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Assessment of selected oxygen metabolism in patients with chronic hepatitis C during treatment with pegylated interferon alfa and ribavirin

Ocena wybranych wskaźników metabolizmu tlenowego u pacjentów leczonych pegylowanym interferonem alfa i rybawiryną

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S u m m a r y

Introduction. In the year 2012 there were several hundred millions patients with chronic hepatitis C (CHC). The etiopathogenesis of this disease is not clear. It was suspected that free radicals may play an important role in the process.

Aim. The aim of the research was to analyse of the indictors of oxygen metabolism among the patients with CHC treated with pegylated interferon alpha (Peg-IFN) and ribavirine (RBV).

Material and methods. The study was carried out on a group of 31 patients (*23 males and 8 females*) with an average age of 41 +/- 11. During the therapy results were controlled four times (before treatment, in the 12th, 24th and 48th week), measuring the level of superoxide anion radicals at rest and after stimulation, the malonic dialdehyde concetration (MDA), the activity of catalase (Cat), glutathione preoxidase (Gpx) and superoxide dismutase.

Results. The results showed an elevated concentration of superoxide anion radicals for patients with chronic hepatitis C. The concentration decreased after the treatment. The MDA concentration was lower after the Peg-IFN + RBV treatment. The activity of antioxidant barrier was a proof of elevated rates of indicator enzymes in the case of patients with CHC and of continuous activity reduction during the treatment with Peg-INF and RBV.

Conclusions. 1. CHC should be described as an oxidative stress disease. 2. Oxidative stress CHC stimulate the antioxidant barrier to generate antioxidative enzymes. 3. Treatment with Peg-IFN and RBV has a positive impact on the oxygen metabolism among patients with CHC, reducing the production of free oxygen radicals and the activity of the antioxidative barrier.

S t r e s z c z e n i e

Wstęp. W 2012 roku nawet kilkaset milionów ludzi na świecie mogło być zakażonych wirusem zapalenia wątroby typu C (ang. *hepatitis C virus* – HCV). Duży odsetek chorych wykształcający przewlekłe zapalenie wątroby (pzw C) oraz progresja choroby ku marskości i pierwotnemu rakowi wątroby stanowią istotny problem medyczny. Etiopatogeneza schorzenia nie została do końca poznana. Podejrzewa się udział wolnych rodników w przebiegu choroby.

Cel pracy. Celem niniejszej pracy była ocena wybranych wskaźników metabolizmu tlenowego u chorych z pzw C leczonych pegylowanym interferonem (Peg-IFN) alfa i rybawiryną (RBV).

Materiał i metody. Badaniem objęto grupę *31 pacjentów* (*23 mężczyzn*, *8 kobiet*) o średniej wieku 44 +/- 11. W trakcie badania oznaczono czterokrotnie (przed leczeniem, po 12, 24 i 48 tygodniach) generowanie anionorodnika ponadtlenkowego (spoczynkowe, stymulowane), stężenie dialdehydu malonowego (MDA) oraz aktywność peroksydazy glutationowej (Gpx), katalazy (Cat) i dysmutazy ponadtlenkowej (SOD).

Wyniki. Uzyskano wyniki badań świadczące o podwyższonym generowaniu anionorodnika ponadtlenkowego u chorych z pzw C. Po zastosowanym leczeniu generowanie zmniejszało się, a w zakresie MDA uzyskano wyniki świadczące o redukcji stężeń. W za-

kresie wskaźników bariery antyoksydacyjnej uzyskano wyniki świadczące o podwyższonej aktywności enzymów wskaźników w pzw C przed leczeniem i systematyczne zmniejszanie się aktywności w trakcie i po zakończeniu terapii skojarzonej Peg-IFN i RBV.

Wnioski. 1. Przewlekłe zapalenie wątroby należy zaliczyć do chorób wolnorodnikowych. 2. Stres oksydacyjny występujący w pzw C stymuluje barierę antyoksydacyjną do wzmożonego generowania enzymów antyoksydacyjnych. 3. Skojarzone leczenie Peg-IFN i RBV wywiera korzystny wpływ na metabolizm tlenowy u chorych z pzw C, redukując generowanie reaktywnych form tlenu (RFT) oraz zmniejszając aktywność bariery antyoksydacyjnej.

