UDC 615.33+615.065
DOI: 10.15587/2519-8025.2021.250223

STUDY OF HEPATOTOXINS INFLUENCE IN VITRO ON BASIC BIOCHEMICAL INDICATORS OF LIVER FUNCTIONAL STATE

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An antimicrobial drug of the fluoroquinolone group, ciprofloxacin, is widely used in Ukraine. However, some researchers have noted the probable hepatotoxicity of this drug with prolonged use or use of high doses of ciprofloxacin. The ***aim*** *of this study was to compare the effects of carbon tetrachloride, as a classical model of hepatocyte injury, with the effects of ciprofloxacin.*

The aim of the study was to investigate the biochemical parameters of the liver when simulating toxic damage to hepatocytes with carbon tetrachloride or ciprofloxacin.

Materials and methods*. The study was carried out on isolated rat hepatocytes, in whose culture medium carbon tetrachloride or ciprofloxacin was added. After incubation in the supernatant and cell homogenate, the activities of marker enzymes of cytolysis were determined: ALT, AST, γ-GTP, LF, LDH, DC and MDA.*

Results*. The introduction of ciprofloxacin into the culture of hepatocytes at a concentration of LC50 caused changes in biochemical parameters similar to those caused by carbon tetrachloride. Thus, an increase in ALT, AST, γ-GTP, LF, LDH, DC and MDA was observed when CCl4 or ciprofloxacin was added to the culture.*

Conclusion*. Incubation of rat hepatocytes with carbon tetrachloride or ciprofloxacin caused an increase in the level of enzymes and lipid peroxidation products. These parameters are indicators of gross changes in cells, which are the result of impaired keto acid formation, impaired redox reactions, impaired glycogen production*

Keywords: *carbon tetrachloride, ciprofloxacin, rat hepatocytes, cytolysis enzymes*

How to cite:
Maloshtan, L., Storozhenko, G., Galuzinska, L., Fylymonenko, V., Sadiq, O. R. (2021). Study of hepatotoxins influence in vitro on basic biochemical indicators of liver functional state. ScienceRise: Biological Science, 4 (29), 15–18. doi: http://doi.org/10.15587/2519-8025.2021.250223

1. Introduction

There is a steady increase in liver disease [1]. This is a consequence of the unfavourable environmental situation, increasing the impact of various chemical agents on humans and more [2]. For the treatment of patients with this pathology there is a wide arsenal of hepatoprotectors of different classes, but some - with little-studied mechanisms of action, the study of features and mechanisms which must be continued after their introduction [3, 4].

In vitro models of liver pathology of various etiologies in pharmacological studies used tetrachloromethane CCl_4 and ciprofloxacin. The first substance is hepatotoxin, which is traditionally used to simulate acute and chronic toxic and drug-induced liver damage [3–5]. Ciprofloxacin is an antibiotic that is usually prescribed because of its broad spectrum of action and good safety profile. However, recent data suggest that it has a tendency to cause idiosyncratic drug-induced liver damage [6]. Liver enzymes have been shown to be impaired after ciprofloxacin in some patients and to improve clinical and biochemical parameters after discontinuation of this antibiotic [6]. An in vitro study of inhibition of human retinal pigment epithelial cell proliferation by ciprofloxacin showed that the concentration of ciprofloxacin, which inhibits growth by 50 % (IC50), is 14.1 μg/ml, and complete inhibition of cell growth was observed at 83 μg/ml [7].

With the defeat of CCl_4 in the membranes of the endoplasmic reticulum of hepatocytes is the formation of free radical products $Cl^{\cdot}$, $CCl_{3\cdot}$, which cause the destruction of the membrane structures of hepatocytes and disruption of synthetic processes in the liver [8].

Ciprofloxacin, like other fluoroquinolones, is known to be associated with low levels (1–3 %) of elevated serum enzymes during therapy [9]. These abnormalities are usually mild, asymptomatic and temporary, and disappear even with continued therapy. However, more importantly, ciprofloxacin has been associated with rare but sometimes severe [10, 11] and even fatal cases of acute liver disease [12].

The aim of the research – comparison of the effects of carbon tetrachloride - as a classical modelling of hepatocyte damage with the effects of ciprofloxacin on cell biochemical parameters.

2. Materials and methods

The experimental part of the work was carried out based on the educational and scientific training center for

medical and biological research of the Educational and Scientific Institute of Applied Pharmacy of the National University of Pharmacy. The experiment was performed on hepatocytes of male Wistar rats weighing 180–220 g, in compliance with the requirements of the NUPh Bioethics Commission (Minutes No. 7 of 20.10.2021) and complied with ARRIVE recommendations and was performed in accordance with the British Animal Act (scientific procedures) 1986, guided by EU Directive 2010/63 / EU on the protection of animals used for scientific purposes.

