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The Effect of Human Embryonic Hepatocytes on Biochemical Indices at Acute Hepatic Failure in the Experiment

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Abstract Acute hepatic failure has been simulated in the rats by single intraperitoneal introduction of CCl4 hepatotrophic toxin in a dose of 1 ml/kg. Human embryonic hepatocytes were extracted from human embryo (spontaneous abortions). Fifty million (50×10^6) fresh preparated hepatocytes were transplanted intraabdominally in the period between 30 and 34 hours after induction of acute hepatic failure. The following biochemical indices of liver functional condition were defined: glucose, crude protein, albumine, urea, creatinine, general bilirubin, AST, ALT, alkaline phosphatase, ammonia. At acute hepatic failure the decrease of some indices (glucose, the crude protein, albumine) and simultaneous increase of the others (urea, creatinine, the general bilirubin, AST, ALT, alkaline phosphatase, ammonia) reflecting changes in the liver functioning has been noted. Human embryonic hepatocytes corrects liver dysfunctions in the rats with acute hepatic failure starting from 7 days after treatment.

Keywords Human embryonic hepatocytes, Acute hepatic failure, Transplantation of hepatocytes, Biochemical indices

1. Introduction

Acute hepatic failure (AHF) is a relevant issue of practical medicine and hepatology [1, 6]. There are many etiological factors leading to its development such as: fulminant forms of viral hepatitis, drug intoxication, severe intoxications by poisons and alcohol, sepsis, massive burns, shock of different etiology, acute suprarenal failure, cardio-surgical interventions, surgical diseases of hepatopancreatobiliary system, metabolic disorders and etc. [2, 9].

The majority of clinicians consider the basis of AHF pathogenesis is an evident humoral and hyperimmune response caused by massive necrosis of hepatocytes. Its severity is directly depends on hepatocytes percentage which lost their functional and reserve abilities and also depends on the duration of the period of their death [5, 8].

Disabled hepatocytes due to the change of membrane proteins structure are apprehended as foreign ones by the immune system and are destroyed. But it is necessary to mention that the liver has big compensatory abilities. As a rule, the AHF development points that more than 75-80% of hepatocytes are disabled [9].

The treatment of AHF is very complicated problem. The most effective treatment method is a liver transplantation, but it has some disadvantages (the lack of donor organs, the necessity of immunosuppressants constant taking, a high risk of severe postoperative complications development) [7].

With the aim of prevention and treatment of AHF the meal of embryonic hepatocytes providing detoxicating effect, activating regenerative processes and improving the prognosis of the disease has been begun to use for the recent years [3, 10]. Earlier we studied a condition of the immune system of animals from ALF receiving HEH [4].

2. Aim

To estimate the dynamics of biochemical indices at the treatment of acute hepatic failure in the rats with the use of human embryonic hepatocytes.

3. Material and Methods

The AHF model was reproduced in the laboratory rats (males) by a single intraperitoneal introduction of CCl4 hepatotrophic toxin in a dose of 1 ml/kg. In the experiment we used mature outbred male rats. The main criteria for the inclusion of animals in the study were: body weight 230-270 g; age 23-24 weeks; wool - smooth, shiny; behavior and general condition - the active dynamics of movement and feed consumption. Before the start of the study, rats meeting

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these criteria were divided into groups using the randomization method. The study protocol was approved by the National Ethics Committee (No. 4 of April 13, 2011), and in the course of the experiment, the requirements of the World Society for the Protection of Animals (WSPA) and the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (Strasbourg, 1986).

Human embryonic hepatocytes were obtained from abortive material with the obligatory consent of the mother, while conducting tests for a wide range of viral diseases.

As a source of donor cells the liver of embryos of non-viable human fetuses, weighing less than 500 grams was obtained from women who artificially terminate the pregnancy for medical reasons at gestational age of 8-11 and 16-20 weeks. The embryo was sterilely transferred to a 250 ml glass bottle with Hanks solution containing the antibiotic gentamicin at a concentration of 500 mcg / ml. Then the embryo was washed three times by Hanks solution with kanamycin (50 mcg / ml).

Human embryonic hepatocytes were extracted from human embryo (spontaneous abortions).

The method of obtaining embryonic hepatocytes was based on the enzymatic-mechanical method of processing the original tissue with minimization of manipulations damaging the embryonic hepatocytes (EH). The essence of the method is to increase the percentage of yield of viable EH with increased morphofunctional properties and reduce the time to receive them.

The method of obtaining the EH included: laparotomy, portal vein cannulation, liver perfusion with nutrient solutions in 2 stages, then the liver was removed from the abdominal cavity, the liver was mechanically processed by chopping the liver with scissors into pieces up to 0.7 mm thick which were transferred into a glass with phosphate-buffered saline 0.25% solution of proteolytic enzyme trypsin, placed on a magnetic stirrer for 15 minutes at + 37°C. After enzymatic treatment, medium 199 is added to inactivate trypsin. Next was performed homogenization and filtration.

