International Journal of BioMedicine 4(2) (2014) 79-81

INTERNATIONAL JOURNAL OF BIOMEDICINE

CLINICAL RESEARCH

The State of the Antioxidant System in Chronic Hepatitis C

Gulnozakhon Z. Aripkhodjaeva

Tashkent Pediatric Medical Institute, Tashkent, Uzbekistan

Abstract

Chronic hepatitis of viral etiology ranks very high in human pathology with respect to its socio-economic and medical significance. In viral hepatitis, membrane destruction occurs via the processes of lipoperoxidation, a valid factor that triggers the mechanism of hepatocyte necrosis. The glutathione system is also involved in the first line of cell defense actions against the effect of the free radicals. In this study, 128 patients with Chronic Hepatitis C (CHC) were examined. The degree of antioxidant defense was determined by the indicators of the activity of the glutathione and glutathione-dependent enzymes. The total, reduced and oxidized glutathione levels were determined by V. G. Chernishov. The activity of the glutathione-dependent enzymes, viz., glutathione peroxidase (GP), glutathione reductase (GR) and glutathione transferase (GT) was measured by the method prescribed by S. N. Vlasova and co-authors (1990). The results of the investigations performed revealed that in CHC patients, deep-seated disorders were observed in the glutathione system manifested by a decrease in the total glutathione levels, its oxidized and reduced forms, changes in the glutathione enzymes and the interrelationships between the intensity of the changes and the degree of the intoxication syndrome.

Keywords: *hepatitis C, glutathione, glutathione-dependent enzymes.*

Introduction

It is well recognized that in viral hepatitis, including CHC, the universal mechanism of the hepatocyte apoptosis developed as a result of the increased production of the active forms of oxygen culminating in an excessive lipoperoxidation of the membrane structures [1-4]. The cell antioxidant defensive system is the main factor of defense during the activation of lipoperoxidation; therefore, the insufficiency of the antioxidant defensive system has become one of the factors of activation for the pro-oxidant system. The first line of the cell defense against the aggressive effect of the free radicals includes the glutathione system comprising the total glutathione level, its reduced and oxidized forms as well as the glutathione-dependent enzymes, viz., glutathione transferase, glutathione reductase and glutathione peroxidase [5,6]. An inhibition of the activity of even one of the glutathione-dependent enzymes may result in the excessive accumulation of the active oxygen forms and consequently damaging of the hepatocyte membranes [7,8].

The purpose of the investigation was to study the state of the antioxidant system in the different variants of CHC.

***Corresponding author:** Gulnozakhon Z. Aripkhodjaeva. Tashkent Pediatric Medical Institute, Tashkent, Uzbekistan. E-mail: gulnozakhon85@mail.ru*

Material and Methods

This study included 128 patients with CHC, between 19 and 45 years of age, at the reactivation stage. Among them 54(42%) were men and 74(58%) were women. Diagnosis was established based on the clinical-laboratory data as well as the results of the PCR (RNA-HCV) and IEA (anti-HCV). The control group was composed of 20 practically healthy subjects having no hepatitis markers. The patients were categorized according to the viral C genotypes as genotype 1 including 47 (36.8%) patients, genotype 2 with 54 (42.1%) patients and genotype 3 having 27 (21.1%) patients. The disease duration extended from 5 to 15 years. The patients, according to the International Classification (Los-Angeles, 1994), were divided into groups based on the expression of the hepatic cytolysis syndrome: Group I with a lower activity level (ALT- from 1.3 to 2.04 mmol/l), Group II having a moderate activity level (ALT- from 2.05 to 3.40 mmol/l) and Group III including those with a marked activity level (ALT- 3.5 mmol/l and more). Related to the level of the viral load, the patients were also divided into three groups: Group I – $2x10^6$ - $2x10^7$ copies/ml, Group II – $2x10^4$ – $2x10^5$ copies/ml and Group III – $2x10^2$ –$2x10^3$ copies/ml.

The states of the antioxidant defensive system were determined by the parameters of the glutathione activity and glutathione-dependent enzymes. The total, reduced

and oxidized glutathione levels were determined by V.G. Chernishov [9]. The activity of the glutathione-dependent enzymes, viz., glutathione peroxidase (GP), glutathione reductase (GR) and glutathione transferase (GT) was measured by the method prescribed by S. N. Vlasova and co-authors [10].Viral loading and the genotype of the C virus were defined by the PCR method of real time, Rotor Gene (Corbet Research, Australia) with "Ribosorb-amplifens" (Russia) kits. Statistical analysis was performed using the statistical software «Statistica 6.0» for Windows. *The mean (M)* and standard error of the mean (SEM) were deduced. Data were analyzed by the Student t-test. A value of $P<0.05$ was considered statistically significant.

