CONTRIBUTION OF PEROXIDE PROCESSES TO PATHOGENETIC MECHANISMS OF EXPERIMENTAL LIVER CIRRHOSIS

A.F. Drygal, Candidate of Medicine, Associate Professor
Yu.V. Grubnik, Doctor of Medicine, Full Professor, Head of a Chair
R.S. Vastyanov, Doctor of Medicine, Full Professor, Head of a Chair
Odessa National Medical University, Ukraine

The results of a series of experimental trials using rats with toxic liver cirrhosis are given. After 12 hours, and in 1, 3, 5 and 7 days after the pathological condition simulation, blood erythrocytes in animals as well as homogenates of liver parenchyma and pancreatic lipid peroxidation were determined by calculation of concentration of lipoperoxidation intermediates and the activity of antioxidant enzymes. The findings suggest that the course of experimental cirrhosis is accompanied by sharp intensification of lipid peroxidation and associated inhibition of the enzymatic activity and non-enzymatic antioxidant protection units, as noted in 5 days with a maximum severity on the day 3. There was shown the involvement of erythrocytes in mediating the pathological process, as well as the liver parenchyma and pancreas. The authors conclude that the complex pathogenetic therapy of liver cirrhosis should include the administration of preparations with expressed antioxidant properties.

Keywords: experimental cirrhosis, lipid peroxidation, antioxidant defence, blood, erythrocytes, liver, pancreas, complex pathogenetic therapy.

http://dx.doi.org/10.18007/gisap.msp.v0i9.1267

Treatment of patients with liver cirrhosis, liver failure and complications, as well as other inflammatory and fibrotic affections of the hepatic parenchyma, is an urgent problem of surgical gastroenterology [1-3]. In the age of significant technological advances that can considerably improve the efficacy of diagnosis and treatment (and also minimally invasive one) of the majority of human diseases including diseases of the gastro-hepatoduodenal area, morbidity and mortality of patients because of the diffuse liver disease (this is a broader category of diseases as to medical classification including cirrhosis and hepatic failure) tend to increase [4, 5].

Economic and social importance of this pathology should be noted in addition to medical, as besides high morbidity and mortality this liver pathology results in significant economic losses related to the financial and economic costs of treatment, rehabilitation, and maintenance treatment of this category of patients, as well as the working age of the patients - 30-40 years [6,7]. A separate medical problem is the clinical progression of liver cirrhosis with signs of portal hypertension with the development of liver cancer, which is also a cause for concern for specialists. [8]

It can be noticed that this situation occurred in the field of biliary hepatology largely due to combination of insufficient notions on the pathogenetic mechanisms of liver failure and cirrhosis, as well as the lack of adequate effective schemes of complex determined pathogenetic therapy of this pathology. Taking this into consideration and having long-term experience in surgical treatment and clinical follow-up of this group of patients, we started basic research in order to clarify the pathogenetic mechanisms of hepatocyte necrosis. Taking into account the systemic disturbances in the patients with liver cirrhosis, rapid development of hepatocellular insufficiency, as well as frequent development of multiple organ dysfunction syndrome with involvement of the pancreas, gall bladder, stomach, vascular component in the pathological process during this disease, we hypothesized the involvement of one of the typical pathological process, which was the inflammation in the pathogenesis of the disease under study [9, 10].

It is known that enhancement of lipid peroxidation is one of the body's response manifestations to the effect of the alternating factor that triggers a systemic inflammatory response. So, the purpose of the present study was to assess the intensity of lipid peroxidation in animals at experimental liver cirrhosis (ELC). An additional objective of the work was a comparative study of lipid peroxidation expression in the parenchyma of the liver and pancreas as well as peroxide disorders in the erythrocytes.

Materials and methods.

Experimental studies were carried out under the conditions of chronic experiment in 80 Wistar rat males weighing 250 to 320 g in accordance with the requirements established in national and international guidelines, rules and regulations on the use of laboratory animals in experimental studies, as well as the requirements of the bioethics Commission of Odessa National Medical University.

