

At the same time, the 72-hour phase of the liver regenerative process after partial organ resection in animals with acetaminophen-induced injury is characterized by maximal activation of aniline *p*-hydroxylation reactions in microsomes (Fig. 1, *A*). This fact is very important considering the potential importance of the CYP450 system in acetaminophen metabolism, which is due to the ability of certain P450 isoenzymes to compensate for changes in the activity of others. We observed this pattern in the activation of N-dealkylation and *p*-hydroxylation reactions during different time intervals of liver parenchyma recovery in animals of the TI/PH group. Specifically, compounds that cause type II spectral changes include nitrogen-containing substances (aniline-type substrate binding as in the case of *p*-hydroxylation reactions), which are capable of coordinating with the heme iron of cytochrome P450 through basic nitrogen atoms (substrate amino groups). In such complexes, the heme iron is in a six-coordinated low-spin state, and the ligand in the sixth coordination position of the heme iron (oxygen-binding site) is replaced by the nitrogen of the substrates [10, 14, 33].

In the study by Yang et al., it is noted that cytochrome P450 isoenzymes exhibit chemo- and regioselectivity, enabling them to metabolize APAP both through an aromatic hydroxylation mechanism, forming the non-toxic catechol 3-hydroxyacetaminophen, and by oxidizing this pharmaceutical xenobiotic to N-acetyl-p-benzoquinoneimine, a hepatotoxic metabolite [11]. We observed that in the microsomal fraction of the liver in rats of the TI/PH group, during the 24-hour period, a maximal increase in N-oxygenase activity (by 55%, $P < 0.001$) was recorded, which was maintained at a high level throughout the entire liver recovery period (Fig. 2). Considering that demethylation is regarded as a type of N-dealkylation of xenobiotics through oxidation [16, 23, 34], the simultaneous increase in N-demethylase and N-oxygenase activities in liver microsomes at the early stages of regeneration, as we have established, likely indicates enhanced production of NAPQI rather than the non-toxic 3-OH-APAP. Cytochrome P450-dependent biotransformation of APAP in phase I predominantly occurs with the involvement of the isoforms CYP1A2, CYP2A6, CYP2C9, CYP2D6, CYP2E1, and CYP3A4. Experimental studies have shown that CYP2E1 is involved in the production and accumulation of the majority of reactive NAPQI in hepatocytes. Then, if dominance in NAPQI generation is not observed in the reaction cycle of the CYP1A2, CYP3A4, and CYP2C9 isoforms, CYP2A6 is characterized by the formation of the non-toxic 3-OH-APAP in a 3:1 ratio [11, 35]. The obtained results regarding the functional redistribution of marker activities of cytochrome P450 in liver microsomes of animals with toxic injury after partial hepatectomy allow for predicting the likeli-

hood of launching competing pathways involved in the metabolic clearance (*p*-hydroxylation) and bioactivation (N-oxidation) of acetaminophen at different time periods of organ recovery.

One of the reasons for the changes we established in the studied enzymatic activities of cytochrome P450 in liver microsomes of animals after partial hepatectomy may be a change in the quantitative content of cytochrome P450. As the obtained results indicate, in the microsomal fraction of the liver of animals subjected to partial hepatectomy (C/PH), only during the 24-hour and 48-hour phases, a decrease in cytochrome P450 content by 65% ($P < 0.01$) and 55% ($P < 0.05$), respectively, was observed compared to the control values at 0 h (Fig. 3), which correlates with a reduction in p-hydroxylase ($r = 0.84$ – 24 h; $r = 0.77$ – 48 h, $P < 0.05$) and N-demethylase ($r = 0.65$ – 24 h, $P < 0.05$) activities during the specified periods. The established fact is likely associated with the suppression of the liver's ability to produce cytochrome P450 due to the immaturity of the synthetic function of regenerated hepatocytes at the early stages of regeneration. Nevertheless, considering the importance of inflammatory responses in initiating the cascade of liver regeneration events, the decrease in cytochrome P450 content at the early stages of the regenerative process in animals of the C/PH group may result from the need to redirect the functional potential of the liver's synthetic apparatus toward the production of acute-phase proteins required to control the systemic inflammatory response under conditions of partial hepatectomy [36-38]. As the liver parenchyma recovers in animals of the C/PH group, the level of microsomal cytochrome P450 returns to control levels (Fig. 3).