Experimental animals were kept in accordance with the established standards of the World Society for the Protection of Animals (WSPA) and the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes. Animals were kept by 4-5 heads in a cage with a sufficient amount of lighting, power, access to water. Statistical processing of research results was carried out on the basis of methods of variation statistics using parametric Student's t-test and non-parametric Mann-Whitney U-test.

For investigation of the general bilirubin, albumine and ammonia a blood sampling was taken from the tail of the rats. Thecontent of some biochemical indices in the blood serum was determined by the unified methods accepted in clinical practice (V.G. Kolb, V.S. Kamyshnikov, 1982). The content of urea was determined by staining reaction with diacetylmonooc sim, creatinine - by staining reaction of Jaffe (the method of Popper and al.), the general bilirubin –by a colorimetric method of Yendrashik, Kleggorn, Groff. In the animals blood serum we determined the following indices: concentration of the crude protein (CP), general bilirubin, urea, glucose, activity of aspartate aminotransferase (AST), alaninaminotranspherase (ALT), alkaline phosphatase (AP). Biochemical researches were carried out with the use of “Vitros” analyzer (Japan).

Animals were divided into groups by 10 in each. The first group was consisted of the rats with the AHF model in which biochemical researches were conducted 3, 7 and 14 days after simulating AHF. The second comparative group was made by the animals with AHF treated by means of HEH without additional introduction of immunosuppressors. Researches were conducted 7 and 14 days after simulating AHF.

4. Results and Discussion

Dynamic biochemical researches in the rats with AHF showed the following results (tab. 1).

Table 1. The dynamics of biochemical indices changes after AHF modeling induced CCl_4 (M±m)

№	Indices	Initial rates (healthy) (n=10)	Animals groups with AHF		
			The 3rd day (n=10)	The 7th day (n=10)	The 14th day (n=10)
1	Glucose, mmol/l	9.95±0.56	4.90±0.16[a]	5.23±0.34[a]	5.71±0.06[ab]
2	Crude protein, g/l	97.54±2.20	48.99±0.85[a]	46.38±0.86[a]	44.21±0.49[ab]
3	Albumin, g/l	47.46±1.64	16.61±0.55[a]	33.97±2.06[a]	29.62±1.82[ab]
4	Urea, mmol/l	10.69±0.64	15.83±0.21[a]	16.44±0.30[a]	15.40±0.21[a]
5	Creatinine, mcmol/l	80.39±4.37	166.58±2.51[a]	138.31±2.77[ab]	142.94±2.12[ab]
6	Bilirubin, mcmol/l	11.87±0.55	47.26±0.84[a]	33.93±2.33[ab]	42.27±1.33[abc]
7	AST, U/l	139.51±5.67	270.81±26.88[a]	230.49±7.03[a]	236.82±7.08[a]
8	ALT, U/l	125.20±3.19	276.21±9.59[a]	239.52±10.72[ab]	245.53±9.17[ab]
9	Alkaline phospha-tase, U/l	117.24±7.24	266.32±13.08[a]	247.92±14.67[a]	229.74±13.95[a]
10	Ammonia, mcmol/l	91.50±2.91	194.57±5.92[a]	207.72±9.25[a]	184.16±8.27[a]

Note: a – reliable to initial rates (healthy), b – reliable to the 3rd dayrates, c – reliable to the7th day rates ($p<0.05$)