The model of liver cirrhosis was reproduced in rats in toxic liver affection with hepatotropic poison - carbon tetrachloride, which had a direct cytolytic effect on the hepatic parenchyma [11]. CCI4 solution was prepared from pure (99.99% purity) drug by adding refined sunflower oil (final solution concentration was 50%) and administered orally using a plastic probe two times a week during 10 weeks. Control animals (n = 9) were orally administered 0.5 mL of 0.9% NaCl saline in similar conditions. The control of ELC formation was performed by laparotomy with biopsy followed by histological examination of biopsy samples in the treated and control groups.

21 (26.3%) of 80 rats died from acute liver failure during the experiment. The remaining 59 rats were euthanized by overdose of etaminal sodium (100 mg/kg/ip) in 12 hours, and in 1, 3, 5 and 7 days after the formation of ELC. The animals’ liver and pancreas were removed, a homogenate of the organ was prepared, after which the tissue samples were homogenised in the medium with 10 mM Tris-HCl buffer (pH = 7.4) at a ratio of 1:9. In order to get solid fraction the homogenate has been centrifuged for 10 min at 3000 g (t = 0±2 ° C). The supernatant was used to determine the concentration of intermediate products of lipoperoxidation - malondialdehyde (MDA), diene conjugates (DC) - and the activity of antioxidant enzymes.
The concentration of lipid peroxidation products and antioxidant enzyme activity in the blood of rats in different periods after reproduction of liver cirrhosis

| Indices under study                          | Control group, n=9 | Values of the studied indices during different periods after reproduction of ELC (M±m), n = 10 |
|---------------------------------------------|--------------------|---------------------------------------------------------------------------------|
|                                             |                    | in 12 hours | in 24 hours | on the 3\textsuperscript{rd} day | on the 5\textsuperscript{th} day | on the 7\textsuperscript{th} day |
| Malondialdehyde, mcmol/l                    | 1.4±0.11           | 2.69±0.18*** | 3.77±0.29*** | 4.41±0.37*** | 3.86±0.26*** | 2.27±0.23*** |
| Diene conjugates, mcmol/l                   | 0.41±0.05          | 0.70±0.07**   | 0.86±0.08**   | 0.97±0.11*** | 0.84±0.07*   | 0.67±0.06*   |
| Catalase, cond. u                           | 1.92±0.13          | 1.31±0.13*** | 1.18±0.12**   | 1.08±0.10**   | 1.21±0.11*** | 1.49±0.14*** |
| SOD units / ml                              | 2.79±0.17          | 1.68±0.16**   | 1.56±0.14**   | 1.48±0.13**   | 1.62±0.17*   | 1.97±0.20*   |
| Total glutathione mM                        | 20.1±0.6           | 15.7±1.1***   | 15.1±1.0**    | 14.4±1.2**    | 15.6±1.3*** | 16.6±1.3***  |
| a-tocopherol, (mcmol / ml)                  | 51.8±3.7           | 38.9±3.8*     | 35.9±3.5**    | 33.4±3.3**    | 36.2±3.7*     | 37.3±3.6*    |

Note: in all Tables * - p <.05, ** - p <.01, and *** - p <.001 - significant differences of the studied indices compared to those values in the control group (ANOVA statistical test)

Results and discussion.

There was a significant accumulation of MDA and DC in the blood of rats with ELC, absolute indices and concentrations of which reached 2.69 ± 0.18 nmol/l and 0.70 ± 0.07 mmol/l, respectively in 12 hours of the process, which was 1.9 times (p < 0.01) and 1.7-fold (p < 0.1) higher than in the control cases (Table 1). Later on, the value of MDA and DC continued to increase, reaching a maximum on the 3rd day of the pathological process when the value of the indices studied exceeded those in the controls by 3.1 times and 2.4 times (in both cases p < 0.01). Subsequently, there was a slight decrease in the value of MDA and DC, the concentration if which remained significantly higher than in controls (p < 0.5, Table 1) on the 7th day.