Regarding the group of animals with acetaminophen-induced injury after partial hepatectomy (TI/PH), we demonstrated an increase in cytochrome P450 content in liver microsomes during the 72-hour regenerative period (24 h – 54%; 48 h – 47%; 72 h – 41%, $P < 0.001$) compared to the values of animals in the TI group at 0 h (Fig. 3). One of the most important features of the components of the monooxygenase system, particularly cytochromes P450, is their ability for selective induction under the action of external stimulus-reactions, in the role of which the substrate-xenobiotics of the induced isoforms predominantly function [39-41]. Since, in our case, the xenobiotic load of APAP preceded partial hepatectomy, the phenomenon of CYP induction can be explained by two mechanisms: intensification of de novo biosynthesis and/or enhancement of the catalytic activity of individual multiple forms of CYP, which statistically significantly aligns with the results of our studies in the TI/PH animal group.

The ability for highly specific induction of CYP is considered a significant component of the adaptive response to foreign compounds entering the cell. This leads to enhanced biotransformation and, typically, suppression of the activity of xenobiotic compounds. In some cases, CYP induction results in the toxification of xenobiotics (protoxicants) through the formation of reactive metabolites that are more toxic than the parent compounds [39-41]. An example of the latter is the selective induction of CYP isoenzymes in relation to acetaminophen, with a predominance of the bioactivation of the pharmaceutical xenobiotic and the formation of the hepatotoxic N-acetyl-*p*-benzoquinoneimine. Considering the inducible nature of acetaminophen metabolism

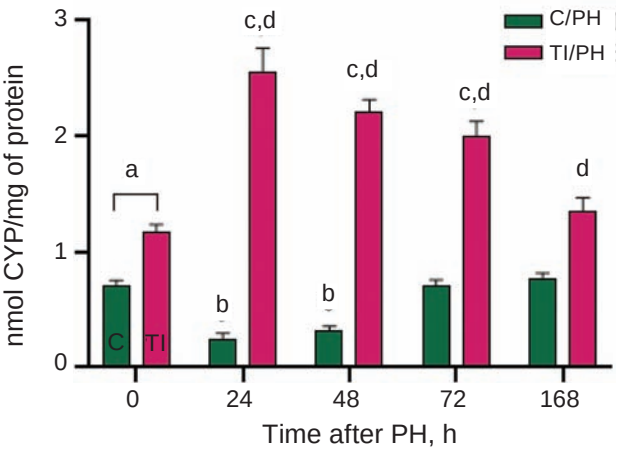

Fig. 3. Cytochrome P450 content in the microsomal fraction of the livers of rats under conditions of partial hepatectomy following acetaminophen-induced toxic injury. C/PH – control animals that underwent partial hepatectomy; TI/PH – rats that underwent partial liver resection after acute acetaminophen-induced toxic injury. a, b, c – 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 compared to the C group at 0 h; c – statistically significant difference of the TI/PH groups compared to the TI group at 0 h; d – statistically significant difference of the TI/PH groups compared to the C/PH groups

under the action of cytochromes P450, the recorded increase in marker monooxygenase activities and CYP content is logical in view of the need for detoxification of high doses of the xenobiotic in animals of the TI/PH group. An additional prerequisite for the increase in cytochrome P450 content in liver microsomes of animals with toxic injury after partial hepatectomy (TI/PH) may be that prior administration of supratherapeutic doses of APAP promotes the accumulation of functionally active CYP3A due to a reduction in the intensity of its proteasomal degradation through polyubiquitination [42].

An analytical review of the literature suggests that the metabolic activation of a xenobiotic compound to form an electrophilic reactive intermediate, as in the case of acetaminophen toxification to NAPQI, can lead to covalent modification of nucleophilic amino acid residues (lysine, serine, threonine, tyrosine, or cysteine) in the CYP apoprotein, alkylation of the heme prosthetic group in situ with its destruction, or coordination with heme iron, which is accompanied by irreversible or quasi-irreversible inactivation of cytochrome P450 [43, 44]. One of the important structural features of cytochrome P450 system enzymes is the coordination of the fifth ligand, a thiolate anion of cysteine (Cys), with the heme iron in the functionally active form of cytochrome P450. Spectral changes caused by the formation of the inactive conformation of CYP450 – cytochrome P420 – are associated with the implementation of two mechanisms: protonation of cysteine thiolate, leading to thiol ligation with heme iron and the formation of a neutral charge, or switching of the proximal ligand from cysteine thiolate to the imidazole group of histidine (His) [45].