INTRODUCTION

According to World Health Organization (WHO) estimation the number of people with confirmed diagnosis of HCV infection around the world is over 180 mln. Other assesments suggest that over 400 mln people is infected with HCV, which is about 6% of the population of the world (1).

HCV infection is characterized by low incidence of spontaneous eliminations. Between 50 and 80% of infected people will reveal chronic hepatitis, developing liver cirrhosis and primary hepatocellular carcinoma in the future (20% after 20 years) (2).

In Poland the number of infected people may reach up to 700 000 depending on an author (3). It is estimated that in the majority of cases the infection is connected with the contact with health service centres. Basic ways of transmission of the virus are: parenteral, sexual and vertical. Special risk groups include drug users (90% infected with the virus in some countries) and hemodialysis patients (according to different sources 30-60% infected) (4). What is important, about 40% of cases are "sporadic infections", when the way of infection is impossible to determine.

Etiopathogenesis of HCV infection has not been clarified up to now. The receptor responsible for the virus penetration into the cell has not been defined unequivocally. The CD81 co-receptor is suspected of cooperation, binding viral E2 protein (envelope) and enabling virions to gather at the cell surface (5). SRBI (scavenger receptor class B type I) co-receptor binding viral HVR1 region (hypervariable region) is also responsible for HCV penetration into the cell. High mutation rate allows to avoid an organism immunologic response. Immunologic system pressure is also responsible for creating viral variants (subtypes and pseudotypes "quasispecies"). It is thought that viral proteins interfere with an organism natural and specific response.

The virus reveals tropism not only to hepatocytes. HCV-RNA was found in mononuclear cells as well. HCV-RNA was revealed in mononuclear cells of blood in 50-80% of the patients. These are the potential reservoirs of infection in further course of the disease. It has not been found whether and how the virus might influence their function (6).

The virus replicates in the cytoplasm of the cell. It is not the only intracellular localization. Viral protein presence has also been documented in mitochondria and nuclei of cells (7).

This localization is important because of the biochemical processes at the molecular level. HCV is considered to influence cell aerobic metabolism disturbing the homeostasis of free radicals production and impairing antioxidative mechanisms in the cell.

The cells of aerobic organisms physiologically produce necessary volumes of reactive oxygen forms – free radicals. One of the basic sites of their production is the inner mitochondrial wall, where the respiratory chain reactions generate superoxide anion radical among others (8).

Atoms or molecules with one or more unpaired electrons are considered as free radicals. Reactive oxygen forms (ROF) in the cell are all products of reactions of excitation and reduction of oxygen having higher biochemical activity than molecular oxygen (O_2). ROF production of in the cell happen through enzymatic reactions (with oxidase from the oxidoreductase group), through xenobiotics oxygenation and through oxygenation of respiratory proteins.

Among the basic ROF we rate: singlet oxygen, ozone, superoxide anion radical, hydrogen peroxide, hydroxyl radical and hydrogen peroxide radical.

Physiologically free radicals production is controlled by a few mechanisms: antioxidant barrier enzymes (catalase, superoxide dysmutase, glutathione peroxidase), natural antioxidants – glutathione and microelements, and antioxidant vitamins (zinc, selenium, vitamins A, E, C). When inflammation begins at the tissue – regardless of its cause – those processes regulation fails, causing the oxidative stress to pathologically arise (9).

Because of their high reactivity free oxygen radicals react with all the basic cell components: lipids, proteins, nucleic acids, carbohydrates. The effects of those reactions are oxygenation of basic cell components, change of their structure and change or loss of their function.

Among harmful effects of ROF influence on a cell we can find: collagen degradation, lipids peroxidation, enzymes inactivation, DNA chain brakes (impairments of the helix causing mutations and neoplasms as a result), erythrocytes' lysis, oxidative phosphorylation inhibition.

The result of abovementioned reactions is impairment of function and structure of the cell, including the cell death.