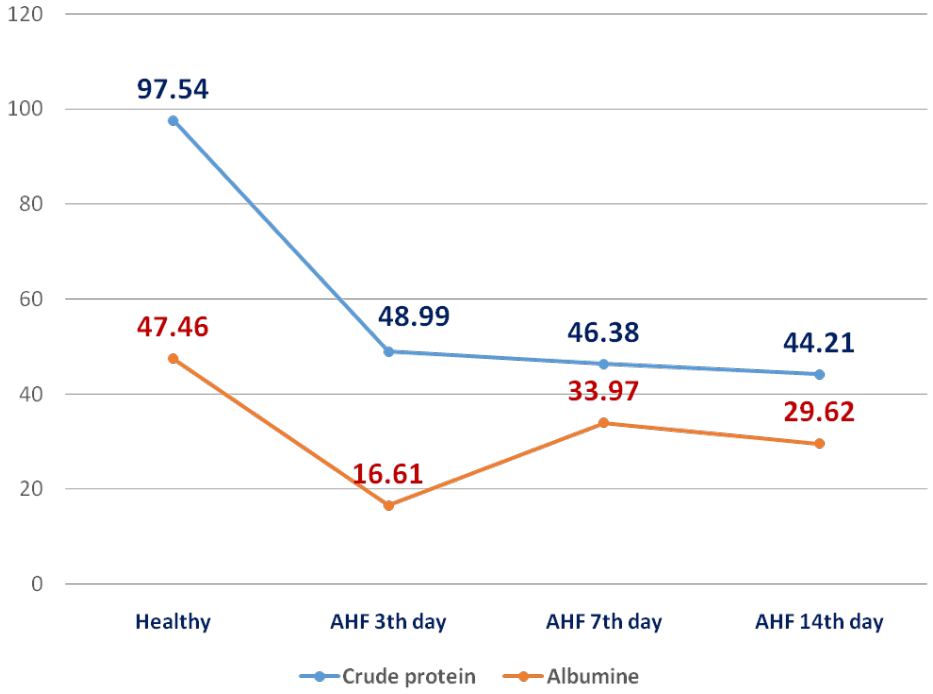

Figure 1. The dynamics of crude protein and albumine (g/l) in the rats with AHF on the 3rd, 7th, 14th day

Concentration of glucose in blood in the 3rd day in comparison with control indices (healthy rats) reliably decreased in 2 times (9.95±0.56 mmol/l - norm, 4.90±0.16 mmol/l - 3 days with AHF). The content of glucose remained at the same level 7 and 14 days later. Level of the crude protein in blood, as well as glucose, significantly decreased: on the 3rd day - in 2.4 times, on the 7th day - in 2.1 times, on the 14th day - in 2.2 times.

Thus, dynamics of glucose and crude protein level changes in blood of the rats was similar with AHF: on the 3rd day their value decreased twice and did not change within 2 weeks.

Other picture has been detected when studying albumine in the blood of animals with AHF. If this index was 47.46±1.64 g/l in the control, then on the 3rd day it decreased in 2.86 times (16.61±0.55 g/l). However, on the 7th day albumine level in compare with the 3rd day increased twice and made up 33.97±2.06 g/l. On the 14th day its level decreased a little - 29.62 ±1.82 (Fig.1).

Therefore, the changes curve of albumine in blood of the rats with liver pathology has wavy character: the peak of decrease falls on the 3rd day, then the level increases on the 7thday then again the decrease is observed. Level of urea is normally equal to 1.69±0.64 mmol/l, and in the rats with AHF on the 3rd day it raised in 1.5 times (15.83±0.21 mmol/l) and practically did not change up to 14 days (Fig.2).

As for creatinine, on the 3rd day its level in the rats with AHF increased in 2.1 times (control - 80.39±4.37 µmol/l, rats with AHF - 166.58± 2.51 µmol/l). But on the 7th day creatinine level in comparison with 3rd day reliably decreased and made up 138.31±2.77 µmol/l. On the 14th day creatinine level increased a little (142.94±2.12 µmol/l). Comparing the dynamics of creatinine and albumine changes level we can see that they are completely opposite each other. We observed a maximal decrease of albumine on the 3rd day with a subsequent reliable increase and creatine, on the contrary, was maximally increased on the 3rd day with its subsequent reliable decrease.

The dynamics of general bilirubin changes in the blood of animals with AHF differed from the above described five indices (glucose, the general protein, albumine, urea, creatinine). Level of the general bilirubin increased more (in 4 times) in comparison with urea and creatinine (in 1.5-2.1 times). On the 3rd day the level of the general bilirubin made up 47.26±0.84 µmol/l (control - 11.87±0.55 µmol/l). On the 7th day the rate of the general bilirubin, in compare with the 3rd day reliably decreased up to 33.93±2.33 µmol/l. But by the 14th day the general bilirubin level in comparison with 7th day again reliably increased up to 42.27±1.33 µmol/l. In other words, the index of the general bilirubin on the 14th day was reliably higher than in the control group on the 7th day, but, at the same, it was reliably lower than on the 3rd day. Such dynamics of the changes was not observed in other cases (Fig.3).