However, when analyzing the results of studies on cytochrome P450 inactivation in the microsomal fraction of the liver under conditions of partial hepatectomy, both in control animals (C/PH) and in rats with acetaminophen-induced injury (TI/PH), the formation of the inactive form of CYP450 – cytochrome P420 – was not detected (Fig. 4). It can be assumed that the preservation of the functionally active conformation of cytochrome P450 in animals of the TI/PH group, even against the background of enhanced NAPQI formation, reflects the implementation of a mechanism of selective induction of cytochromes P450 to counteract acetaminophen accumulation under conditions of administration of its toxic doses prior to partial liver tissue resection.

Conclusions. Thus, the course of the regeneration process after partial liver resection in animals that were not exposed to the effect of toxic injury at the early stages of parenchymal recovery (24 and 48 h) is accompanied by the suppression of aniline *p*-hydroxylase and dimethylaniline N-demethylase activities of cytochrome P450, along with a simultaneous decrease in its content in microsomes against the background of a compensatory increase in CYP450 N-oxygenase activity during the 24-hour phase.

At the same time, liver tissue recovery after partial hepatectomy in animals with toxic injury is characterized by an increase in cytochrome P450 content, along with concurrent activation of aromatic hydroxylation, N-dealkylation, and N-oxidation reactions throughout the entire regenerative period. During the 24-hour and 48-hour periods, N-demethylation and N-oxidation processes predominate, with an intensification of *p*-hydroxylation

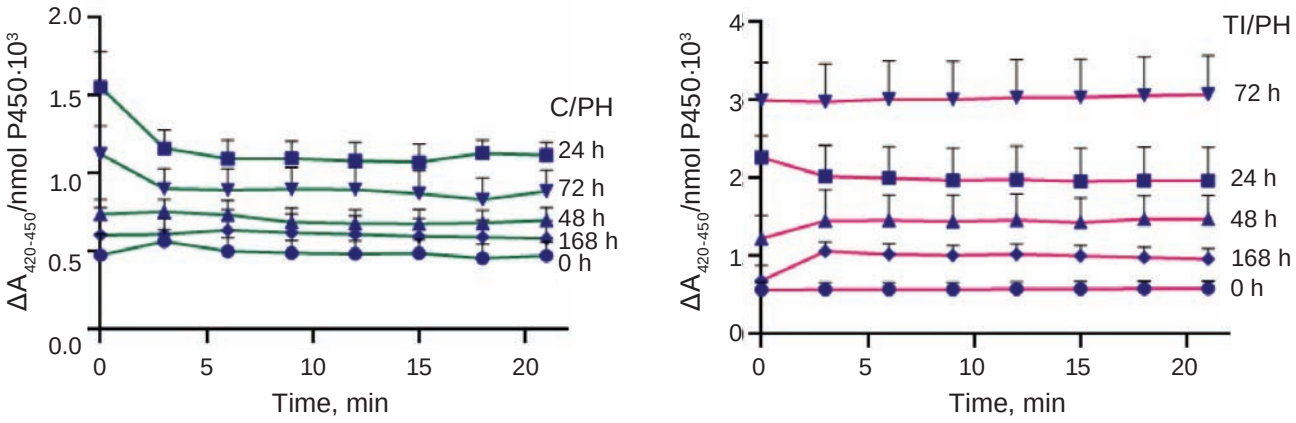

Fig. 4. The rate of cytochrome P450 inactivation in the microsomal fraction of the livers of rats under conditions of partial hepatectomy following acetaminophen-induced toxic injury

reactions exclusively during the 72-hour period. The functional redistribution of microsomal monooxygenase activities of cytochrome P450 in animals with toxic injury after partial liver resection provides an opportunity to forecast the likelihood of initiating competing pathways of acetaminophen detoxification and/or toxification through *p*-hydroxylation or N-oxidation at different time intervals during the regeneration process.

From a biochemical perspective, the remote intensification of cytochrome P450 *p*-hydroxylation reactions during the 72-hour phase, against the background of activated N-oxidation processes at the early stages of liver parenchyma recovery, may serve as a marker of the functional state of the liver detoxification system under conditions of partial hepatectomy following toxic injuries, which is of critical importance for the correction of a number of pathological conditions associated with acute drug-induced liver failure.