There are three basic mechanisms of defense for the organism against free radicals:

1) prevention – of the ROF reactions with biologically active agents,

2) intervention – interruption of free radicals reactions,
3) elimination – of ROF reactions effects.

Among the enzymes decomposing the precursors of hydroxyl radical – superoxide anion radical and hydrogen peroxide, we find enzymes using dismutation properties (disproportionate) of ROF. This group comprises enzymes of an antioxidant barrier – Cat, Gpx, SOD (10).

Currently the most effective method of treatment for chronic hepatitis C is a combined therapy with pegylated interferon alpha (Peg-IFN) and ribavirine (RBV).

Interferons are species specific pleiotropic cytokines released by cells as a reaction to viral infection. Currently two subtypes of alpha interferon are used in the therapy: 2a and 2b. Effectiveness of interferon monotherapy was low (15-20%). Adding of poliethylene glycol molecule (pegylation) to the interferon molecule caused a change in its physical and chemical properties, increasing the drug effectiveness. Pegylated interferon is more water soluble, has a better bioavailability, pegylated particle is chemically neutral and protects the drug against opsonisation and phagocytosis. Also it elongates the half-life (less frequent drug injections) (11).

Currently the effectiveness of the treatment with Peg-IFN alpha with ribavirine allows to reach satisfactory effect in 50% of the patients (12).

The virus eradication not always is possible to achieve. Positive is to achieve lasting suppression of viral replication and as a result inhibition of necroticoinflammatory processes in the liver. It prevents the progression to liver cirrhosis and primary hepatocellular carcinoma.

AIM

The aim of this work is to assess an aerobic metabolism rates in patients with chronic hepatitis C and evaluation of the influence of the treatment with pegylated interferon alpha 2a and ribavirine to chosen rates of aerobic metabolism.

MATERIAL AND METHODS

The study comprised *31 patients* (*8 women and 23 men*) aged 19 to 58 (on average 44 +/- 11). The group consisted of the patients hospitalised at the Infectious Diseases Clinic of Military Medical Division and at City Units in Biegański's Provincial Specialised Hospital in Łódź (Wojewódzki Specjalistyczny Szpital im. dr. Wł. Biegańskiego w Łodzi).

Control group comprised 22 healthy adults, adjusted according to the sex and age.

The patients qualified to the study were diagnosed with chronic hepatitis C (CHC) according to the serological tests (presence of antibodies anti-HCV), biochemical test (increased ALT, AST activity, minimum 2 times higher than normal), presence of HCV-RNA and histopathology. Liver tissue sampled during liver biopsy was assessed with 5-grade Batts and Ludwig scale has minimum S2 (inflammation).

Disqualifying criteria were autoimmunologic diseases, haematologic disorders, unstable liver failure, heart failure, unstable ischaemic heart disease, pregnancy, breast feeding, other severe devastative diseases.

TREATMENT

All the patients were treated with 180 ug of Peg-IFN alpha 2 subcutaneously once weekly for 48 weeks. Daily they were treated with 1000 or 1200 mg of RBV (depending on body weight).

The samples for the study were collected in the quantity of 2 ml from the ulnar vein to the vaccutainers with K3 EDTA. Blood samples were taken during the tests essential to the monitoring of the treatment. Assessment of each parameter was performed before start of the treatment, after 12, 24 and 48 weeks of treatment, which is after completion of the combined therapy.

In the subsequent samples we tested:
- in neutrophiles of blood – production of superoxide anion radical at rest and after stimulation,
- in erythrocytes of blood – concentration of malonic dialdehyde (MDA), catalase activity (Cat), glutathione peroxidase activity (Gpx), superoxide dismutase activity (SOD).

Photocolorimetric method was implemented to the tests. Superoxide anion radical activity was tested with Bellavita method, MDA concentration – with Placer method, SOD activity – with Misdry and Fridroviha method, Gpx activity – with Lindsay method in Little and O'Brien modification, Cat activity – with Beers and Sizer method.

