UDC 615.273:576.5

Influence of biologically active substances on synthesis function and cellular destruction of hepatocytes *in vitro*

A.V. Shkuropat[1,2], V.A. Shvets[1], I.V. Golovchenko[1], Ya.M. Prosiannikova[1]

[1]Kherson State University;
[2]PI "Kherson Academy of Continuing Education"; e-mail: shvetsvika88@gmail.com

The aim of the study was to reveal the effect of biologically active substances on the synthesis activity and cellular destruction of hepatocytes in vitro. Liver sections were prepared for investigation and placed in culture vials with DMEM nutrient medium with 15% calf serum, glucose, and antibiotics (streptomycin and penicillin). Liver sections were incubated for 14 days with interleukin-2 (roncoleukin) at a concentration of 5000 IU/ml and 7500 IU/ml, and erythropoietin (epobiocrine, "Biopharma", USA) at a concentration of 13 IU/ml (high concentration), 6.5 IU/ml (medium concentration) and 1.3 IU/ml (low concentration) and without stimulation (control cultures). Synthesis activity and cellular destruction of hepatocytes were studied by determining the protein content, alanine aminotransferase and aspartate aminotransferase activity in the supernatant of liver organ cultures on the 7th and 14th days of incubation. It was found that culturing organotypic cultures with IL-2 did not affect the synthesis function of hepatocytes, but reduced aspartate aminotransferase activity throughout the culture period. At a concentration of 7500 IU/ml IL-2 showed a weak hepatotoxic effect. It was found that erythropoietin at a medium concentration had a hepatoprotective effect, at a high concentration it suppressed the synthesis activity of hepatocytes and contributed to the destruction of the cytoplasmic membrane of cells. At low concentrations, erythropoietin increased the synthesis activity of liver cells but caused an increase in the activity of aminotransferases, this may indicate both mass cell death and intensification of amino acid transamination processes. It was established that interleukin and its inhibitor cause biological effects when incubated with organotypic cultures.
Key words: interleukin-2; erythropoietin; organ cultures of the liver; biochemical parameters of the liver.

INTRODUCTION

The search for various approaches in the treatment of hepatitis is an urgent problem today. Mortality rates from hepatitis of various etiologies occupy a leading position around the world, being in the top ten among the causes of death [1–3]. In terms of severity, complications of liver disease include liver fibrosis/cirrhosis, hepatocellular carcinoma, etc. The most effective way to treat these pathologies is a liver transplant. The question of finding new treatment approaches arises due to the existing shortage of donor organs, the frequency of transplant rejection, and the high cost of treatment [2, 4, 5]. One of such approaches is cell therapy methods.

There are several directions of cell therapy to treat liver diseases, for example, the recovery of liver tissue using liver stem cells. Gumerova et al., Pallett et al., Mazur et al., have works in this direction [1, 4, 6]. Another direction is the creation of systems for purification of blood plasma of patients with hepatitis using cultures of hepatocytes of different cell lines.

The problem of any direction of cellular therapy of liver diseases will be the creation of an appropriate microenvironment of hepatocytes in vitro and the stimulation of the processes of proliferation, differentiation or individual functions of liver cells. The creation of a microenvironment of liver cells to prevent the loss of their characteristic phenotype is an equally important issue [6–9].

Organotypic cultures are appropriate for studying the parameters of liver cells in vitro. This method of culturing allows to preserve cell

diversity and relationships between cultured cells and tissue matrix, i.e. reproducing the tissue phenotype [5, 6]. As a result, cells almost do not lose their phenotype and it is possible to study the morphological and functional changes of cells in their response to various agents. Since cells of organotypic cultures do not practically lose their phenotype, preserve the microenvironment, cellular diversity of individual tissues, the morphological and functional changes in such cultures will be close to those in vivo [4, 10, 11].