Results

The analysis of the results obtained showed the absence of any reliable interrelationship between the changes in the glutathione parameters and the activity of the glutathione-dependent enzymes with the marked cytolysis syndrome and the level of the viral load in the CHC patients studied. A significant disorder was observed in the variational rows, which is evidently a result of the multifactor processes affecting the activity of the glutathione system. The following phase of our investigation was the study of a possible interrelationship existing between the state of the glutathione system and the intensity of the intoxication syndrome in these patients. In the patients studied, the syndrome of intoxication was evaluated by the expression of the following symptoms: periodical weakness, stable weakness, rapid fatigability after a normal physical load, increased irritability, poor memory, depression, lack of being in good spirits after a night's sleep, sleep disorders, rheumatic pains in the muscles and febrile chills. With regard to the number of symptoms registered, the patients were classified under three groups: Group I patients possessing 1-3 symptoms, Group II including those with 4-6 symptoms and Group III with those having 7-10 clinical symptoms of intoxication.

The results of the investigations performed showed that in patients with CHC, changes in the parameters of the glutathione system correlated with the intensity of the intoxication syndrome, being incoherent in character (Table 1). As evident from the data presented in the Table 1, the total glutathione levels and their reduced variants were reliably lower than the characteristics expressed by the control group. The lowest parameters were registered in those patients with the marked intoxication syndrome. The changes in the parameters of the oxidized glutathione revealed analogue direction, although reliability in the changes was noted only in the Group III individuals. In contrast to the glutathione levels, a variety was observed with respect to the changes in the activity of the glutathione-dependent enzymes. Thus, the GR activity increased in the CHC patients having the least clinical intoxication, although with the progression of the intoxication syndrome it began to reduce, whereas in the Group III patients this reduction already showed a reliable character. The GP activity was reliably lower than the control values in all the patients studied and the expression of these changes increased with the progression of the intoxication syndrome. The GT parameters in the Group I patients having the least number of clinical symptoms of intoxication revealed no reliable differences from the control; however, with the increase in the number of symptoms registered in patients from Groups II and III a reliable decrease in the enzyme activity was observed.

The reduction in the glutathione level was determined to a significant degree by a change in the activity of the enzymatic systems, regulating the correlation of its oxidized and reduced forms. To realize their antioxidant functions, the glutathione-dependent enzymes used reduced glutathione [8,11]. In patients with CHC, a marked depression was noted in the GP activity, the key enzyme in the process of utilization of the active forms of oxygen and the products of peroxidation, which play a pivotal part in the defense of the cell membrane structures [12]. Against this background of the reduction in the glutathione levels and GP activity in the patients studied, an increase was observed in the GR activity. The GR provided the bio-regeneration of the reduced glutathione and the stimulation of its activity may be considered as a compensatory reaction for the maintenance of the glutathione level required to realize the adaptation processes in the patient's body [6,13,14].

Thus, the results of the investigations performed in this study showed that in CHC deep-seated disorders were evident in the glutathione system. They were manifested by a reduction in the total glutathione levels, its oxidized and reduced forms, an inhibition of the activity of the glutathione-dependent enzymes and damage to the homeostatic functions of the antioxidant processes, all of which evidently contributed to the production of the irreversible changes in the liver tissue.

Table 1.

Parameters of the glutathione system in CHC patients with the intoxication syndrome

Parameters	Control Group n=20	Degree of the intoxication syndrome		
		Group I n=32	Group II n=48	Group III n=48
GR, mmol/NADFN/Hb	2.84±0.02	3.30±0.11*[3]	2.45±0.20[1,2]	1.14±0.21*[1,2]
GP, mmol/GSSG/Hb	583.3±6.63	434.85±62.45*[3]	363.30±36,44*[3]	233.17±23,78*[1,2]
GT, mmol/GSN/Hb	2.43±0.03	2.22±0.29	1.24±0.07*[1,3]	0.89±0.11*[1,2]
Glutathione total, mmol/ml	45.33±1.53	28.20±1.90*[3]	25.47±1.61*[3]	19.15±2.18*[1,2]
Glutathione reduced, mmol/ml	43.17±1.55	26.20±1.40*[3]	23.77±1.34*[3]	18.03±2.12*[2]
Glutathione oxidized, mmol/ml	2.16±0,09	2.00±0.50	1.70±0.31	1.10±0.20*

