INTEGRAL ESTIMATION OF OXIDATIVE STATUS IN PATIENTS WITH ACUTE TOXIC HEPATITIS AND CHRONIC ALCOHOLIC LIVER DISEASE

A.Y. Shchupak, O.A. Lebedko, V.V. Yukhno

Far-eastern State Medical University, Ministry of Health of the Russian Federation, Khabarovsk, Russian Federation

BACKGROUND Acute toxic hepatitis associated with acute poisoning with alcohol-containing disinfectants remains a medical and social problem.

MATERIAL AND METHODS With an aid of chemiluminescence, we performed the integrated assessment of the oxidative status in the blood serum and homogenized liver biopsy tissue of 62 patients with the diagnosis «severe acute toxic hepatitis associated with the use of alcohol-containing disinfectants».

RESULTS The research showed that at the onset of a disease, patients with acute toxic hepatitis had an expressed activation of free radical oxidation of the blood serum and biopsy tissue. This was indicated by almost double increase in the intensity of free radicals generation (Ssp). This significantly increased production of peroxide (Sind-1) and hydroxyl radicals (Slum) — 2.5 and 1.86 times, respectively; also, it increased concentration of lipid hydroperoxides (h) almost three times, evidencing activation of the initial stage of lipid peroxidation
There was no statistically significant fall of figures indicating the liver parenchymal oxidative status 30 days after the admission. The same situation was observed 6 months after the beginning of the study.

CONCLUSION Analyzing chemiluminescence scans of blood serums up to 30 days from admission, it is possible to conclude indirectly on a condition of the oxidative status in a liver parenchyma of patients.

Keywords: acute toxic hepatitis, oxidative status, alcohol-containing disinfectants.

CL — chemiluminescence

FRO — free radical oxidation

PHMG — polyhexamethylene guanidine hydrochloride

INTRODUCTION

In recent years , the development of acute toxic hepatitis due to use of true alcohol surrogates has been one of the urgent issues in many regions of the Russian Federation. Thus, in 2006-2008, physicians and toxicologists faced with acute diseases accompanied by severe jaundice which led more than 12,400 people to a hospital. The uniformity of the clinical picture in most cases in different regions of Russia showed a single etiological factor of toxic hepatitis, which led to the conclusion about the equivalence of alcohol-containing liquids which caused the disease [1]. As shown by analytical studies, the main toxic substance was disinfectant polyhexamethylene guanidine hydrochloride (PHMG) [2].

The number of hospital patients only in Khabarovsk Center of Acute Poisoning in a period covering November, 2006 – January, 2008 was 150 people. [3]

According to some researchers, the severity of the disease and its protraction were associated with cholestasis, which was confirmed by histologic examination of the liver [4]. The diagnosis was complicated by the fact that in the majority of victims, the poisoning developed in the course of various stages of alcoholic liver disease [5].

It is known that the disturbance of free radical oxidation (FRO) is one of the leading pathogenetic element for liver diseases of various etiologies [6], and its symptoms objectively reflect the severity of tissue damage and the condition of the body's systems [7]. In the literature, there is evidence of free radical oxidation failure in chronic ethanol viral hepatitis *B, C* [8, 9]. At the same time, there is no data on oxidative status in toxic hepatitis associated with the use of alcohol-based disinfectants.

Aim of study: to examine changes in oxidative status in blood serum and homogenized hepatic byopsy materials in acute toxic hepatitis associated with the use of alcohol-containing disinfectants in patients with chronic alcoholic liver disease.

MATERIAL AND METHODS

Upon receiving the approval of the Ethics Committee to conduct investigations,

as well as voluntary informed consent of patients enrolled in the study using the method of chemiluminescence (CL), we performed an integrated assessment of oxidative status in blood serum and homogenized hepatic byopsy materials in 62 patients aged 24-70 years (42.31±1.09) with a diagnosis of "severe acute toxic hepatitis associated with alcohol-containing disinfectants consumption", treated at the Department for Acute Poisonings of the State Clinical Hospital No.10, Khabarovsk. Registration of CL was performed with the fluorescent spectrometer *LS* 50*B «PERKIN ELMER»*. Before the beginning of the disease, patients had drunk alcohol-based liquids, containig PHMG. Chronic alcoholic liver disease was a premorbid factor. For diagnostic purposes, the blood serum was examined in all patients on day 1 and 30 (with additional analysis 180 days later); percutaneous needle biopsy of the liver was performed as well using a special automatic gun *BARD* (USA), and biopsy needles *GALLINI* (Italy) of a diameter *G-16*. Columns of biopsy tissue (20x2 mm) were fixed in 10% neutral formalin solution. Histologic sections of 4-5 microns were prepared on a rotary microtome *Leica RM* 2135 and stained with hematoxylin, eosin, picro-fuchsin (Van Gieson's stain).