The study of the pleiotropic action of cytokine drugs in vitro and in vivo is relevant because it allows to create and influence the existing microenvironment of cells [3, 6, 12]. Interleukin-2 (IL-2) is a pro-inflammatory cytokine that directly regulates the formation of effector immune cells [12]. However, there are data in the scientific literature that indicate other, non-immune, effects of IL-2 [13, 14]. Erythropoietin is a hormone that regulates not only the formation of erythrocytes, but also has other pronounced pleiotropic effects. Thus, it was shown that erythropoietin caused a decrease in the activity of ALT, TNF-α and MDA in rats with hepatic ischemia, i.e.it had a hepatoprotective effect[15]. In the study [10], it was found that erythropoietin at medium and high concentrations inhibited the division of mouse fibroblasts in vitro, but at all concentrations it contributed to faster cell adaptation.

The aim of the study was to determine the effect of biologically active drugs on the synthetic activity and cellular destruction of hepatocytes in vitro.

METHODS

The study was performed within the initiative theme of the Department of Human Biology and Immunology "Study of the impact of cytokines in vitro", state registration number 0120U101313. Organotypic cultures of mouse liver were studied to assess the effect of IL-2 and erythropoietin onthe hepatocyte function. Males of white laboratory outbred mice were selected for the study. The study followed general ethical principles for the care and use of laboratory animals: "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 2005).

After euthanasia, liver sections were made and placed in culture vials with DMEM nutrient medium with 15% calf serum, glucose and antibiotics (streptomycin and penicillin). Liver sections were incubated for 14 days with IL-2 (roncoleukin, PJSC "Biotech", USA) at concentrations of 5000 IU/ml, 7500 IU/ml, erythropoietin (epobiocrine, "Biopharma", USA) at concentrations of 13 IU/ml (high concentration), 6.5 (medium concentration) and 1.3 IU/ml (low concentration) and without any stimulation (control cultures).

The culture supernatant wasexamined for protein content to assess the synthetic function of the liver. Studies were performed on the 7th and 14th days of incubation. Protein was determined by biuret method ("Genesis", Ukraine). The determination of the activity of aminotransferases (alanine aminotransferase – ALT and aspartate aminotransferase – AST) in the culture supernatant was studied by the unified Reitman and Frenkel's method ("Diagnosticum", Hungary) to assess the degree of destruction of hepatocytes [13, 14].

Numerical data were processed by methods of variation statistics using software packages "Microsoft Excel 2010" and "Statistica 9.0". Significance of differences between the indicators of the studied organ cultures was determined using a two-sample Wilcoxon test.

RESULTS AND DISCUSSION

Synthetic liver function was assessed by determining the amount of protein in the cell culture supernatant. When culturing liver organotypic cultures with IL-2 at concentrations of 5000 and 7500 IU/ml, no significant changes in protein concentration compared to the control

ones were observed (Fig. 1). On day 14 of culturing, the protein concentration with IL-2 stimulation in vitro decreased in comparison with the day 7 ($P \leq 0.05$), however, the protein concentration also decreased in control cultures ($P \leq 0.05$). Thus, the protein concentration with and without IL-2 stimulation was approximately at the same level.

In the study of the amount of protein in organotypic cultures of the liver when cultured with erythropoietin, a dose-dependent change was observed compared to the control cultures. The amount of protein produced by liver cells under a low concentration of erythropoietin (1.3 IU/ml) was higher compared to control cultures ($P \leq 0.05$). At a medium (6.5 IU/ml) and a high concentration (13 IU/ml) of erythropoietin the amount of protein produced by liver cells was lower compared to control cultures ($P \leq 0.05$). However, culturing with medium erythropoietin concentration had a more pronounced effect of reducing the amount of protein compared to control cultures. On the 14th day of culturing, the protein concentration in cultures cultured with low erythropoietin concentration decreased compared to day 7, but the indicator remained higher than in control cultures ($P \leq 0.05$). When culturing liver organotypic cultures with medium erythropoietin concentration the protein concentration increased significantly on day 14 of culturing compared to day 7 of culturing and started exceeding similar indicators of control cultures. On the 14th day of culturing stimulation with erythropoietin at a high concentration led to a decrease in the protein concentration in the culture compared to day 7, thus, these indicatorsdid not differ from the control ones.

Markers of cellular tissue destruction of the liver are the level of activity of aminotransferases, namely alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The level of aminotransferase activity is a specific marker of the state of liver cells. ALT and AST are enzymes that catalyze the transfer of amino groups between amino acids [14]. As it is an intracellular enzyme, an increase in ALT and AST activity in the cell culture supernatant will be associated with an increase in cell destruction. ALT is a cytoplasmic enzyme and its activity increases with the damage to the cytoplasmic membrane. AST is a mitochondrial enzyme, its activity increases with the mass death of hepatocytes and the release of mitochondrial enzymes from cells [4, 10].