The statistical analysis comprised 24 patients who completed 48-week therapy. 7 patients were disqualified from treatment because of side effects or unsatisfactory treatment results. The analysis used dependent sample, which is the same group at different subsequent points of time. For the statistical significance level 0.05 ($p < 0.05$) was assumed. Program Statistica 7 was used for the statistical analysis.

RESULTS

All the patients were infected with HCV genotype 1. In 28 cases genotype 1b was found, and in 2 patients – genotype 1a (tab. 1).

Table 1. HCV genotypes in the tested group.

	Viral genotype		
	1a	1b	3a
Number of patients	2	28	1

Histological advancement in the liver sample obtained during the liver biopsy was assessed with Batts and Ludwig scale was G_1S_2 to G_4S_4. At the majority of cases (19 patients) medium intensity of changes was found (G_2S_2). The histological results are presented in table 2.

After 48 weeks of Peg-IFN and RBV treatment 21 patients achieved ETR (end of treatment response). 17 patients (56% of the patients who started treatment) achieved SVR (sustained virological response) after 6 months.

Table 2. Liver biopsy results at the tested group.

Inflammation (G) Fibrosis (S) (Batts and Ludwigs scale)	Number of patients (n = 31)
G_1S_2	2
G_1S_3	1
G_2S_2	19
$G_2S_{2/3}$	1
G_3S_2	3
G_3S_3	1
$G_3S_{3/4}$	3
G_4S_3	1

Three patients were disqualified because of side effects (leucopaenia) induced by interferon. Three patients were disqualified from further treatment after 12 weeks because of lack of effects of treatment EVR-negative (early virological response). The results are presented in table 3.

Table 3. HCV-RNA test results in a tested group of patients with CHC in three periods of time during pegylated interferon alpha 2a and ribavirine therapy (IU/ml = 7.8 viral copies/ml).

	Group	Number of patients (n)	(%)
A	Group of the patients with no HCV-RNA after 48 weeks of treatment (ETR-positive)	21	70
B	Group of patients with HCV-RNA present after 48 weeks of treatment (ETR-negative)	3	10
C	Group of patients in which the treatment was ceased due to the lack of positive treatment prognosis (EVR-negative)	3	10
D	Patients who has the treatment ceased due to side effects	3	10
E	Patients who achieved SVR	17	56

ETR (end of treatment reaction) – RNA-HCV after the treatment completion:
ETR-positive – RNA HCV elimination
ETR-negative – lack of RNA-HCV elimination
EVR (early virological responce) – RNA-HCV test in 12th week of treatment:
EVR-positive – no RNA-HCV or decrease in viral load by 2 log
EVR-negative – no minimal 2 lgo RNA-HCV decrease
SVR (sustained virological responce) – RNA-HCV test after 24 weeks after the treatment completion:
SVR-positive – no RNA-HCV
SVR-negative – RNA-HCV present

Aerobic metabolizm rates

Superoxide anion radical production at rest, before treatment was 9.24 to 23.160 (nmolO*$_2$/min/kom); on average 13.621 +/- 3.188 (nmolO*$_2$/min/kom). These results were higher than in a control group 6.239 to 16.670 (nmolO*$_2$/min/kom); on average 6.76 +/- 0.520 (nmolO*$_2$/min/kom). After 24 weeks lower production of superoxide anion radical was found (4.290 to 11.3 (nmolO*$_2$/min/kom); on average 7.988 +/- 1.933 (nmolO*$_2$/min/kom). The lowest levels were fund after 48 weeks of treatment (4.340 to 11.09 (nmolO*$_2$/min/kom); on average 7.988 +/- (nmolO*$_2$/min/kom). These results were higher than in a control group ($p < 0.05$), and lower than the results before treatment ($p < 0.05$). Superoxide anion radical production at rest in the patients with CHC treated with Peg-IFN and RBV are presented in table 3 and figure 1.