Under these conditions, the blood of rats showed a significant reduction in the activity of antioxidant enzymes - catalase, SOD, glutathione and...
α-tocopherol, indices of the absolute activity were minimal during 1 - 3 days since the moment of ELC reproduction (p < 0.1). Subsequently the activity of the studied enzymes did not restore until the 7th day of the experiment (p <0.5, Table 1).

A concentration of the intermediate products of lipid peroxidation in the red blood cells had a similar tendency: intense pathobiochemical changes in the red blood cells were revealed during 1-5 days of the ELC course with the highest concentration of MDA and DC on the 3rd day of the pathological process, when the studied indices exceeded those in control cases in both cases (p < 0.01, Table 3). The maximum expression of the intermediate products of lipid peroxidation accumulation was observed on the 1st day of ELC (p < 0.01) with a slight decrease in the studied indices on the 3rd (p < 0.01) and 5th (p < 0.5) days of the experiment. On the 7th day of the experiment the values of the studied indices did not differ in the experimental and control groups (p> 0.5).

A course of ELC induced a significant increase of MDA and DC concentration in the liver tissue, which was by 85% and 129% greater respectively already in 12 hrs after reproduction of the pathological state in comparison with those of control animals (p < 0.01, Table 3). Maximum intensity of the changes marked was recorded after 24 hours from the moment of the ELC reproduction (p < 0.01) and has been observed for 3 days (with the exception of MDA concentration), later the studied indices were similar both in the experimental and control groups (p> 0.5).

Thus, our results after a critical analysis allow us formulate the following basic points concerning the pathophysiological mechanisms of ELC. First, the ELC course is accompanied by the increased lipid peroxidation manifested in the accumulation of intermediate products of lipid peroxidation and decreased enzymatic activity, and non-enzymatic antioxidant protection units. These facts are evident in Table 3.

| Indices under study | Control group, n=9 | Values of the studied indices during different periods after reproduction of ELC (M±m), n = 10 |
|---------------------|--------------------|---------------------------------------------------|
| Malon dialdehyde, mcmol / l | 2.87±0.19 | 4.82±0.33 *** | 5.11±0.41 *** | 4.59±0.29 ** | 3.61±0.21 * | 3.14±0.23 * |
| Diene conjugates, mcmol / l | 0.47±0.05 | 0.85±0.08 *** | 0.92±0.08 *** | 0.67±0.09 * | 0.55±0.08 | 0.43±0.04 * |
| SOD units / ml | 1.79±0.17 | 1.05±0.08 ** | 0.98±0.08 ** | 1.29±0.08 * | 1.44±0.12 * | 1.74±0.14 * |
| Glutathione peroxidase, units/g | 2.71±0.19 | 1.47±0.12 ** | 1.31±0.11 *** | 1.82±0.13 * | 2.04±0.16 | 2.30±0.18 |
| Glutathione reductase, units/g | 2.59±0.14 | 1.67±0.12 ** | 1.43±0.12 *** | 1.71±0.13 * | 2.19±0.17 | 2.54±0.21 |
are consistent with the known views [19-21] on the pathogenetic role of intensification of lipid peroxidation in a number of pathological processes and inflammation under the action of heat, radiation factor and other alternating influences. Secondly, the data obtained demonstrates the involvement of the blood cell unit, namely erythrocytes, in pathogenetic mechanisms of the hepatocellular destruction; there is increased concentration of lipid peroxidation products and reduction of the antioxidant enzyme activity in the erythrocytes unidirectional with the blood plasma. Summing up these results and suggestions it becomes obvious that there is generalization of the pathological process in liver cirrhosis, which explains both its acceleration and magnitude of the abnormal cell changes, that should necessarily be taken into account in the clinical conditions when determining the appropriate treatment strategy for these patients.