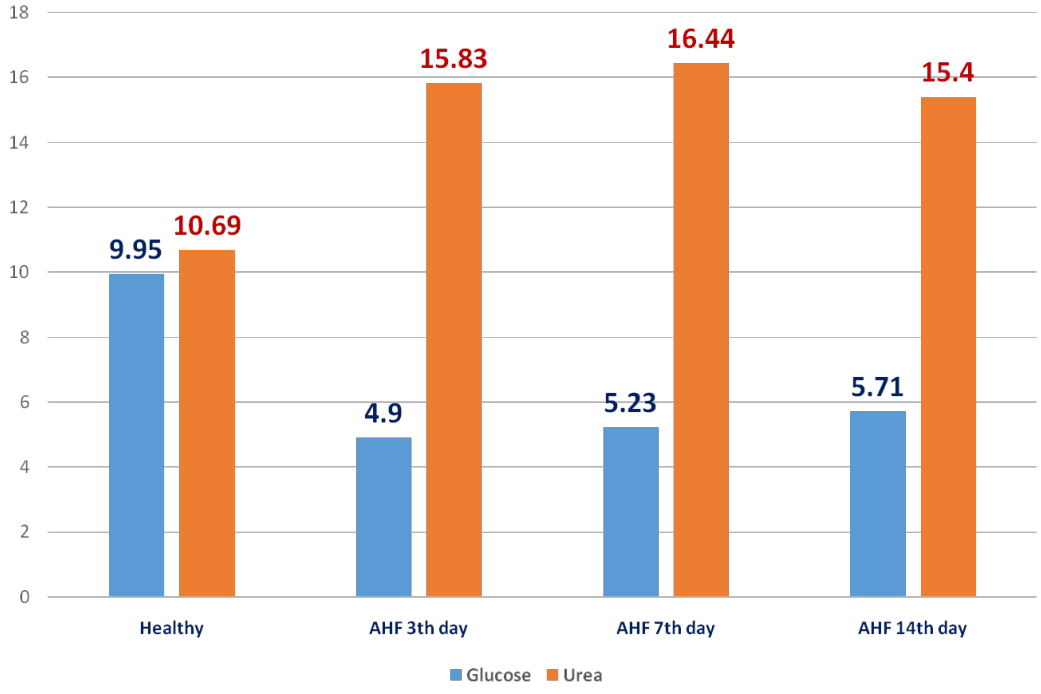

Figure 2. The dynamics of glucose and urea (mmol/l) in the rats with AHF on the 3rd, 7th, 14th day

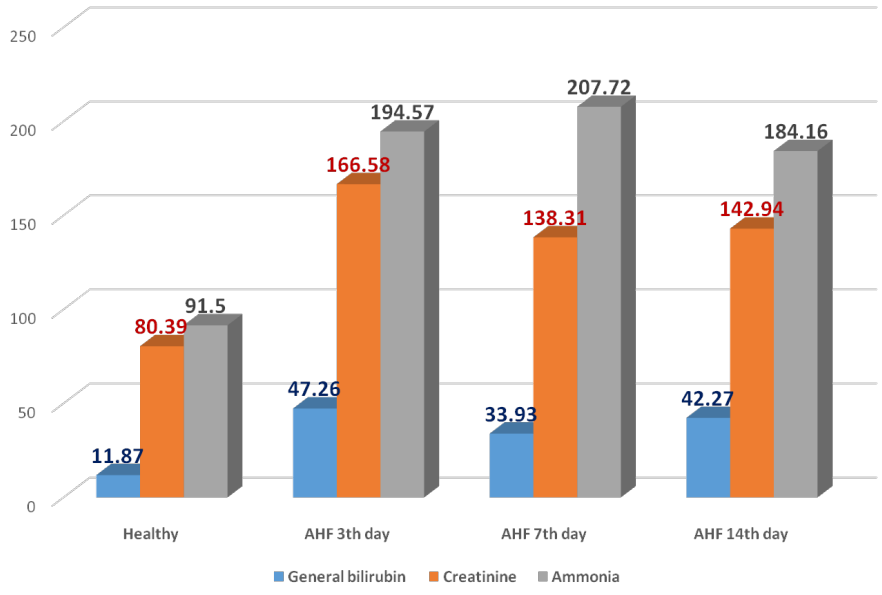

Figure 3. The dynamics of general bilirubin, creatinine, ammonia (mcmol/l) in the rats with AHF on the 3rd, 7th, 14th day

Dynamics of AST changes coincides with dynamics of urea changes level in the rats with AHF. The AST index in the was 139.51±5.67 U/l and on the 3rd day after AHF induction its level increased in 1.94 times (270.81±26.88 U/l). On the 7th day AST was 1.65 times higher than the control and on the 14th day –in 1.70 times. The reliable difference between AST values on the 3rd, 7th and 14th days was not revealed as well as in the urea.

Dynamics of ALT level changes differed a little from AST (tab. 1). On the 3rd day ALT index, as well as AST raised in 2.1 times (125.20±3.19 U/l - norm, 276.21±9.59 - pathology). But on the 7th day the ALT level in comparison with the 3rd day reliably decreased up to 239.52±10.72 U/l and remained at the same level up to 14th day (245.53±9.17 U/l).

The Alkaline Phosphatase (AP) in the rats on the 3rd day after AHF modeling raised in 2.27 times (control

-117.24±7.24 U/l, AHF - 266.32±13.08 U/l). Further the tendency to the decrease was observed: the 7th day - 247.92±14.67 U/l, the 14th day - 229.74±13.95 U/l. However, the reliable difference between AP indices was not revealed on the 3rd, the 7th and the 14th days (Fig.4).

In general, the same dynamics of changes was found when studying ammonia in the blood of animals with AHF. On the 3rd day its level increased in 2.13 times (norm - 91.50±2.91 U/l, pathology - 194.57±5.92 U/l). On the 7th day ammonia level in comparison with control values was in 2.3 times higher, and on the 14th day - twice, at the same time on all terms of studying we did not reveal a reliable difference between values.