Isolation of hepatocytes was performed by the Petrenko method [13]. CCl_4 and ciprofloxacin were used to reproduce the toxic lesion on hepatocyte culture. Hepatocyte culture was incubated in Dulbecco's Modified Egles Minimal Essential Medium (DMEM, Sigma) under standard conditions of 5 % CO_2 / 95 % O_2 at 37 °C [14]. Toxins were added in concentrations corresponding to LC_{50} (for CCl_4 – 0,0065 μmol / ml of culture medium, for ciprofloxacin – 0,2218 μmol/ml of culture medium). After incubation, hepatocyte survival was assessed.

Supernatant or cell homogenate was used to assess biochemical parameters. The evaluation was performed 48 hours after incubation with hepatotoxins, and the biochemical status of hepatocytes in culture was evaluated. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), malonic dialdehyde (MDA), diene conjugates (DC), alkaline phosphatase (APh), lactate dehydrogenase (LDH), and gamma-glutamyltranspeptidase (γ-GTP). To determine ALT, AST, LF, LDH, γ-GTP used sets of CJSC "Diacon-DS", and the determination of MDA, DC was performed according to standard methods [15, 16].

One-way Anova analysis of variance and Student's t-test were used to compare the obtained data. Differences between groups were considered statistically significant at $p \leq 0.05$.

3. Research results and their discussion

Model liver damage by various toxins is accompanied by cytolytic syndrome and intensification of lipid peroxidation (LPO) [17], in the serum of animals there is an increase in the activity of cytolysis marker enzymes such as ALT, AST, APh, γ-GTP and others. In addition, the rate of formation of LPO products (DC, MDA, etc.) is increasing.

ALT-enzyme that catalyzes transamination (transfer of the amino group from L-alanine to α-ketoglutaric acid). The enzyme is present in the cytosolic fraction of hepatocytes. It enters the bloodstream by destroying the internal structure of hepatocytes and increasing the permeability of cell membranes, which is characteristic of liver pathology [18, 19].

AST-enzyme that inversely catalyzes transamination (transfer of an amino group from L-aspartic acid to α-ketoglutaric acid). Most AST is detected in mitochondria (80 %), 20 % – in the cytosolic fraction. In inflammatory processes, including hepatitis, AST is released from the cytosol and enters the bloodstream [18, 19].

γ-GTP is an enzyme involved in the formation of glutathione (contains residues of 3 amino acids – glutamic, cysteine and glycine), which plays an important role in redox reactions. Violation of its formation leads to loss of hematopoietic function of the liver. Determination of γ-GTP is often carried out in relation to APh, AST [20].

The enzyme LDH is an element of the glycolytic cycle that inversely catalyzes the oxidation of L-lactate to pyruvic acid. The greatest activity of this enzyme is observed in the liver, kidneys, blood cells. Elevated levels of the enzyme LDH in the serum are a diagnostic indicator of various types of liver pathology (toxic lesions, various forms of hepatitis, mechanical liver damage, etc.) [20].

The APh enzyme of the phosphomonoesterase group is a catalyst for the cleavage of phosphoric acid from organic compounds. In the liver it is localized in the microvilli of the bile ducts, the sinusoidal membrane, to a lesser extent in the cytosol of hepatocytes. Increased serum APh indicates the development of cholestasis, infectious or tumor processes, hepatitis, cirrhosis, etc. [17].

Under the conditions of any liver lesion there is an increased formation of LPO products, which leads to cellular lesions [5, 18, 19, 20]. Peroxide radicals react with fatty acid molecules, which leads to the formation of hydroperoxides and new peroxide radicals [21]. Some of the toxic metabolites are DC and MDA. These substances easily react with amino groups (proteins, nucleic acids, lipids) to form products that increase the viscosity and stiffness of membranes, reduce cell lability and accelerate the aging process [5, 20].

The effect of CCl_4 on the level of biochemical parameters was studied at LC50 equal to 0.0065 μmol / ml.

When the toxin was introduced into the hepatocyte culture, an increase in the activity of transferases and the activity of markers of LPO intensity was observed (Table 1).

Table 1

The degree of change in biochemical parameters in cell culture under the influence of CCl_4

Indicator	Intact series	CCl_4
ALT (U/l)	2.0±0.071	3.2±0.086 *
AST (U/l)	3.8±0.051	7.9±0.086 *
γ-GTP (U/l)	2.0±0.066	4.0±0.071 *
APh (U/l)	6.0±0.100	12.0±0.093 *
LPG (U/l)	10.8±0.141	20.9±0.135 *
DC (10^{-3} c.u./ mg of protein)	2.01±0.027	3.79±0.021 *
MDA (μm/l)	7.11±0.017	13.81±0.024 *

*Note: * – deviation of the indicator is significant in relation to the group of intact control, $p \leq 0.05$; CCl_4 – carbon tetrachloride at a concentration of 0.0065 μmol/ml*

In the series of cell culture under the influence of CCl_4 at a concentration of 0.0065 μmol / ml, there was a significant increase in biochemical parameters compared to the intact series of cells, which was expressed in an increase in ALT 1.6 times, AST – 2.08 times, γ- GTP – 2.0 times, APh – 2.0 times, LDH – 1.9 times, DC – 1.89 times, MDA – 1.94 times. The obtained results indicate an increase in the level of transferases and LPO products after the introduction of CCl_4 into the culture of hepatocytes, because the values of the studied indicators probably increased in comparison with the intact series.