Thirdly, we have shown the accompanying processes of accelerated lipid peroxidation and inhibition of antioxidant protection expression, which take place directly in the liver tissue. In our opinion this data explains the rapid development of large volume and, as a rule, irreversibility of the pathological process of the cellular destruction in liver cirrhosis. And finally, fourthly, taking into account the anatomical proximity, common physiological functioning and disorders similar to the liver parenchyma, which were manifested in shifting the dynamic equilibrium in the "POL-antioxidant system" towards the increased lipid peroxidation, we were able to clearly register the accumulation of lipid peroxidation products and inhibition of the antioxidant protection processes expression in the parenchyma of the pancreas somewhat less pronounced than in the liver tissue.

Summarizing the data, pathophysiological mechanisms of development of multiple organ dysfunction syndrome in liver cirrhosis, the development of liver fibrosis, portal hypertension and/or liver failure become obvious. Taking into account the facts of intensification of lipid peroxidation and resulted inhibition of antiradical protection activity, inclusion of drugs with antioxidant properties facilitating and/or preventing the process of the hepatocellular destruction is important for making up schemes of complex pathogenetic reasonable pharmacotherapy of liver cirrhosis. It may also provide crucial protective effect in preventing the development of hepatic insufficiency.

References:

1. A comparative study of early vs. delayed laparoscopic cholecystectomy in acute cholecystitis., R.P. Yadav, S. Adhikary, C.S. Agrawal et al., Kathmandu Univ. Med. J. (KUMJ). – 2009., Vol. 7, No. 25., pp. 16-20. http://dx.doi.org/10.3126/kumj.v7i11.1759
2. Surgical outcomes of open cholecystectomy in the laparoscopic era, A.S. Wolf, B.A. Nijssse, S.M. Sokal et al., Am. J. Surg. – 2009., Vol. 197, No. 6., pp. 781-874. http://dx.doi.org/10.1016/j.amjsurg.2008.05.010
3. Zakharash M.P., Zaverny L.G., Stel’makh A.I., Zakharash Yu.M., Bekmuradov A.R., Kalashnikov A.A., Butenko D.I. Surgical tactics in acute cholecystitis and its complications in patients with increased operational and anesthetic risk. Kharkiv Surg. School. - 2007; 4(27), pp. 92-96 (In Russian).
4. Grubnik V.V., Koval’chuk A.L., Zagorodnyuk O.N., Grubnik Yu.V. Endovascular surgery in the complex treatment of patients with cholelithiasis with concomitant liver cirrhosis., Ukr. J. Surg. 2009; 5., pp. 58-60 (In Russian).
5. Pavlovsky M.P., Kolomiytsev V.I., Shakhova T.I. Modern diagnostic-curing algorithm in case of complicated acute calculous cholecystitis. Military Health Problems 2007; 20., pp. 56-62 (In Ukrainian).
6. Modern methods of surgical correction of ascitic syndrome in cirrhosis., Borisov A.E., Andreev G.N., Zemlyanoy V.P. et al. – Saint Petersburg, 2000. – 222 p. (In Russian).
7. Pathogenesis, diagnosis and treatment of liver cirrhosis complicated with refractory ascites., Andreev G.N., Borisov A.E., Ibadilin A.S. et al. – Novgorod., 1999. – 191 p. (In Russian).
8. Yeramishantsyev A.K., Manuk’yan G.V. "Today" and "tomorrow" of portal hypertension surgery: a view at
   the problem. Ann Surg. Hepatol 1998; 2., pp. 72-75 (In Russian).
9. Free-radical processes and inflammation (pathogenic, clinical and therapeutic aspects), Sologub T.V., Roman'tsov M.G., Kremen N.V. et al. – Moskva, "Academy of Natural Sciences" Publisher", 2008. - 350 p (In Russian).
10. Oxidative stress. Pro-oxidants and antioxidants., Menshikova Ye.B., Lakin V.Z., Zenkov N.K. et al. – Moskva, “Word” Publisher, 2006. - 556 p (In Russian).
11. Galperin E.I. Liver cirrhosis and ascites induction in the experiment. Exp. Surg 1960; 1., pp. 46-49 (In Russian).
12. Andreeva L.L., Kozhemyakin L.A., Kishkin A.A. Modification of method for determining the lipid peroxide in the test with thio Barbital acid. Labs 1998; 11., pp. 41-46 (In Russian).
13. Kogan V.S., Orlov O.N., Prilipko L.L. Problem of analysis of endogenous lipid peroxidation products. - Moskva, Medicine, 1988. - 287 p. (In Russian).
14. Dubinina Ye.Ye., Salnikova L.A., Yefimova L.F. The activity of superoxide dismutase and erythrocyte isoenzyme spectrum of human plasma. Labs 1983; 10., pp. 30-33 (In Russian).
15. Moin V.M. A simple and specific method for determining the activity of glutathione peroxidase in erythrocytes. Labs 1986; 12., pp. 724-727 (In Russian).
16. Vlasova S.N., Shabunina Ye.L., Pereslegina I.A. The activity of glutathione-dependent enzymes of red blood cells in chronic liver diseases in children. Labs 1990; 8., pp. 19-21 (In Russian).
17. Kisilevich R.Sh., Skvarko S.I. Determination of vitamin E in the blood. Labs 1972; 8., pp. 473-475 (In Russian).
18. Rudakova-Shilina M.K., Matykhova N.P. Evaluation of antioxidant system. Labs 1982; 1., pp. 19-22 (In Russian).
19. Gozhenko A.I., Nasibullin B.A., Kohn Yu.S. On the state of redox processes in the mucosa of duodenal ulcer in the dynamics of the process. Odessa Med. J. 1998; 1., pp. 31-32 (In Ukrainian).
20. Mikaelyan E.M., Shaldzhyan A.L., Mkhitarian V.G. Lipid peroxidation in erythrocyte membranes and blood under stress. J. Exp. Clin. Med. 1984; 24(2), pp. 123–130 (In Russian).
21. Morsy M.A. Protective effect of lisinopril on hepatic ischemia/
reperfusion injury in rats., M.A. Morsy., Ind. J. Pharmacol. - 2011., Vol. 43, No. 6., pp. 652-655.