On day 7 IL-2 at a concentration of 7500 IU/ml caused an increase in ALT activity compared to the indicators of control cultures ($P \leq 0.05$; Fig. 2). When culturing liver organotypic

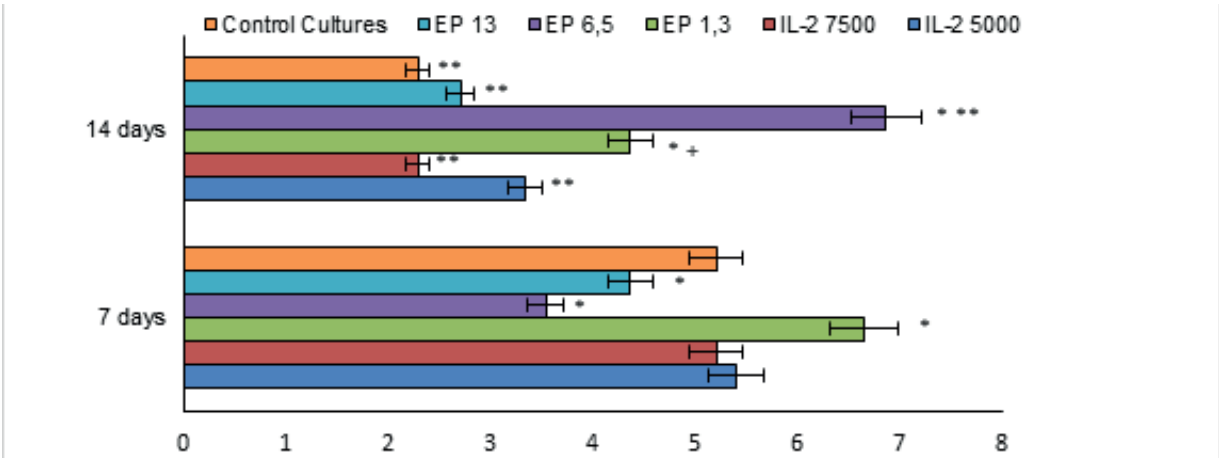

Fig. 1. The protein concentration of the supernatant of liver organ cultures under conditions of stimulation of IL-2 and erythropoietin (g/l). Note: *statistically significant difference between the indicators of different liver organ cultures within one day of the study at $P \leq 0.05$; **statistically significant difference between the indicators of the same organ cultures on different days of the study at $P \leq 0.05$

cultures with IL-2 at a concentration of 5000 IU/ml, no changes in ALT activity compared to control cultures were observed. On day 14 there was a decrease in ALT activity ($P \leq 0.05$) under IL-2 stimulation in both concentrations and without stimulation, but there was a to show higher indicators in cultures under stimulation in comparison with control cultures.

After 7 days of culturing IL-2 stimulation of organ cultures of the liver caused a significant decrease in AST activity ($P \leq 0.05$; Fig. 3), however, further culturing led to a change in AST activity under IL-2 stimulation. On the 14th day of culturing, stimulation of IL-2 at a concentration of 5000 IU/ml caused an increase in AST activity compared to culturing on the 7th day ($P \leq 0.05$), whereas stimulation of IL-2 at a concentration of 7500 IU/ml, led to an increase of AST activity in organotypic cultures of the liver, but this difference did not reach the level of statistical significance. The AST activity of control cultures during 14-day culturing remained unchanged. On the 14th day of the study the AST activity of liver organotypic cultures, cultured with IL-2 at a concentration of 5000 IU/ml, exceeded indicators of control cultures ($P \leq 0.05$). While indicators of the cultures, cultured with IL-2 at a concentration of 7500 IU/ml, on the 14th day of culturing remained lower than those of control cultures.

