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ORIGINAL ARTICLE

Role of Cryopreserved Placenta Extract in Prevention and Treatment of Paracetamol-Induced Hepatotoxicity in Rats

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Abstract

Background/Aim: Drug-induced liver injury is one of the major causes of acute liver failure. Under current circumstances of the pandemic of COVID-19, the use of paracetamol which has a proven hepatotoxic effect has increased. This prompts the search for novel agents with hepatoprotective properties. The purpose of this article was to evaluate the hepatoprotective activity of cryoextract of the placenta (CEP) on the model of paracetamol-induced hepatitis.
Methods: The study was performed on 28 male rats. Acute drug liver damage was modelled by intragastric administration of paracetamol twice at a dose of 1250 mg/kg.
Results: The development of paracetamol-induced hepatitis in rats was accompanied by a 71.3 % increase ($p < 0.001$) in the content of active products of thiobarbituric acid (TBA-AP) in liver homogenates as compared with intact animals. Besides, there was a 2.1-fold ($p < 0.001$) increase of ALT activity, a 58.8 % increase ($p < 0.001$) of AST activity and a 4.2-fold ($p < 0.001$) increase of the concentration of total bilirubin as compared with intact rats. The use of cryopreserved placenta extract showed significant hepatoprotection in a rat model of paracetamol-induced hepatitis. This was demonstrated by a 2.3-fold ($p < 0.01$) increase of the antioxidant-prooxidant index, a significant ($p < 0.001$) decrease of activity of ALT (by 44.0 %) and AST (by 29.6 %), as well as by a decrease of direct bilirubin level by 52.5 % ($p < 0.001$) in animals treated with CEP as compared with rats without treatment.
Conclusion: The development of acute paracetamol-induced hepatitis in rats was associated with activation of lipid peroxidation processes in liver tissues, while CEP showed marked hepatoprotective activity in paracetamol-induced hepatitis in rats.

Key words: Cryopreserved placenta extract; Paracetamol; Liver injury; Hepatoprotection.

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Introduction

Drug-induced liver injury (DILI) is one of the most common side effects of many medications. It is primarily related to the physiological role of the liver in the elimination of xenobiotics, as well as to the spread use of generic drugs which are not always of sufficient quality.[1, 2] DILI accounts for about 10 % of the total number of patients with liver disease. The global prevalence of DILI ranges from 2.4 cases per 100,000 population per year in Great Britain to 34.2 cases per 100,000 population per year in Spain.[3, 4] The relevance of the DILI problem is confirmed by the functioning

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of networks focusing their activity on control and prevention of development of drug-associated liver injuries, eg the database "Drug Induced Liver Injury Network" in the system of the Food and Drug Administration (USA), the system "LiverTox (USA), the "HepaTox" system (China), etc.[5-7]

Metabolic transformation and subsequent conjugation and excretion of hydrophilic products of drug biotransformation with bile and urine take place in liver. Among the drugs that most frequently cause the development of DILI, non-steroidal anti-inflammatory drugs (39 %) rank first, followed by antibacterial drugs (29 %), immunosuppressant (9 %) and antiplatelet drugs (7 %), antidiabetic drugs (4 %) and others (21 %).[8] Taking paracetamol, troglitazone, valproate, antibiotics and anticancer drugs was the most frequently associated with death in patients with DILI.[9-11]

Paracetamol or acetaminophen is the most widely used over-the-counter analgesic-antipyretic in the world.[12, 13] The relevance of the use of this drug has increased especially in the context of the pandemic of the new viral infection COVID-19. It is also worth noting that paracetamol is often used as a component of combined painkillers and anti-inflammatory drugs – [paracetamol + ibuprofen], [paracetamol + diclofenac sodium], [paracetamol + metamizole sodium], etc. The ability of paracetamol to covalently bind to mitochondrial proteins of hepatocytes and a number of enzymes (glutamine synthetase, glutamine dehydrogenase, carbonic anhydrase III, glutamate dehydrogenase, glycine-N-methyltransferase) is the basis of its hepatotoxic effect.[12, 13]

To date, according to the State Register of Medicinal Products of Ukraine, more than 100 drugs with hepatoprotective activity are registered on the pharmaceutical market of Ukraine, but none of them can fully satisfy the clinicians' needs. Authors' attention was drawn to the national-produced drug "Cryocell – placenta cryoextract" as a potential biotechnological agent with a hepatoprotective effect. Placenta cryoextract (CEP) was obtained by scientists of the Institute of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine.[14-16] In previous studies, it was established that the therapeutic and preventive administration of CEP normalised metabolic processes in the liver and restored its functional state due to antioxidant and membrane-stabilising effects, which weakened the cytolytic syndrome caused by the administration of D-galactosamine and restored the protein-synthesising function of the liver.[17-19] In addition, it was shown that CEP has an energy-stabilising effect on hepatocytes of rats with simulated tetrachloromethane liver damage.[20-22]

The aim of the present study was to analyse the hepatoprotective activity of placenta cryoextract on the model of paracetamol-induced hepatitis.