Thus, in the rats with AHF various changes in level of the investigated indices were observed. Level of glucose, general protein and albumine sharply fell and concentration of urea, creatinine, bilirubin, AST, ALT, alkaline phosphatase and ammonia, on the contrary, significantly increased.

It was interesting to study what was the effect of HEH to biochemical indices in the rats with AHF (tab. 2).

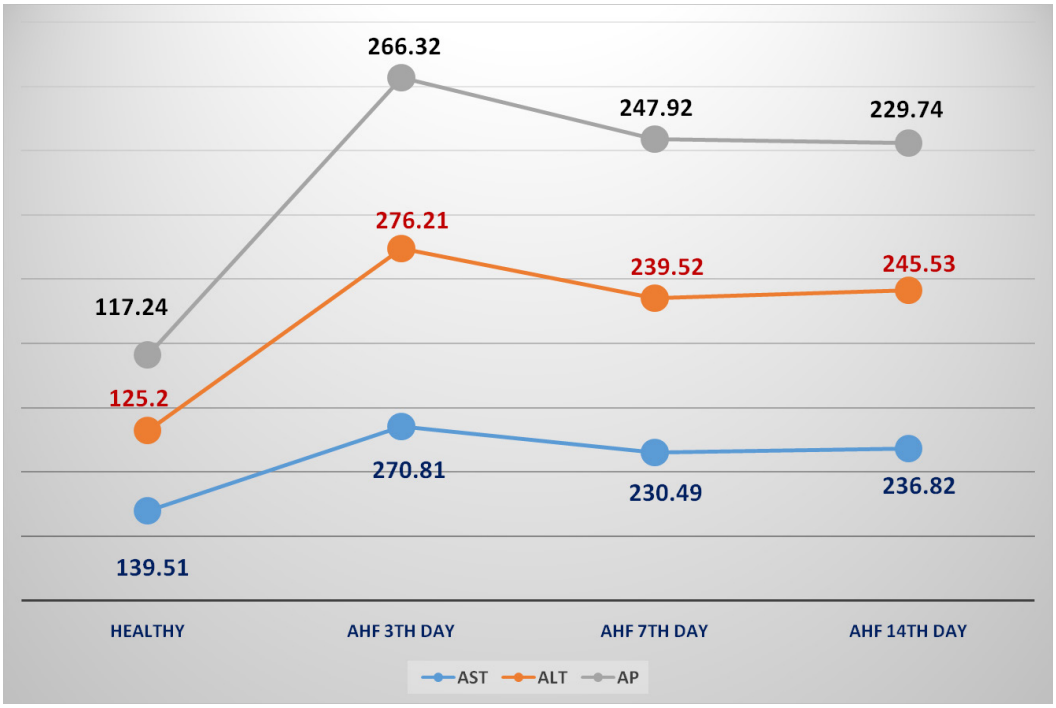

Figure 4. The dynamics of AST, ALT and AP (U/l) in the rats with AHF on the 3rd, 7th, 14th day of the experiment

Table 2. Dynamics of changes in biochemical indicators after modeling AHF, induced by CCl4 and treated with HEH (M±m)

№	Indices	Initial rates (healthy) (n=10)	Rats with AHF		Rats with AHF+HEH	
			7th days (n=10)	14th days (n=10)	7th days (n=10)	14th days (n=10)
1	Glucose, mmol/l	9.95±0.56	5.23±0.34[a]	5.71±0.06[a]	5.28±0.43[a]	5.98±0.16[a]
2	Crude protein, g/l	97.54±2.20	46.38±0.86[a]	44.21±0.49[a]	46.65±0.71[a]	51.30±0.75[ab]
3	Albumi-ne, g/l	47.46±1.64	33.97±2.06[a]	29.62±1.82[a]	35.46±1.89[a]	35.92±1.90[ab]
4	Urea, mmol/l	10.69±0.64	16.44±0.30[a]	15.40±0.21[a]	13.59±0.39[ab]	12.96±0.50[ab]
5	Creatinine, mcmol/l	80.39±4.37	138.31±2.77[a]	142.94±2.12[a]	112.96±3.43[ab]	104.19±2.82[ab]
6	Total bilirubin, mcmol/l	11.87±0.55	33.93±2.33[a]	42.27±1.33[a]	30.19±2.12[a]	27.20±1.25[ab]
7	AST, U/l	139.51±5.67	230.49±7.03[a]	236.82±7.08[a]	190.44±4.86[ab]	161.71±4.56[ab]
8	ALT, U/l	125.20±3.19	239.52±10.72[a]	245.53±9.17[a]	181.98±3.83[ab]	171.66±3.68[ab]
9	Alkaline phosphatase, U/l	117.24± 7.24	247.92±14.67[a]	229.74±13.95[a]	189.56±6.66[ab]	173.64±5.12[ab]
10	Ammonia, mcmol/l	91.50±2.91	207.72±9.25[a]	184.16±8.27[a]	167.03±6.36[ab]	153.58±4.86[ab]