The ratio of AST and ALT enzymes in organotypic liver cultures under conditions of IL-2 stimulation shows a relative increase in ALT activity with a decrease in AST activity on the 7th day of culturing. On the 14th day of the study in organotypic cultures cultured with IL-2 at a concentration of 5000 IU/ml, the ratio of AST/ALT was approximately 1, whereas in cultures cultured with IL-2 at a concentration of 7500 IU/ml there was a relative predominance of ALT activity. As ALT activity did not differ from that of the control on the 7th day of the experiment, when cultured with IL-2 at a concentration of 5,000 IU/ml, a decrease in AST activity compared to ALT activity may be associated with its less formation. Although, when cultured with IL-2 at a concentration of 7500 IU/ml, ALT activity exceeded the indicatorsof control cultures, AST activity showed lower indicators than those of control cultures, which may indicate both the release of cytoplasmic enzymes in cell culture supernatant due to cytoplasmic membrane destruction and reducing the formation of AST. Culturing of liver organotypic cultures with erythropoietin had the following effect on aminotransferase activity. ALT activity treated with a medium concentration of erythropoietin in vitro, both on the 7th day and the 14th day of culturing did not differ from that in control cultures. At low and high concentrations of erythropoietin on the 7th day of culturing there was a decrease in ALT activity ($P \leq 0.05$), but on the 14th day the level of ALT activity increased and exceeded similar

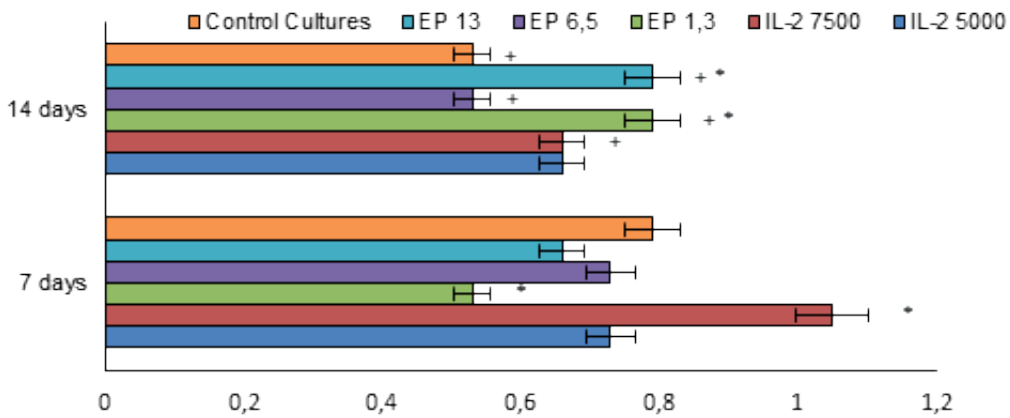

Fig. 2. Activity of alanine aminotransferase of the supernatant of organ cultures of the liver under conditions of stimulation of IL-2 and erythropoietin (μmol/(h □

indicators of control cultures ($P \leq 0.05$).

TheAST activity under the influence of erythropoietin when culturing of liver organotypic cultures varied dose-dependently. Under erythropoietin low concentrations there was an increase in the AST activity on both the 7th and 14th day ($P \leq 0.05$). Under erythropoietin medium concentrations, theAST activity did not differ from the activity AST of control cultures throughout the culture period; at high concentrations, erythropoietin caused a significant decrease in AST activity compared to control cultures ($P \leq 0.05$).

The ratio between AST and ALT activity in culturing liver organotypic cultures with erythropoietin at different concentrations was as follows: erythropoietin at medium concentrations had little effect on aminotransferase ratio, i.e. AST activity was virtually equal to ALT activity throughout the study period. When cultured with low concentrations of erythropoietin on the 7th day, there was a significant predominance of AST activity over ALT, it may indicate the destruction of liver cells and the entry of the fraction of mitochondrial enzymes in the culture supernatant. On the 14th day, there was an increase in the activity of both aminotransferases, so that their ratio was approximately 1. This indicates an increase in the release of both cytoplasmic and mitochondrial enzymes due to the destruction of biological membranes and cell destruction [2, 10]. When cultured with erythropoietin in high concentrations, a decrease in the ratio of aminotransferases was observed, i.e. a decrease in the AST activity and an increase in the ALT activity. ALT is a cytoplasmic enzyme, its activity increases due to a hepatotoxic effect of non-mitochondrial nature [4, 10].