Methods

The study was performed on 28 male rats weighing 200–220 g which were divided into 4 groups[21]:

I – intact rats (n = 7);

II – rats (n = 7) with paracetamol-induced hepatitis (control group);

III – rats (n = 7) with paracetamol-induced hepatitis, which were injected intramuscularly (im) cryoextract of the placenta (CEP) in a dose of 0.16 mL/kg 5 times;[24, 25]

IV – rats (n = 7) with paracetamol-induced hepatitis, which were administered a derivative of the amino acid L-cysteine – acetylcysteine (ACC) intraperitoneally (ip) in a dose of 150 mg/kg.[21, 26]

Acute medical damage to the liver was simulated by intragastric (ig) administration of paracetamol at a dose of 1250 mg/kg once a day for 2 consecutive days.[21] CEP and ACC were administered 60 minutes after each administration of paracetamol (2 administrations) and further for 3 consecutive days after simulating hepatitis (a total of 5 administrations). The animals were withdrawn from the experiment 72 hours after the second injection of paracetamol and sacrificed under general anaesthesia.

Biochemical research methodology

The research material was whole blood and rat liver homogenates. To obtain the liver homogenate, the liver was perfused with a cold (+4 °C) isotonic 1.15 % KCl solution and homogenised at 3000 rpm (teflon/glass) in a buffer solution at a ratio of 1:10 (weight/volume: 250 mg + 2.25 mL of 1.15 % KCl solution), obtaining a 10.0 % homogenate.

Content of active products of thiobarbituric acid (TBA-AP) in liver homogenates was determined

spectrophotometrically according to Asakawa et al at the wavelength of λ = 535 nm.[27] The molar extinction coefficient of the red-coloured complex, which is 1.56×10^5 mol^{-1}/cm^{-1} expressed in μmol/kg tissue, was considered for further analysis. Catalase activity in liver homogenates was determined spectrophotometrically by light absorption at a wavelength of λ = 410 nm and expressed as mcat/kg of tissue.[28] Antioxidant-prooxidant index (API) was calculated according to the formula: API = (Catalase activity × 100) / TBA-AP content.[28]

Alanine aminotransferase (ALT) activity and aspartate aminotransferase (AST) activity in peripheral blood was determined spectrophotometrically according to the method of Reitman and Frankel.[30] De Ritis ratio was calculated as the AST to ALT ratio.[28] Concentration of bilirubin in peripheral blood was determined spectrophotometrically by the reaction of diazophenylsulphonic acid with direct bilirubin.[29, 31]

Statistical analysis

Differences between groups were assessed with the use of parametric or nonparametric criteria, as applicable. Normally distributed independent values were compared with the paired t-test, while other than normally distributed values were compared with a Mann-Whitney test. A p-value of < 0.05 was considered statistically significant. The averages of the normally distributed values are reported as mean ± standard error of the mean or mean (95 % confidence interval). The averages of other than normally distributed values are reported as medians [interquartile ranges].[32]

Bioethical compliance

All experimental studies on laboratory animals were performed in accordance with the requirements of Good Laboratory Practice and in compliance with the basic provisions of the Council of Europe Convention on the Protection of Vertebrate Animals Used in Experiments and Other Scientific Purposes of 18 March 1986, European Parliament and Council Directive 2010/63/EU of 22 September 2010 on the protection of animals. The comprehensive research program was considered and approved by the Committee on Bioethics at the Institute of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine (Protocol No 5 of 22 November 2022).

Results

A significant increase ($p < 0.001$) in the content of TBA-AP in liver homogenates by 71.3 % compared to the values of intact rats was noted (Table 1). ACC administration resulted in decrease of TBA-AP level by 18.6 % ($p = 0.04$). Administration of CEP resulted in almost complete recovery of the TBA-AP level in liver homogenates to the level of 9.3 ± 1.48 μmol/kg of tissue (as compared with 9.4 ± 0.68 μmol/kg of tissue in intact animals).