Note: a – reliable to initial rates (healthy), b - reliably to indices of rats with AHF the same period not received HEH ($p < 0.05$)

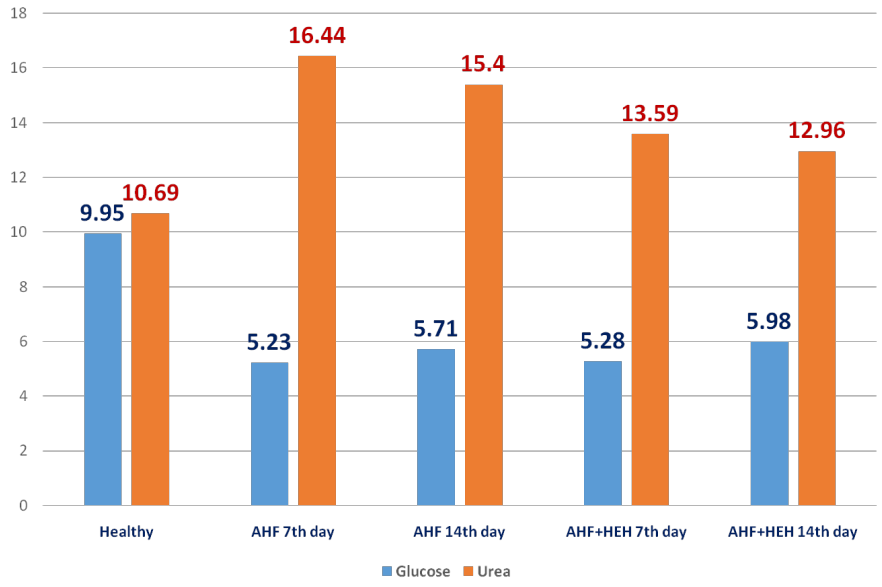

Figure 5. The dynamics of glucose and urea (mmol/l) in the rats with AHF modeling and treated by HEH on the 3rd, 7th, 14th day of the experiment

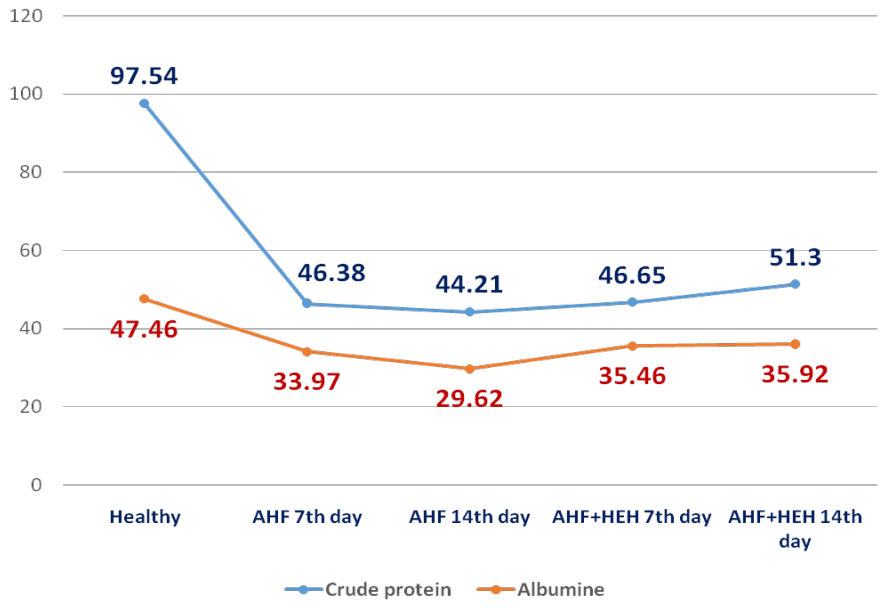

Figure 6. The dynamic of crude protein and albumine (g/L)I n the rats with AHF modeling and treated by HEH on the 3rd, 7th, 14th day of the experiment

The glucose level in the rats blood with AHF which were treated by HEH 7 and 14 days after modeling AHF did not differ from the values obtained from untreated animals. In other words, HEH has no impact on the reduced glucose level in blood of rats with liver pathology (Fig.5).

The level of the crude protein (reduced at AHF) in the rats receiving HEH did not change on the 7th day, but on the 14th day was reliably increased (AHF - 44.21± 0.49 g/l, AHF+HEH - 51.30±0.75 g/l). The same picture was observed at the analysis of albumine level in the blood of the rats with AHF: on the 7th day in the treated by HEH this reduced index did not change and on the 14th day –reliably raised (ALF - 29.62±1.82 g/l, ALF+HEH - 35.92±1.90 g/l) (Fig.6).