*Note: *- P<0.05 vs control; [1]- P<0.05 vs Group I; [2]- P<0.05 vs Group II; [3] – P<0.05 vs Group III.*

Conclusions

1. In this study, the patients with CHC revealed a reliable reduction in the total glutathione levels, its oxidized and reduced forms and incoherent changes in the activity of the glutathione-dependent enzymes, viz., GR, GP and GT.

2. In the patients with CHC in this study, no reliable interrelationship was evident between the degree of changes in the glutathione parameters and the glutathione-dependent enzymes with a viral load and intensity of the cytolytic syndrome.

3. In the groups of the patients studied a reliable correlation was found between the degree of changes occurring in the glutathione parameters and the glutathione-dependent enzymes and the intensity of the intoxication syndrome.

References

1. Beloborodova EI, Savchenko IV, Beloborodova EV, Rachkovskiy MI, Burkovskaya VA, Abdrashitov RF, et al. Metabolic aspects of the prediction of outcome in chronic viral hepatitis. Klin Med (Mosk) 2005; 83(2):53-6. [Article in Russian].
2. Levent G, Ali A, Ahmet A, Polat EC, Aytac C, Ayse E, et al. Oxidative stress and antioxidant defense in patients with chronic hepatitis C patients before and after pegylated interferon alfa-2b plus ribavirin therapy. J Transl Med 2006, 4:25.
3. Boya P, de la Pena A, Beloqui O, Larrea E, Conchillo M, Yurdana Castelruiz Y, et al. Antioxidant status and glutathione metabolism in peripheral blood mononuclear cells from patients with chronic hepatitis C. J Hepatol 1999; 31(5):808-14.
4. Paracha UZ, Fatima K, Alqahtani M, Chaudhary A, Abuzenadah A, Damanhouri G, et al. Oxidative stress and hepatitis C virus. Virol J 2013; 10:251
5. Urazova OI, Kravets EB, Novitskiy VV, Rogaleva AV, Vasileva OA, Kuznetsova VN, et al. Activity of lipid peroxidation and glutathione system in the blood lymphocytes of the patients with diffusive toxic goiter. Bull Siber Med 2008; 4:47-51. [Article in Russian].
6. Chesnokova NP, Ponukalina EV, Bizenkova MN. Molecular-cellular mechanisms of inactivation of free radicals in the biological systems. Usp Sovr Estes 2006; 7:20-36. [Article in Russian].
7. Aripov OA. Molecular mechanisms of the cellular apoptosis in hepatitis and methods of their correction. Abstract of ScD Thesis. Tashkent; 2010.
8. Glushkov SI. Disorder of the glutathione system and its role in the pathogenesis of acute intoxications with xenobiotics with various mechanisms of toxic effect. Abstract of ScD Thesis. Saint-Petersburgh; 2006. [Abstract in Russian].
9. Chernishov VG. Determination of the reduced and oxidized glutathione in the erythrocytes of pregnant women. Lab Delo 1983: 3:31-33. [Article in Russian].
10. Vlasova SN, Shabunina EI, Pereslegina IA. The activity of the glutathione-dependent enzymes of erythrocytes in chronic liver diseases in children Lab Delo 1990; 8:19-22. [Article in Russian].
11. Delanin NV, Gerasimov AM. Mechanisms of body antioxidant defensive system in change of the regime of oxygen supplying. Materials of International Scientific Conference. Grodno1993; p.18-19. [Abstract in Russian].
12. Mirova GD, Romanova NN. Factor of nonspecific defense in viral hepatitis B. All-Union conference:"Molecular mechanisms of the development of infectious diseases". Zvenigorod 1990; p.68. [Abstract in Russian].
13. Vladimirov YuA. Free radicals in the biological systems. Soros Obraz J 2000; 6(12):13-19.
14. Zenkov NK, Lapkin VZ, Menshikova EB. Oxidized stress. Biochemical and pathophysiological aspects. M: MAIK, Nauka, Interperiodika; 2001. [Book in Russian].