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АКТИВНІСТЬ ЕНЗИМІВ ЦИТОХРОМУ P450 В ПЕЧІНЦІ ЩУРІВ ЗА УМОВ ТОКСИЧНОГО УРАЖЕННЯ ТА ЧАСТКОВОЇ ГЕПАТЕКТОМІЇ

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Унікальна здатність печінки до репаративної регенерації відіграє вирішальну роль у відновленні її гомеостатичного потенціалу. Однак, у деяких клінічних ситуаціях, наприклад, через наявність ушкоджень, спричинених токсикантами медикаментозної природи, регенеративна відповідь може бути порушена. Безконтрольне використання нестероїдного протизапального препарату парацетамолу (ацетамінофен, APAP) є однією з провідних причин гострої печінкової недостатності. Робота присвячена оцінці *p*-гідроксилазної, N-деметилазної та N-оксигеназної активності цитохрому P450 (CYP), а також вмісту CYP у мікросомальній фракції печінки щурів за умов часткової гепатектомії після гострого ацетамінофен-індукованого токсичного ураження. Білих нелінійних щурів розподілили на дві групи: з частковою гепатектомією (резекція 2/3 тканин печінки) та з частковою гепатектомією після перорального введення APAP протягом 2 діб у дозі 1250 мг/кг. Експериментальні дані аналізували на 0 (контроль), 24, 48, 72 і 168 год після гепатектомії. Регенераційний процес на ранніх етапах після часткової гепатектомії у тварин, які не зазнали дії APAP-індукованого ураження супроводжується пригніченням активності анілін *p*-гідроксилази та диметиланілін N-деметилази, одночасно зі зменшенням вмісту цитохрому P450 на тлі компенсаторного підвищення активності N-оксигенази. Відновлення тканин печінки після часткової гепатектомії у тварин з APAP-індукованим ушкодженням характеризувалося підвищенням вмісту цитохрому P450 з одночасною активацією реакцій ароматичного гідроксилювання, N-деалкілування та N-окислення протягом усього періоду регенерації. Отримані дані свідчать про ініціацію конкуруючих шляхів детоксикації та/або токсифікації ацетамінофену в різні часові проміжки процесу репаративної регенерації печінки.

Ключові слова: цитохром P450, *p*-гідроксилювання, N-деметилювання, N-окислення, часткова гепатектомія, ацетамінофен, токсичне ураження, мікросомна фракція, печінка.

References

1. Rodimova S, Mozherov A, Elagin V, Karabut M, Shchechkin I, Kozlov D, Krylov D, Gavrina A, Bobrov N, Zagainov V, Zagaynova E, Kuznetsova D. Effect of hepatic pathology on liver regeneration: the main metabolic mechanisms causing impaired hepatic regeneration. *Int J Mol Sci.* 2023; 24(11): 9112.
2. Bae B, Kang K, Song SK, Chung CW, Park Y. Is partial hepatectomy a curable treatment option