A significant decrease of catalase activity by 35.3 % ($p < 0.01$) as compared to that of intact rats (2.2 ± 0.24 mcat/kg of tissue) was noted. In CEP administration, this index increased by 18.2 % ($p = 0.03$) and in ACC administration, it increased by 59.1 % ($p < 0.01$) as compared with rats with paracetamol-induced hepatitis. A significant decrease in the value of the API by 62.2 % was noted ($p < 0.001$). The use of CEP, as well as of ACC, resulted in a significant 2.3-fold and 1.9-fold increase ($p < 0.01$) of API, respectively (Table 1).

In acute paracetamol-induced hepatitis in rats, there was a statistically significant ($p < 0.001$) 2.1-fold increase ALT activity, as well as an increase ($p < 0.001$) of AST activity by 58.8 % as compared with indices of intact rats. A disproportionate increase in the activity of aminotransferases led to a statistically significant ($p = 0.02$) decrease of the De Ritis ratio by 26.7 % as compared with the values in intact animals (Table 2).

The use of ACC led to a uniform decrease in the activity of ALT and AST in the peripheral blood of rats with paracetamol-induced hepatitis by 28.0 % ($p < 0.001$) and 25.9 % ($p < 0.01$), respectively, as compared to the parameters of animals without treatment. However, when the imbalance in the activity of aminotransferases caused by liver damage persisted, the De Ritis ratio was 26.7 % lower ($p = 0.03$) as compared with the untreated animals (Table 2). A significant ($p < 0.001$) decrease in the activity of ALT (by 44.0 %) and AST (by 29.6 %) was noted in group III when CEP was used, as compared to the respective indices of animals of the control group. In contrast to ACC (group IV), the use of CEP (group III) was associated with a statistically significant ($p = 0.01$) increase of De Ritis ratio (by 27.3 %).

In paracetamol-induced hepatitis in rats a statistically significant ($p < 0.001$) 4.2-fold increase of

the concentration of total bilirubin was observed as compared with intact rats (Table 3). Indirect bilirubin increased 5.2-fold, while direct bilirubin increased by 2.7 times compared to the values of intact rats and was, respectively, 14.1 ± 0.6 μmol/L compared with 47.0 ± 1.8 μmol/L. The level of direct bilirubin significantly ($p < 0.001$) decreased both with the use of CEP (by 52.5 %) and with the use of ACC (by 55.3 %).

Table 1: The effect of CEP on the biochemical lipid peroxidation and antioxidant system in tissue homogenates in paracetamol-induced hepatitis in rats (M ± m (95 % CI), n = 28)

The studied index, units of measurement	Experimental group			
	Group I Intact rats	Group II Paracetamol hepatitis	Group III Paracetamol + CEP	Group IV Paracetamol + ACC
n	7	7	7	7
TBA-AP, μmol/kg tissue	9.4 ± 0.7 (8.1 – 10.8)	16.1 ± 1.0 (14.1 – 18.2) $p_{1-2} < 0.001$	9.3 ± 1.5 (6.4 – 12.2) $p_{1-3} = 0.90$ $p_{2-3} < 0.01$	13.1 ± 0.8 (11.5 – 14.7) $p_{1-4} < 0.01$ $p_{2-4} = 0.04$ $p_{3-4} = 0.04$
Catalase, mcat/kg of tissue	3.4 ± 0.2 (3.0 – 3.9)	2.2 ± 0.2 (1.7 – 2.7) $p_{1-2} < 0.01$	2.6 ± 0.2 (2.2 – 3.1) $p_{1-3} = 0.03$ $p_{2-3} = 0.20$	3.5 ± 0.3 (2.9 – 4.1) $p_{1-4} = 0.90$ $p_{2-4} < 0.01$ $p_{3-4} = 0.03$
Antioxidant-prooxidant index	38.1 ± 4.5 (29.3 – 46.9)	14.4 ± 2.4 (9.7 – 19.2) $p_{1-2} < 0.001$	32.6 ± 5.4 (22.0 – 43.2) $p_{1-3} = 0.50$ $p_{2-3} < 0.01$	27.5 ± 2.8 (21.9 – 33.0) $p_{1-4} = 0.07$ $p_{2-4} < 0.01$ $p_{3-4} = 0.40$