The results of biochemical studies of the supernatant of organotypic cultures of the liver indicate that IL-2 did not affect the synthetic function of hepatocytes. At a concentration of 5000 IU/ml IL-2had no effect on the destruction of liver cells, at a concentration of 7500 IU/ml had a weak hepatotoxic effect, as indicated by increased ALT activity on day 7 of the study. AST activity decreased at both concentrations of IL-2, which may indicate a decrease in its synthesis in liver cells under the influence of IL-2.

Erythropoietin at a concentration of 13 IU/ml inhibited the synthetic function of hepatocytes and increased ALT activity, but significantly reduced the amount of AST. This may indicate an effect of hepatocytes on the plasma membrane and the release of cytoplasmic enzymes into the cell culture supernatant. Erythropoietin at a concentration of 6.5 IU/ml increased the synthetic function of liver cells and did not show hepatotoxic function on cells of organotypic cultures. Erythropoietin at a concentration of 1.3 IU/ml increased the synthetic activity of liver cells, but on the 7th day of the study there was an increase in mitochondrial enzyme activity in the supernatant of cultures, and on the 14th day there was an increase of cytoplasmic enzyme activity. It may be caused by the destruction of plasma membranes and the mass death of hepatocytes. However, according to [13, 14], an increase in the activity of both AST and ALT may be associated not only with cell destruction but also with an increase in the intensity of amino acid transamination processes and precedes fatty degeneration of the liver.

CONCLUSIONS

1. Culturing of organotypic cultures with IL-2 did not affect the synthetic function of hepatocytes but reduced AST activity throughout the culture period. At a concentration of 7500 IU/ml IL-2 showed a weak hepatotoxic effect.

2. Erythropoietin at medium concentration had a hepatoprotective effect, at high concentration, it suppressed the synthetic activity of hepatocytes and contributed to the destruction of the cytoplasmic membrane of cells. At low concentrations, erythropoietin increased the synthetic activity of liver cells but caused an increase in the activity of aminotransferases, which may indicate both mass cell death and the intensification of amino acid transamination processes.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

А.В. Шкуропат[1,2], В.А. Швець[1], І.В. Головченко[1], Я.М. Просяннікова[1]

ВПЛИВ БІОЛОГІЧНО АКТИВНИХ ПРЕПАРАТІВ НА ФУНКЦІЮ СИНТЕЗУ ТА КЛІТИННУ ДЕСТРУКЦІЮ ГЕПАТОЦИТІВ IN VITRO

[1]Херсонський державний університет;
[2]КЗ «Херсонська академія неперервної освіти»; e-mail: shvetsvika88@gmail.com

Вивчали вплив біологічно активних препаратів на синтетичну активність та клітинну деструкцію гепатоцитів in vitro. Для дослідження робили зрізи печінки та поміщали у культуральні флакони з живильним середовищем DMEM з додаванням 15%-ї телячої сироватки, глюкози та антибіотиків (стрептоміцин та пеніцилін). Інкубували протягом 14 діб з інтерлейкіном-2 (ІЛ-2, ронколейкін) у концентрації 5000 і 7500 МО/мл, еритропоетином (епобіокрин, «Biopharma», США) у високій концентрації (13 МО/мл), середній (6,5 МО/мл) та низькій (1,3 МО/мл), а також без стимуляції (контрольні культури). Досліджували синтетичну активність та клітинну деструкцію гепатоцитів за вмістом білка, активністю аланінамінотрансферази (АлАТ) та аспартатамінотрансферази (АсАТ) у супернатанті органних культур печінки на 7-й та 14-й день інкубації. З'ясували, що культивування органотипових культур з ІЛ-2 не вплинуло на синтетичну функцію гепатоцитів, проте зменшило активність АсАТ протягом усього періоду культивування. У концентрації 7500 МО/мл ІЛ-2 виявляв слабку гепатотоксичну дію. Встановлено, що еритропоетин у середній концентрації чинив гепатопротекторну дію, у високій – пригнічував синтетичну активність гепатоцитів та сприяв руйнуванню цитоплазматичної мембрани клітин, у низькій концентрації збільшував синтетичну активність клітин печінки, однак, викликав збільшення активності амінотрансфераз. Це може свідчити як про масову загибель клітин, так і про інтенсифікацію процесів трансамінування амінокислот. Було встановлено, що інтерлейкін та його інгібітор викликають біологічні ефекти при інкубуванні з органотиповими культурами.