for hepatocellular carcinoma accompanied by cirrhosis? A meta-analysis and cure model analysis. *Ann Hepatobiliary Pancreat Surg.* 2022; 26(1): 47-57.
3. Córdoba-Jover B, Arce-Cerezo A, Ribera J, Pauta M, Oró D, Casals G, Fernández-Varo G, Casals E, Puntes V, Jiménez W, Morales-Ruiz M. Cerium oxide nanoparticles improve liver regeneration after acetaminophen-induced liver injury and partial hepatectomy in rats. *J Nanobiotechnology.* 2019; 17(1): 112.
4. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury. Acetaminophen. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases. Available at https://www.ncbi.nlm.nih.gov/books/NBK548162/ (Updated 2016 Jan).
5. Popiołek I, Piotrowicz-Wójcik K, Porebski G. Hypersensitivity Reactions in Serious Adverse Events Reported for Paracetamol in the EudraVigilance Database, 2007–2018. *Pharmacy (Basel).* 2019; 7(1): 12.
6. Liao J, Lu Q, Li Z, Li J, Zhao Q, Li J. Acetaminophen-induced liver injury: Molecular mechanism and treatments from natural products. Front Pharmacol. 2023; 14: 1122632.
7. Yoon E, Babar A, Choudhary M, Kutner M, Pyrsopoulos N. Acetaminophen-induced hepatotoxicity: a comprehensive update. *J Clin Transl Hepatol.* 2016; 4(2): 131-142.
8. Gilgenkrantz H, Collin de l'Hortet A. Understanding Liver Regeneration: From Mechanisms to Regenerative Medicine. *Am J Pathol.* 2018; 188(6): 1316-1327.
9. Mazaleuskaya LL, Sangkuhl K, Thorn CF, FitzGerald GA, Altman RB, Klein TE. PharmGKB summary: pathways of acetaminophen metabolism at the therapeutic versus toxic doses. *Pharmacogenet Genomics.* 2015; 25(8): 416-426.
10. Golovenko MYa, Larionov VB, Valivodz IP. Spectral characteristics of cytochrome P450 in the interaction with propoxazepam and its metabolite. *Med Clin Chem.* 2023; 25(2): 12-19. (In Ukrainian).
11. Yang Y, Wong SE, Lightstone FC. Understanding a substrate's product regioselectivity in a family of enzymes: a case study of acetaminophen binding in cytochrome P450s. *PLoS One.* 2014; 9(2): e87058.
12. Kopylchuk GP, Shmarakov IO, Buchkovska IM. Superoxide anion radical and nitric oxide generation intensity in mouse liver after partial hepatectomy. *Biological Systems.* 2011; 3(3): 206-211. (In Ukrainian).
13. Kopylchuk HP, Nykolaichuk IM, Lylyk IS. Indexes of citrulline metabolism in rat liver under the toxic injury against the background of alimentary protein deficiency. *Ukr Biochem J.* 2020; 92(1): 113-119.
14. Shymanskyi IO, Ketsa OV, Marchenko MM, Veliky MM. Liver cytochrome P450-hydroxylation system of tumor-bearing rats under the influence of ω-3 polyunsaturated fatty acids and vitamin D_3. *Ukr Biochem J.* 2018; 90(4): 36-44.
15. Shmarakov IO, Borschovetska VL, Blaner WS. Hepatic Detoxification of Bisphenol A is Retinoid-Dependent. *Toxicol Sci.* 2017; 157(1): 141-155.
16. 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 Sci Gastrointest Disord*. 2022; 5(4): 39-48.
17. Röder A, Hüsken S, Hutter MC, Rettie AE, Hanenberg H, Wiek C, Girhard M. Spotlight on CYP4B1. *Int J Mol Sci.* 2023; 24(3): 2038.
18. Hora S, Wuestefeld T. Liver Injury and Regeneration: Current Understanding, New Approaches, and Future Perspectives. *Cells.* 2023; 12(17): 2129.
19. Hadjittofi C, Feretis M, Martin J, Harper S, Huguet E. Liver regeneration biology: Implications for liver tumour therapies. *World J Clin Oncol.* 2021; 12(12): 1101-1156.
20. Stavropoulou E, Pircalabioru GG, Bezirtzoglou E. The Role of Cytochromes P450 in Infection. *Front Immunol.* 2018; 9: 89.
21. Fujino C, Sanoh S, Tateno C, Ohta S, Kotake Y. Coordinated cytochrome P450 expression in mouse liver and intestine under different dietary conditions during liver regeneration after partial hepatectomy. *Toxicol Appl Pharmacol.* 2019; 370: 133-144.
22. Huang J, Rudnick DA. Elucidating the metabolic regulation of liver regeneration. *Am J Pathol.* 2014; 184(2): 309-321.
23. Eh-Haj BM. Metabolic N-dealkylation and N-oxidation as elucidators of the role of alkylamino moieties in drugs acting at various receptors. *Molecules*. 2021; 26(7): 1917.