In the study of changes in biochemical parameters in cell culture under the influence of ciprofloxacin, the antibiotic was used at a concentration of LC50 0.2218 μmol/ml.

The introduction of ciprofloxacin into hepatocyte culture caused changes in biochemical parameters characteristic of liver pathology (Table 2).

Table 2

The degree of change in the main biochemical parameters in cell culture under the influence of ciprofloxacin

Indicator	Intact series	CIP
ALT (U/l)	1.4±0.093	3.1±0.086 *
AST (U/l)	4.0±0.081	8.2±0.080 *
γ-GTP (U/l)	1.9±0.086	3.8±0.108 *
APh (U/l)	5.9±0.108	11.9±0.075 *
LPG (U/l)	11.0±0.068	21.9±0.116 *
DC (10^{-3} c.u./ mg of protein)	1.69±0.014	3.2±0.012 *
MDA (μm/l)	6.8±0.014	12.41±0.019 *

*Note: * – deviation of the indicator is significant in relation to the group of intact control, p≤0.05; CIP – ciprofloxacin at a concentration of 0.2218 μmol/ml*

Under the influence of ciprofloxacin on hepatocyte culture at a concentration of 0.2218 μmol / ml, the following changes in biochemical parameters were observed: ALT increased 2.2 times, AST – 2.1 times, γ-GTP – 2.0 times, APh – in 2.0 times, LDH – 1.99 times, DC – 1.89 times, MDA – 1.83 times. All indicators are probably higher compared to the control. Such changes indicate the intensification of LPO processes and intensification of the cytolysis process.

Changes in biochemical parameters that occurred in the group where the antibiotic was introduced, compared to the intact series were plausible.

4. Discussion of research results

Experimental data indicate that the introduction into the incubation environment of hepatocytes CCl_4 caused a significant increase in the level of enzymes markers of cytolysis compared with control cells. These data are in good agreement with the results obtained by other researchers [5, 18], and indicate the partial death of hepatocytes with the release of their contents into the culture medium [20]. The addition of high doses of ciprofloxacin caused an increase in hepatocytes such as ALT and AST, which indicates hepatocyte damage [18, 19]. In addition, the action of ciprofloxacin increases the rate of formation of LPO – DC and MDA products in hepatocytes, which can also cause cell damage [17, 18]. Having conducted a comparative study of two xenobiotics CCl_4 and ciprofloxacin in terms of their effect on the biochemical parameters of rat hepatocytes, it can be concluded that ciprofloxacin has fewer toxic properties compared to the studied parameters. We came to this conclusion due to the fact that the dose of CCl_4 (0.0065 μmol / ml) was 100 times lower than the dose of ciprofloxacin – 0.2218 μmol / ml. Also, the dose of ciprofloxacin was several times higher than the therapeutic dose in humans.

In general, the hepatotoxicity study of high doses of ciprofloxacin revealed effects similar to the toxic lesions of CCl_4 hepatocytes. Given the effectiveness of ciprofloxacin treatment and the normalization of liver function, after discontinuation of this drug [6], to avoid severe liver damage, the use of hepatoprotectants may be suggested in case of long-term use or high doses of this antibiotic.

Study limitations. The study is limited to the use of a single dose of xenobiotics on a single model object – hepatocytes, which opens up the prospect of further research to study the dose-dependent effect of selected substances.

Prospects for further research. There are insufficient data in the literature on the hepatotoxic properties of different doses of ciprofloxacin, there are only isolated reports of liver damage in patients taking this antibiotic. Therefore, the direction of further research is to study the different doses, duration of action of this antibiotic and search for appropriate hepatoprotectors.

5. Conclusions

The obtained results indicate that the introduction into the incubation medium of hepatocytes ciprofloxacin in the concentration of LC_{50} there were changes in biochemical parameters characteristic of liver pathology. There was an increase in ALT, AST, γ-GTP, APh, LDH, DC and MDA when introduced into the culture of hepatocytes CCl_4 or ciprofloxacin. Thus, the data in Tabs. 1 and 2 indicate that when introduced into the culture of CCl_4 cells or ciprofloxacin, there is an increase in LPO products and marker enzymes of cytolysis: ALT increased from 1.6 to 2.2 times, AST – 2, 1 time, γ-GTP – 2.0 times, APh – 2.0 times, LDH – from 1.9 to 2.0 times, DC – 1.89 times, MDA – from 1.83 to 1, 94 times. Increased levels of enzymes and LPO products are indicators of gross changes in cells, which are the result of impaired keto acid formation, impaired redox reactions, the process of glycogen formation, impaired membrane permeability.

Conflict of interests

The authors declare there is no conflict of interests.

Financing

The study was performed without financial support.

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Received date 02.11.2021
Accepted date 14.12.2021
Published date 30.12.2021

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