Indices $_{1, 2, 3, 4}$ indicate the number of the group whose characteristics were compared; n – number of rats; CI: confidence interval; ACC – amino acid L-cysteine – acetylcysteine; CEP – cryoextract of the placenta; TBA-AP – active products of thiobarbituric acid;

Table 2: The effect of CEP on the activity of aminotransferases in the peripheral blood in rats with paracetamol hepatitis (M ± m (95 % CI), n = 28)

The studied index, units of measurement	Experimental group			
	Group I Intact rats	Group II Paracetamol hepatitis	Group III Paracetamol + CEP	Group IV Paracetamol + ACC
n	7	7	7	7
ALT, μmol/(mL×h)	1.2 ± 0.1 (1.0 – 1.4)	2.5 ± 0.1 (2.3 – 2.7) $p_{1-2} < 0.001$	1.4 ± 0.1 (1.2 – 1.6) $p_{1-3} = 0.15$ $p_{2-3} < 0.001$	1.8 ± 0.1 (1.7 – 1.9) $p_{1-4} < 0.001$ $p_{2-4} < 0.001$ $p_{3-4} < 0.01$
AST, μmol/(mL×h)	1.7 ± 0.1 (1.5 – 1.9)	2.7 ± 0.13 (2.4 – 2.9) $p_{1-2} < 0.001$	1.9 ± 0.1 (1.7 – 2.1) $p_{1-3} = 0.24$ $p_{2-3} < 0.001$	2.0 ± 0.2 (1.6 – 2.3) $p_{1-4} = 0.29$ $p_{2-4} < 0.01$ $p_{3-4} = 0.79$
De Ritis ratio (AST/ALT)	1.5 ± 0.2 (1.2 – 1.8)	1.1 ± 0.1 (1.0 – 1.2) $p_{1-2} = 0.02$	1.4 ± 0.1 (1.2 – 1.5) $p_{1-3} = 0.41$ $p_{2-3} = 0.01$	1.1 ± 0.1 (0.9 – 1.3) $P_{1-4} = 0.03$ $P_{2-4} = 0.89$ $P_{3-4} = 0.03$

Indices $_{1, 2, 3, 4}$ indicate the number of the group whose characteristics were compared; p_{2-1} is the level of statistical significance of difference between the groups; n – number of rats; CI: confidence interval; ACC – amino acid L-cysteine – acetylcysteine; CEP – cryoextract of the placenta; ALT – Alanine aminotransferase; AST – Aspartate aminotransferase;

Table 3: Effect of CEP on the concentration of bilirubin in the peripheral blood of rats against the background of paracetamol hepatitis (M ± m (95 % CI), n = 28)

The studied index, units of measurement	Experimental group			
	Group I Intact rats	Group II Paracetamol hepatitis	Group III Paracetamol + CEP	Group IV Paracetamol + ACC
n	7	7	7	7
Total bilirubin, μmol/L	14.4 ± 0.8 (12.8 – 16.1)	61.1 ± 2.2 (56.8 – 65.5) $p_{1-2} < 0.001$	50.1 ± 0.8 (48.6 – 51.7) $p_{1-3} < 0.01$ $p_{2-3} < 0.001$	27.7 ± 1.2 (25.3 – 30.1) $p_{1-4} < 0.001$ $p_{2-4} < 0.001$ $p_{3-4} < 0.001$
Direct bilirubin, μmol/L	5.3 ± 0.4 (4.6 – 12.0)	14.1 ± 0.6 (12.9 – 15.4) $p_{1-2} < 0.001$	6.7 ± 0.4 (5.9 – 7.5) $p_{1-3} = 0.02$ $p_{2-3} < 0.001$	6.3 ± 0.4 (5.5 – 7.1) $p_{1-4} = 0.10$ $p_{2-4} < 0.001$ $p_{3-4} = 0.48$
Indirect bilirubin, μmol/L	9.1 ± 0.7 (7.8 – 10.5)	47.0 ± 1.8 (43.5 – 50.5) $p_{1-2} < 0.001$	43.4 ± 1.0 (41.6 – 45.3) p1-3 < 0.001 p2-3 = 0.11	21.4 ± 1.00 (19.5 – 23.4) p1-4 < 0.001 p2-4 < 0.001 p3-4 < 0.001

Indices $_{1, 2, 3, 4}$ indicate the number of the group whose characteristics were compared; n – number of rats; CI: confidence interval; ACC – amino acid L-cysteine – acetylcysteine; CEP – cryoextract of the placenta;

Discussion

Presented study has shown that simulation of paracetamol-induced hepatitis in rats was accompanied by activation of lipid peroxidation processes in liver tissues. This was demonstrated by a statistically significant increase in the content of TBA-AP in liver homogenates by 71.3 % compared to the values of intact rats.