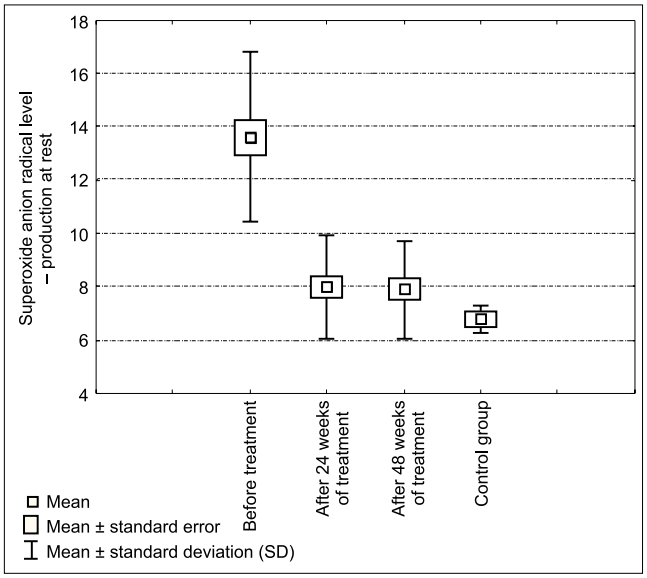

Fig. 1. Superoxide anion radical level – production at rest in the patients with CHC treated with Peg-IFN alpha and ribibavirine.

Superoxide anion radical stimulated production, before treatment was 15.04 to 30.150 (nmolO*$_2$/min/kom); on average 21.196 +/- 4.248 (nmolO*$_2$/min/kom). These results were higher than in a control group 9.32 to 28.410 (nmolO*$_2$/min/kom); on average 17.09 +/- 1.08 (nmolO*$_2$/min/kom) ($p < 0.05$). After 24 weeks lower production of superoxide anion radical was found (5.96 to 15.270 (nmolO*$_2$/min/kom); on average 10.673 +/- 2.199 (nmolO*$_2$/min/kom). Results after 24 weeks were significantly lower than in a control group and after 48 weeks of treatment ($p < 0.05$). The lowest levels were fund after 48 weeks of treatment (11.41 to 17.170 (nmolO*$_2$/min/kom); on average 14.571 +/- 1.757 (nmolO*$_2$/min/kom). These results were lower than in a control group ($p < 0.05$), and lower than the results before treatment ($p < 0.05$). Superoxide anion radical stimulated production at rest in the patients with CHC treated with Peg-IFN and RBV is presented in table 4, 5 and figure 2.

Table 4. Superoxide anion radical level – production at rest in the patients with CHC treated with Peg-IFN alpha and ribavirine.

Characteristic	Superoxide anion radical level – production at rest (nmolO*$_2$/min/kom)			
	Hiatus		X	SD
	Min.	Max.		
Control group	6.239	16.670	6.760	0.520
Before treatment	9.240	23.160	13.621	3.188
After 24 weeks of treatment	4.290	11.300	7.988	1.933
After 48 weeks of treatment	4.340	11.090	7.908	1.835

Table 5. Superoxide anion radical level – production on stimulation in the patients with CHC treated with Peg-IFN alpha and ribavirine.

Characteristic	Superoxide anion radical level – production on stimulation (nmolO*$_2$/min/kom)			
	Hiatus		X	SD
	Min.	Max.		
Control group	9.32	28.410	17.09	1.08
Before treatment	15.040	30.150	21.196	4.248
After 24 weeks of treatment	5.960	15.270	10.673	2.199
After 48 weeks of treatment	11.410	17.170	14.571	1.757