Ключові слова: інтерлейкін-2; еритропоетин; органні культури печінки; біохімічні показники печінки.

REFERENCES

1. Mazur HM, Merlavsky VM, Manko BO, Manko VV. mPTP opening differently affects electron transport chain and oxidative phosphorylation at succinate and NAD-dependent substrates oxidation in permeabilized rat hepatocytes. Ukr Biochem J. 2020; 92(4): 14-23.
2. Iwakiri YI, Groszmann RJ. The hyperdynamic circulation of chronic liver diseases: from the patient to the molecule. Hepatology. 2006; 43(2 Suppl 1):122-31.
3. Handa P, Kowdley KV. Chemokines: potent mediators of hepatic inflammation and fibrosis in chronic liver diseases. Ann Hepatol. 2014; 13(1):152-4.
4. Golovchenko I, Bodnar A, Chaban I, Minenko O. Features of changes in enzymes of aminotansferrase in blood of women 18-21 years under conditions of use of different fitness species. Bull Chernihiv State Pedagog Inst Named after T. Shevchenko. Ser Pedagog Sci Phys Educat Sports. 2017;147(1):79-85.
5. Wang Y, Zhang A, Ye Z, Xie H, Zheng S. Bone marrow-derived mesenchymal stem cells inhibit acute rejection of rat liver allografts in association with regulatory T-cell expansion. Transplant Proc. 2009;41(10): 4352-6.
6. Pallett LJ, Davies J, Colbeck EJ, Robertson F, Hansi N, Easom NJW, et al. IL-2 high tissue-resident T cells in the human liver: Sentinels for hepatotropic infection. J Exp Med. 2017;214(6):1567-80.
7. Eliopoulos N, Zhao J, Forner K, Birman E, Young YK, Bouchentouf M. Erythropoietin gene-enhanced marrow mesenchymal stromal cells decrease cisplatin-induced kidney injury and improve survival of allogeneic mice. Mol Ther. 2011;19(11):2072-83.
8. Golovchenko IV, Hayday MI. Correlation indices of cerebral hemodinamics and electricalactivity in children with impaired motor skills. Fiziol Zh. 2016;62(1):74-82.
9. Shkuropat AV. Coherent relations in EEGs of adolescents with partial hearing loss underconditions of an orthostatic test. Neurophysiology. 2018; 50(5), 365-71.
10. Golovchenko I, Shkuropat A. Features of electrolyte exchange in the blood of women 18-21 years old in the conditions of using different types of fitness. Sci Bull Natl Sci (Biol Sci). 2020;28:33-43.
11. Golovchenko IV, Shkuropat AV. Features of cerebral circulation under conditions of motor and sensor deprivation. Fiziol Zh. 2020; 66(4):30-6.
12. Herr F, Lemoine R, Gouilleux F, Meley D, Kazma I, Heraud A, Velge-RousselF, Baron Chr, Lebranchu Y. IL-2 phosphorylates STAT5 to drive IFN-γproduction and activation of human dendritic cells. J Immunol. 2014;192(12): 5660-70.
13. Shvets V, Shkuropat A, Prosiannikova Y, Golovchenko I. Effect of Interleukin-2 on the humoral link of immunity during physical activity. J Physic Educ Sport. 2020;20(Suppl Iss 6): 3153-9.
14. Shvets VA, Shkuropat AV. Effect of interleukin-2 on antioxidant system and lipid peroxidation during physical activity. Cherkasy Univ Bull: Biol Sci Ser. 2020;2:

107-15. [Ukrainian].
15. Maksymenko O, Shkuropat A, Hasiuk O. Biological effect of erytropoetine in different concentrations on the cell culture. Ukr Zh Med Biol Sport. 2017;4(6):185-8. [Ukrainian].
16. Golovchenko IV, Gayday NI. Spatial patterns of correlations between amplitudes of the main EEG rhythms in children in the norm and with central disorders of motor activity. Neurophysiology. 2015;47(6): 459-71.

Received 12.07.2022