24. Rydberg P, Jørgensen MS, Jacobsen TA, Jacobsen AM, Madsen KG, Olsen L. Nitrogen inversion barriers affect the N-oxidation of tertiary alkylamines by cytochromes P450. *Angew Chem Int Ed Engl.* 2013; 52(3): 993-997.
25. Hlavica P. N-oxidative transformation of free and N-substituted amine functions by cytochrome P450 as means of bioactivation and detoxication. *Drug Metab Rev.* 2002; 34(3): 451-477.
26. Kim SD, Morgan L, Hargreaves E, Zhang X, Jiang Z, Antenos M, Li B, Kirby GM. Regulation of Cytochrome P450 2a5 by Artemisia capillaris and 6,7-Dimethylesculetin in Mouse Hepatocytes. *Front Pharmacol.* 2021; 12: 730416.
27. Li X, Yu D, Jie H, Zhou H, Ye H, Ma G, Wan L, Li C, Shi H, Yin S. Cytochrome P450 1A2 Is Incapable of Oxidizing Bilirubin Under Physiological Conditions. *Front Pharmacol.* 2019; 10: 1220.
28. Zhou SF, Wang B, Yang LP, Liu JP. Structure, function, regulation and polymorphism and the clinical significance of human cytochrome P450 1A2. *Drug Metab Rev.* 2010; 42(2): 268-354.
29. Gunes A, Dahl ML. Variation in CYP1A2 activity and its clinical implications: influence of environmental factors and genetic polymorphisms. *Pharmacogenomics.* 2008; 9(5): 625-637.
30. Caparrotta TM, Antoine DJ, Dear JW. Are some people at increased risk of paracetamol-induced liver injury? A critical review of the literature. *Eur J Clin Pharmacol.* 2018; 74(2): 147-160.
31. Mak PJ, Denisov IG. Spectroscopic studies of the cytochrome P450 reaction mechanisms. *Biochim Biophys Acta Proteins Proteom.* 2018; 1866(1): 178-204.
32. Dai ZR, Ai CZ, Ge GB, He YQ, Wu JJ, Wang JY, Man HZ, Jia Y, Yang L. A Mechanism-Based Model for the Prediction of the Metabolic Sites of Steroids Mediated by Cytochrome P450 3A4. *Int J Mol Sci.* 2015; 16(7): 14677-14694.
33. Peng CC, Cape JL, Rushmore T, Crouch GJ, Jones JP. Cytochrome P450 2C9 type II binding studies on quinoline-4-carboxamide analogues. *J Med Chem.* 2008; 51(24): 8000-8011.
34. Markey SP. Pathways of Drug Metabolism. Principles of Clinical Pharmacology (Second Edition). Academic Press, 2007. P. 143-162.
35. Yang Y, Pan L, Lightstone FC, Merz KM Jr. The Role of Molecular Dynamics Potential of Mean Force Calculations in the Investigation of Enzyme Catalysis. *Methods Enzymol.* 2016; 577: 1-29.
36. Tanaka M, Miyajima A. Liver regeneration and fibrosis after inflammation. *Inflamm Regen.* 2016; 36: 19.
37. Morgan ET, Li-Masters T, Cheng PY. Mechanisms of cytochrome P450 regulation by inflammatory mediators. *Toxicology.* 2002; 181-182: 207-210.
38. Morgan ET. Regulation of cytochrome p450 by inflammatory mediators: why and how? *Drug Metab Dispos.* 2001; 29(3): 207-212.
39. Zhu BT. On the general mechanism of selective induction of cytochrome P450 enzymes by chemicals: some theoretical considerations. *Expert Opin Drug Metab Toxicol.* 2010; 6(4): 483-494.
40. Loerracher AK, Braunbeck T. Inducibility of cytochrome P450-mediated 7-methoxy-coumarin-O-demethylase activity in zebrafish (*Danio rerio*) embryos. *Aquat Toxicol.* 2020; 225: 105540.
41. Manikandan P, Nagini S. Cytochrome P450 Structure, Function and Clinical Significance: A Review. *Curr Drug Targets.* 2018; 19(1): 38-54.
42. Ohtsuki Y, Sanoh S, Santoh M, Ejiri Y, Ohta S, Kotake Y. Inhibition of cytochrome P450 3A protein degradation and subsequent increase in enzymatic activity through p38 MAPK activation by acetaminophen and salicylate derivatives. *Biochem Biophys Res Commun.* 2019; 509(1): 287-293.
43. Hollenberg PF, Kent UM, Bumpus NN. Mechanism-based inactivation of human cytochromes p450s: experimental characterization, reactive intermediates, and clinical implications. *Chem Res Toxicol.* 2008; 21(1): 189-205.
44. Mirzaei MS, Ivanov MV, Taherpour AA, Mirzaei S. Mechanism-Based Inactivation of Cytochrome P450 Enzymes: Computational Insights. *Chem Res Toxicol.* 2021; 34(4): 959-987.
45. Sun Y, Zeng W, Benabbas A, Ye X, Denisov I, Sligar SG, Du J, Dawson JH, Champion PM. Investigations of heme ligation and ligand switching in cytochromes P450 and P420. *Biochemistry.* 2013; 52(34): 5941-5951.