As it was mentioned above, the level of urea at AHF raised (see Fig. 2). At additional introduction of HEH on the 7th day of the experiment we observed a reliable decrease of urea level (AHF - 16.44 ±0.30 mmol/l, AHF+HEH - 13.59±0.39 mmol/l). A reliable decrease of urea level was also registered on the 14th day (AHF- 15.40± 0.21 mmol/l, AHF+HEH - 12.96±0.50 mmol/l).

As for the increased level of the general bilirubin in the rats with AHF treated by HEH its level practically did not change (AHF - 33.93±2.33 µmol/l, AHF+HEH - 30.19±2.12

¼¬¼«½/l) on the 7th day. But on the 14th day the level of general bilirubin reliably decreased (AHF - 42.27±1.33 µmol/l, AHF+HEH -27.20±1.25 µmol/l) (Fig.7).

The increased level of AST in the rats with AHF treated by HEH reliably decreased (AHF - 236.82± 7.08 U/l, AHF+HEH - 190.44±4.86 U/l) on the 7th day. The same regularity remained up to the 14th day: (AHF+HEH - 190.44 ±4.86 U/l).

Analogous result were obtained at the analysis of other enzyme - ALT. Reliable decrease of its level we observed on the 7th day (AHF - 239.52±10.72 U/l, AHF+HEH - 181.98 ±3.83 U/l) and remains up to 14 days (AHF- 245.53±9.17 U/l, AHF+HEH - 171.66 ±3.68 U/l) (Fig.8).

As at above described enzymes (AST, ALT), the increased level of APon the 7th day after treatment reliably decreased (AHF- 247.92±14.67 U/l, AHF+HEH - 189.56±6.66 U/l) and this pattern remained up to the 14th day (AHF- 229.74±13.95 U/l, AHF+HEH - 173.64±5.12 U/l).

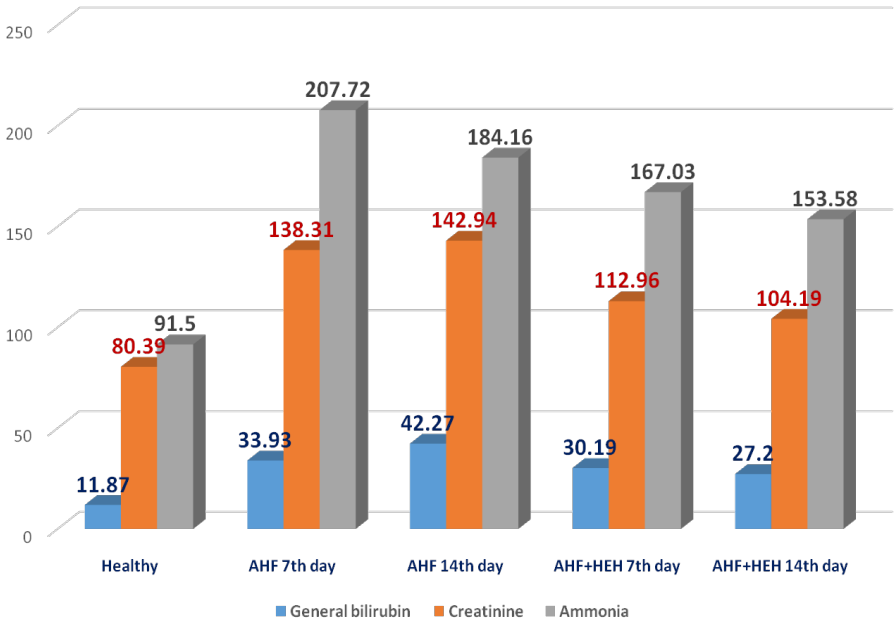

Figure 7. The dynamics of general bilirubin, creatinine and ammonia (mcmol/l) in the rats with AHF modeling on the 3rd, 7th, 14th day of the experiment