Information about authors:
1. Alexander Dzygal - Candidate of Medicine, Associate Professor, Odessa National Medical University; address: Ukraine, Odessa city; e-mail: adzygal@eximneft.com
2. Yuri Grubnik - Head of a Chair, Doctor of Medicine, Full Professor, Odessa National Medical University; address: Ukraine, Odessa city; e-mail: adzygal@eximneft.com
3. Ruslan Vastyanov - Head of a Chair, Doctor of Medicine, Full Professor, Odessa National Medical University; address: Ukraine, Odessa city; e-mail: adzygal@eximneft.com

INTERNATIONAL UNION OF COMMERCE AND INDUSTRY

Union of commercial enterprises, businessmen, scientists, public figures and politicians from different countries. The union combines the social and commercial elements of functioning.

- Promotion of international consolidation and cooperation of business structures
- Promotion of development of commercial businesses of various kinds
- Assistance in settlement of relations and businessmen with each other and with social partners in business environment
- Assistance in development of optimal industrial, financial, commercial and scientific policies in different countries
- Promotion of favorable conditions for business in various countries
- Assistance in every kind of development of all types of commercial, scientific and technical ties of businessmen of different countries with foreign colleagues
- Promotion of international trade turnover widening
- Initiation and development of scientific researches, which support the effective development of businesses and satisfy the economic needs of the society
- Expert evaluation of activities in the field of settlement of commercial disputes, establishment of quality standards and defining of factual qualitative parameters of goods and services
- Legal and consulting promotion of business
- Establishment and development of activities of the international commercial arbitration
- Exhibition activities
- Holding of business and economic forums

www.iuci.eu