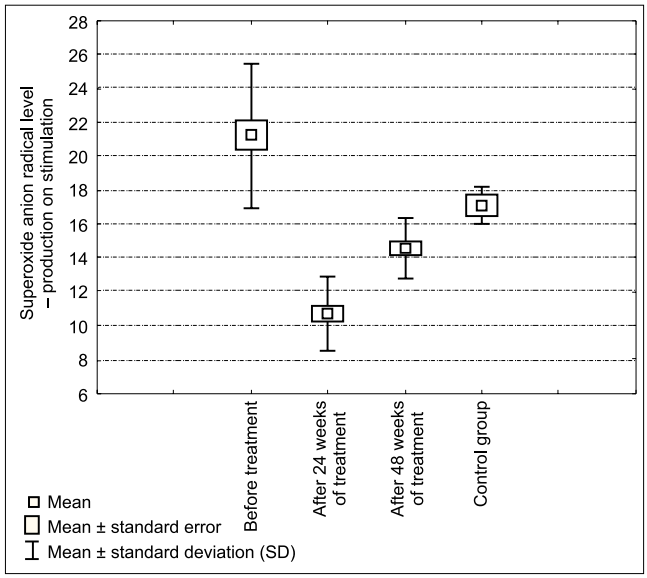

Fig. 2. Superoxide anion radical level – production on stimulation in the patients with CHC treated with Peg-IFN alpha and ribavirine.

Malonic dialdehyde

Before treatment the malonic dialdehyde concentration was 0.223 to 0.450 μmol/g Hb; on average 0.321 +/- 0.056 μmol/g Hb. After 12 weeks of treatment MDA concentration was 0.103 to 0.589 μmol/g Hb; on average 0.300 +/- 0.10 μmol/g Hb.

After 24 weeks of treatment MDA concentration was 0.118 to 0.592 μmol/g Hb; on average 0.216 +/- 0.110 μmol/g Hb. After 48 weeks of treatment MDA concentration was 0.11 to 0.740 μmol/g Hb; on average 0.312 +/- 0.137 μmol/g Hb.

MDA concentration after 12 weeks of treatment is significantly lower ($p > 0.05$) than in a control group and insignificantly lower ($p > 0.05$) than the results before treatment.

After 24 weeks MDA concentration is significantly lower ($p < 0.05$) than in a control group, before treatment and after 12 weeks of treatment. After 48 weeks of treatment MDA concentration is lower ($p < 0.05$) than in a control group and before treatment but significantly higher ($p > 0.05$) than after 24 weeks.

Malonic dialdehyde concentration in patients with CHC treated with Peg-IFN and ribavirine is presented in table 6 and figure 3.

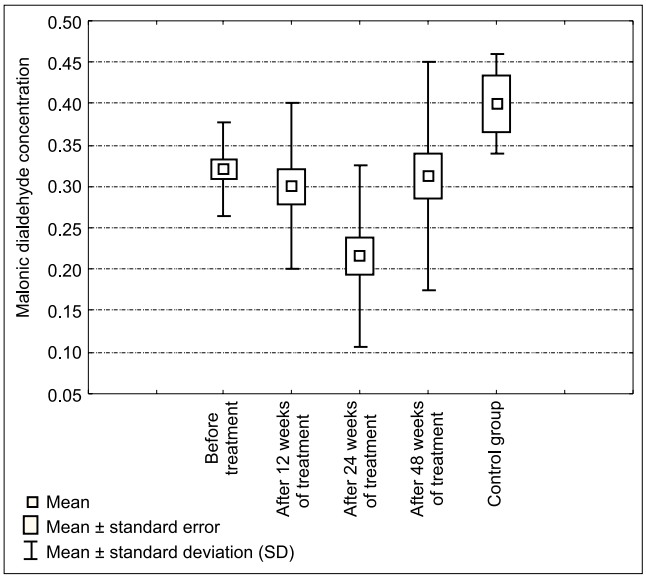

Fig. 3. Malonic dialdehyde concentration in the patients with CHC treated with Peg-IFN alpha and ribavirine.

Catalase

Catalase activity before treatment was 17.430 to 41.96 U/g Hb. Cat activity subsequently in 12, 24 and 48 week after treatment: 27.738 +/- 8.00 U/g Hb vs 20.965 +/- 14.259 U/g Hb vs 18.538 +/- 5.651 U/g Hb. In the tested group Cat activity was significantly higher than in a control group. After 12 weeks insignificant elevation was fund in comparison with the group before treatment ($p > 0.05$) and significant elevation in comparison with a control group ($p < 0.05$).

After 24 weeks of treatment Cat activity was lower than in a control group and significantly lower than in previous tests ($p < 0.05$).