The control group consisted of 11 patients aged from 28 to 70 years with cholelithiasis and minimal changes in the liver parenchyma (steatosis *A* 0-I + fibrosis *F* 0-I), who had already underwent planned endoscopic cholecystectomy.

During the stay in hospital, patients underwent complex therapy, developed by FSI "Scientific-Practical Toxycology Center" of the Federal Agency for Healthcare and Social Development. The complex pathogenetic therapy included Hepasol (500 ml, i.v. drop infusion, 1-2 times per 24h) as the primary hepatoprotective and stabilizer of membranes. The obligatory antibiotic to suppress intestinal microflora was Ciprofloxacin 500 mg 2 times per 24h for 7 days. Intravenous infusion were administered to manage water and electrolyte balance, acid-base status.

The study of quantitative traits was performed by comparing average values of two sample sets with calculation of Student's t-criterion and significance level *(p)*. For multiple comparisons the Newman-Keuls criterion was calculated.

RESULTS AND DISCUSSION

The diagnostic results are presented in Table 1. The findings indicate that estimated parameters in serum of the control group almost did not differ from normal values. At the onset of the disease in patients with acute toxic hepatitis, the pronounced activation of free radical oxidation in the liver parenchyma occurred. This is indicated by almost 2-fold increased (relative to the control group) intensity of free radical generation (Ssp). This significantly increased production of peroxide *(Sind*-1) and hydroxyl radicals *(Slum)* — 2.5 and 1.86 times, respectively; and almost 3 times increased concentration of lipid hydroperoxide *(h),* which indicates activation of the initial stage of lipid peroxidation. In addition, analysis of the CL images of hepatic byopsy materials showed a statistically significant (relative to the comparison group) 1.73-times decrease in antioxidant antiradical protection.

Thirty days after admission, indicators of oxidative status in the liver parenchyma were statistically significantly increased relative to those in the control group, and its statistically significant decrease relative to values upon admission did not occur. We should note a particularly high rate of peroxide radicals accumulation of

(Sind-1) and concentration of lipid hydroperoxide *(h)* — the figures remained 2.39 and 2.48 times higher, respectively.

Analysis of CL images of blood serum over time showed the same pattern as in the parenchyma of the liver: improvement of all indicators of oxidative status relative to values in the control group upon admission to hospital and the lack of statistically significant reduction 30 days later.

The analysis based on Spearman's rank *(r)* correlation coefficient showed the presence of strong direct links between all the studied parameters of oxidative status in blood serum and gepatic byopsy tissues of patients at the onset of the disease (for *Ssp r=0.82, p<0.001; Sind-1 r=0.75, p<0.001; h r =0.43, p<0.05; Slum r=0.71, p<0.001; Sind-2 r=0.45, p<0.05; H (peroxide resistance of substrate) r=0.43, p<0.05),* and a month after hospital admission (Table. 2).

Table 1

Indicators of chemiluminescence (in relative units) in the development of acute toxic hepatitis *(M*±m)

Indicators	Reference values	Control group (*n*=11)	Upon arrival (*n*=62)	After 30 days (*n*=62)	After 6 months (*n*=35)
LIVER PARENCHYMA					
Ssp		0.117±0.008 P1<0.01	0.228±0.01 P4>0.05	0.189±0.017 P2<0.01	–
Sind-1		0.420±0.090 P1<0,01	1.045±0.07 P4>0.05	1.005±0.080 P2<0.01	–
h		0.260±0.090 P1<0.01	0.712±0.030 P4>0.05	0.647±0.050 P2<0.01	–
Slum		0.111±0.016 P1<0.05	0.207±0.020 P4>0.05	0.179±0.018 P2<0.05	–
Sind-2		0.405±0.080 P1<0.05	0.699±0.040 P4>0.05	0.643±0.060 P2<0.05	–
H		0.343±0.080 P1<0.05	0.624±0.040 P4>0.05	0.564±0.050 P2<0.05	–
BLOOD SERUM					
Ssp	0.110±0.006	0.112±0.008 P1<0.01	0.144±0.006 P4>0.05 P5>0.05	0.130±0.006 P2<0.05 P6>0.05	0.131±0.003 P3<0.05
Sind-1	0.258±0.028	0,257±0,030 P1<0,05	0.436±0.022 P4>0.05 P5>0.05	0.422±0.023 P2<0.05 P6>0.05	0.387±0.028 P3<0.05
h	0.100±0.005	0.101±0.010 P1<0.05	0.172±0.016 P4>0.05 P5<0.05	0.159±0,014 P2<0.05 P6>0.05	0.138±0.005 P3<0.05
Slum	0.101±0.009	0.102±0.008 P1<0.05	0.126±0.012 P4>0.05 P5>0.5	0.119±0.009 P2<0.05 P6>0.05	0.134±0.002 P3<0.05
Sind-2	0.281±0.026	0.286±0.024 P1<0.01	0.440±0.023 P4>0.05 P5<0.05	0.405±0.026 P2<0.05 P6<0.05	0.342±0.009 P3<0.05
H	0.202±0.026	0.200±0.020 P1<0.01	0.367±0.027 P4>0.05 P5<0.05	0.341±0.027 P2<0.05 P6<0.05	0.252±0.018 P3<0.05