ACC administration resulted in decrease of TBA-AP level by 18.6 %. Administration of CEP resulted in almost complete recovery of the TBA-AP level in liver homogenates. The assessment of the level of catalase (reflecting the activity of the antioxidant system) in liver homogenates showed that the development of acute paracetamol hepatitis was accompanied by a significant decrease of catalase activity by 35.3 % ($p < 0.01$) as compared to that of intact rats. In CEP administration, this index increased by 18.2 % ($p = 0.03$) and in ACC administration, it increased by 59.1 % ($p < 0.01$) as compared with rats with paracetamol-induced hepatitis. An integral assessment of the state of the prooxidant-antioxidant system in liver homogenates showed that in paracetamol-induced hepatitis, a statistically significant decrease in the value of the API by 62.2 % was noted ($p < 0.001$). The use of CEP, as well as of ACC, resulted in a significant 2.3-fold and 1.9-fold increase ($p < 0.01$) of API, respectively, indicating a slightly more pronounced ability of CEP to restore the balance of the pro-oxidant-antioxidant system of liver tissues.

The assessment of the activity of aminotransferases in peripheral blood showed that in acute paracetamol-induced hepatitis in rats, there was a significant 2.1-fold increase ALT activity, as well as an increase of AST activity by 58.8 % as compared with indices of intact rats. A disproportionate increase in the activity of aminotransferases led to a statistically significant ($p = 0.02$) decrease of the De Ritis ratio by 26.7 % as compared with the values in intact animals. A low of De Ritis ratio can be observed during the activation of gluconeogenesis processes via a glucose-alanine shunt with participation of ALT. This process which is necessary for maintenance of an adequate blood glucose level, results in elevation of activity of transaminases. Also, low De Ritis ratio may imply a decrease of liver function.[33]

The use of ACC led to a uniform decrease in the activity of ALT and AST in the peripheral blood of rats with paracetamol-induced hepatitis by 28.0 % and 25.9 %, respectively, as compared to the parameters of animals without treatment. However, when the imbalance in the activity of aminotransferases caused by liver damage persisted,

the De Ritis ratio was 26.7 % lower (p = 0.03) as compared with the untreated animals. A significant decrease in the activity of ALT (by 44.0 %) and AST (by 29.6 %) was noted in group III when CEP was used, as compared to the respective indices of animals of the control group. In contrast to ACC, the use of CEP was associated with a significant increase of De Ritis ratio (by 27.3 %), indicating the recovery of metabolic balance in liver.[28]

Studies of liver pigment metabolism showed that in paracetamol-induced hepatitis in rats a significant (p < 0.001) 4.2-fold increase of the concentration of total bilirubin was observed as compared with intact rats. This increase was mainly due to indirect bilirubin, which increased 5.2-fold, while direct bilirubin increased by only 2.7 times compared to the values of intact rats and was. An increase in the level of total bilirubin mainly by an increase in the indirect bilirubin portion is indicative functional membrane-bound transport system disorder involved in capturing of indirect bilirubin.[28]

The use of CEP led to decrease in the concentration of indirect bilirubin in a smaller extent than the use of ACC. However, the ability to reduce the level of direct bilirubin in the peripheral blood of rats with paracetamol-induced hepatitis of the two studied agents was similar: the level of direct bilirubin significantly decreased both with the use of CEP (by 52.5 %) and with the use of ACC (by 55.3 %).

Conclusion

The development of acute paracetamol-induced hepatitis in rats was associated with activation of lipid peroxidation processes in liver tissues, as demonstrated by a statistically significant increase of TBA-AP content in liver homogenates as compared with intact animals. Also, there was a statistically significant rise in the activity of both ALT and AST as well as a statistically significant increase of total bilirubin concentration as compared with intact rats.

The cryopreserved placenta extract showed marked hepatoprotective activity in paracetamol-induced hepatitis in rats. This was demonstrated by a statistically significant increase of the antioxidant-prooxidant index, a statistically significant decrease of the ALT and AST activity, as well as a statistically significant decrease of the level of direct bilirubin in rats treated with CEP as compared with non-treated animals.

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Conflict of interest

None.

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