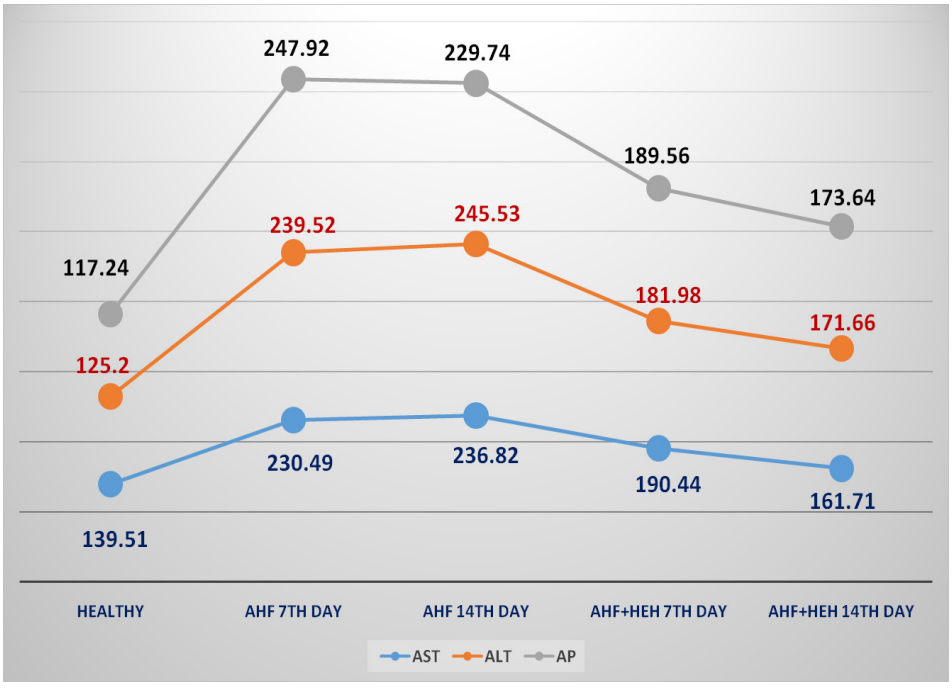

Figure 8. The dynamics of AST, ALT and AP (u/L) in the rats with AHF modeling and treated by HEH on the 3rd, 7th, 14th day of the experiment

Positive results were also received at the analysis of ammonia level in the rats with AHF treated by HEH. On the 7th day there was a reliable decrease of the increased ammonia level (AHF- 207.72± 9.25 μmol/l, AHF+HEH - 167.03±6.36 μmol/l). The same pattern was also registered on the 14th day of our research (AHF- 184.16±8.27 μmol/l, AHF+HEH - 153.58±4.86 μmol/l).

Thus, we found different terms of modulation of the changed biochemical indices in the animals with AHF treated by HEH. First, treatment did not affect glucose level in blood. Secondly, normalization of the crude protein, albumine and general bilirubin occurred only from the 14th day of the investigation. Thirdly, normalization of such indices as urea, creatinine, AST, ALT, AP and ammonia was observed from the 7th day. It should be noted that in no case the value of the studied biochemical indices returned to initial values.

5. Conclusions

In the animals with AHF the decrease of some biochemical indices (glucose, the crude protein, albumine) and simultaneous increase of the others (urea, creatinine, the general bilirubin, AST, ALT, alkaline phosphatase, ammonia) which reflect changes in liver functioning was noted. Under the influence of HEH the normalization of biochemical characteristics in the rats with AHF occurs starting with the 7th day after treatment.

Abbreviations

AHF - acute hepatic failure
HEH - human embryonic hepatocytes
AST - aspartate aminotransferase
ALT - alaninaminotranspherase
AP - alkaline phosphatase

REFERENCES

[1] Groyzik L.A. Transplantation of tinned izolated cells of a liver at an acute liver failure//The abstract of the thesis of the candidate of medical sciences. - 1985. - M - 25 pages.

[2] Lepekhova S.A., Sanogenez of a liver failure under the influence of xenotransplantation of cells of a liver and a spleen//The abstract of the thesis of the Dr.Sci.Biol. - 2010. - Irkutsk. - 32 pages.

[3] Subbota N.P., Gryshchenko V.I. Cells and tissues of human embrionsas source of cryotinned transplant material//Problem of a cryobiology. - 1991. - No. 1. - Page 25-29.

[4] Khadjibayev A.M., Urazmetova M.D., Madaminov A.A., etc. Comparative assessment of the impact of the allogenic and xenogenic isolated hepatocytes on the immune status of rats with an acute liver failure//the Messenger of experimental medicine. - 2011.-No. 2. - Page 48-51.

[5] Shagidulin M. Yu., Onishchenko N.A., Krashenninikov V.M. Transplantation of hepatocytes as method of treatment of hepatic insufficiency: experimental and clinical experience//Messenger of transplantology and artificial organs, 2010. - No. 2. - Page 53-60.

[6] Baine A.M., Hori T., Chen F. et al. Fulminant liver failure models with subsequent encephalopathy in the mouse //Hepatobiliary Pancreat. Dis. Int. - 2011. - Vol.10. - N6. - P. 611-619.

[7] Strain A.G. Hepatocyte transplantation //Progress in gastroenterology and hepatology. - Part III. Hannover (Germany) - 2002. - P. 8-9.

[8] Tunon M.J., Alvarez M., Culebras J.MK. et al. An overview of animal models for investigating the pathogenesis and therapeutic strategies in acute hepatic failure //World J. Gastroenetrol. - 2009. Vol.15. - N25. - P. 3086-3098.

[9] Vergani D., Mieli-Vergani G. Aetiopathogenesis of autoimmune hepatitis //World J. Gastroenterol. - 2008. - Vol.14. - N21. - P. 3306-3312.

[10] Zhou P., Lessa N., Estrada D.C. et al. Decellularized liver matrix as a carrier for the transplantation of human fetal and primary hepatocytes in mice //Liver Transpl. - 2011. - Vol.17. - N4. - P. 418-427.