Notes: P1 — statistically significant difference between indictors of the control group and upon admission; P2 — statistically significant difference between indicators of the control group and 30 days later; P3 — statistically significant difference of indicators between the comparison group and 6 months later; P4 — statistically significant difference of indicators uon admission and 30 days later; P5 — statistically significant differences of indicators upon admission and 6 months later; P6 — statistically significant difference of indicators 30 days later and 6 months later

Table 2

Correlation coefficients *(R)* and its levels of significance *(p)* between the indices of blood serum chemiluminescence and liver homogenates in acute toxic hepatitis upon admission

	LIVER HOMOGENATE											
BLOOD SERUM		*Ssp*		*Sind*-1		*h*		*Slum*		*Sind*-2		*H*	
		R	*p*	*R*	*p*	*R*	*p*	*R*	*p*	*R*	*p*	*R*	*p*
	Ssp	**0.82**	<0.001	**0.67**	<0.001	0.43	>0.05	**0.71**	<0.001	**0.65**	<0.001	**0.59**	<0.001
	Sind-1	**0.78**	<0.001	**0.75**	<0.001	0.0	>0.05	**0.68**	<0.001	**0.61**	<0.001	**0.59**	<0.001
	h	**0.42**	<0.05	**0.48**	<0.05	**0.43**	<0.05	**0.46**	<0.05	**0.32**	<0.05	**0.34**	<0.05
	Slum	**0.72**	<0.001	**0.68**	<0.001	0.43	>0.05	**0.71**	<0.001	**0.65**	<0.001	**0.60**	<0.001
	Sind-2	**0.38**	<0.05	**0.46**	<0.05	0.40	>0.05	**0.48**	<0.05	**0.45**	<0.05	**0.39**	<0.05
	H	**0.32**	<0.05	**0.45**	<0.05	0.41	>0.05	**0.38**	<0.05	**0.47**	<0.05	**0.43**	<0.05

The difference in the intensity of free radicals generation *(Ssp),* as well as concentration of peroxide *(Sind*-1) and hydroxyl radicals *(Slum)* compared with the onset and 30 days after admission was statistically significant. All parameters studied in the serum of patients 6 months after the study were significantly higher relative to values in the control group.

Antioxidant antiradical activity *(Sind*-2) 6 months after the study was significantly reduced in comparison with the primary examination, *H* was increased.

Thus, even 6 months after the beginning of the study, oxidative status indicators in blood serum remained higher relative to values of the control group [10].

CONCLUSIONS

1. In patients with acute toxic hepatitis associated with the use of alcohol-based disinfectants and also having chronic alcoholic liver disease, activation of free radical oxidation occurs, as indicated by a two-fold increase in the intensity of generation of free radicals in the analysis of CL images of hepatic byopsy materials and inhibition of antioxidant anti-radical protection.

2. There is a direct correlation between CL-indicators of oxidative status of blood serum and liver homogenates in patients with acute toxic hepatitis associated with consumption of alcohol-containing disinfectants.

3. CL images of blood serum made upon admission and 30 days later in patients with acute toxic hepatitis associated with consumption of alcohol-based disinfectants in the course of chronic liver disease alcohol allow to conclude indirectly on the state of oxidative status in the liver parenchyma.

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ALEKSANDR YURYEVICH SHCHUPAK, CANDIDATE OF MEDICAL SCIENCES,
HEAD OF THE DEPARTMENT FOR CLINICAL TOXICOLOGY AND EMERGENCY MEDICINE
FAR-EASTERN STATE MEDICAL UNIVERSITY
E-MAIL: SCHUPAKALEX@MAIL.RU