Testing after 48 weeks showed lower levels than in a control group, before treatment and after 12 weeks ($p < 0.05$). Despite the levels being lower after 24 weeks, the difference was not statistically significant ($p > 0.05$).

Catalase activity in patients with CHC treated with Peg-IFN i RBV is presented in table 7 and figure 4.

Glutathione peroxidase

Gpx activity before treatment, after 12, 24 and 48 weeks was in turn: 68.754 +/- 20.116 U/g Hb vs 61.645 +/- 17.942 U/g Hb vs 44.791 +/- 11.174 U/g Hb vs 51.051 +/- 15.402 U/g Hb. In a control group Cat activity was 47.090 +/- 6.840 U/g Hb.

Activity in a tested group before treatment was significantly elevated in comparison to a control group. After 12 weeks it was significantly elevated in comparison to a control group and significantly lower than in the test before treatment ($p < 0.05$).

After 24 weeks Gpx activity was significantly lower than before treatment, after 12 weeks and in a control group ($p < 0.05$).

After 48 weeks Gpx activity was insignificantly elevated in comparison to the results after 24 weeks ($p > 0.05$) and significantly lower than in the other groups (control group, after 12 and 24 weeks).

Table 6. Malonic dialdehyde concentration in the patients with CHC treated with Peg-IFN alpha and ribavirine.

Characteristic	Malonic dialdehyde (μmol/g Hb)			
	Hiatus		X	SD
	Min.	Max.		
Control group	0.304	0.520	0.400	0.137
Before treatment	0.223	0.450	0.321	0.056
After 12 weeks of treatment	0.103	0.589	0.300	0.100
After 24 weeks of treatment	0.118	0.592	0.216	0.110
After 48 weeks of treatment	0.1100	0.740	0.312	0.137

Glutathione peroxidase activity in patients with CHC treated with Peg-IFN and RBV is presented in table 8 and figure 5.

Table 7. Catalase activity in the patients with CHC treated with Peg-IFN alpha and ribavirine.

Characteristic	Catalase (U/g Hb)			
	Hiatus		X	SD
	Min.	Max.		
Control group	16.623	28.134	21.400	2.10
Before treatment	17.430	41.690	28.361	6.687
After 12 weeks of treatment	16.200	44.110	29.738	8.001
After 24 weeks of treatment	9.290	78.570	20.965	14.25
After 48 weeks of treatment	8.950	32.930	18.538	5.651

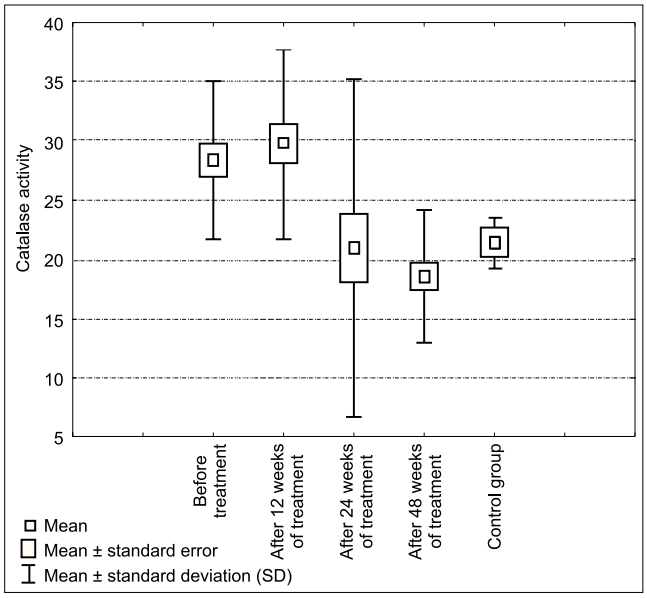

Fig. 4. Catalase activity in the patients with CHC treated with Peg-IFN alpha and ribavirine.

Superoxide dismutase

Superoxide dismutase activity before treatment was 2386.95+/- 790.533 U/g Hb and was insignificantly higher than in a control group 2145 +/- 127 U/g Hb ($p < 0.05$). Activities after 12, 24 and 48 weeks were in turn:

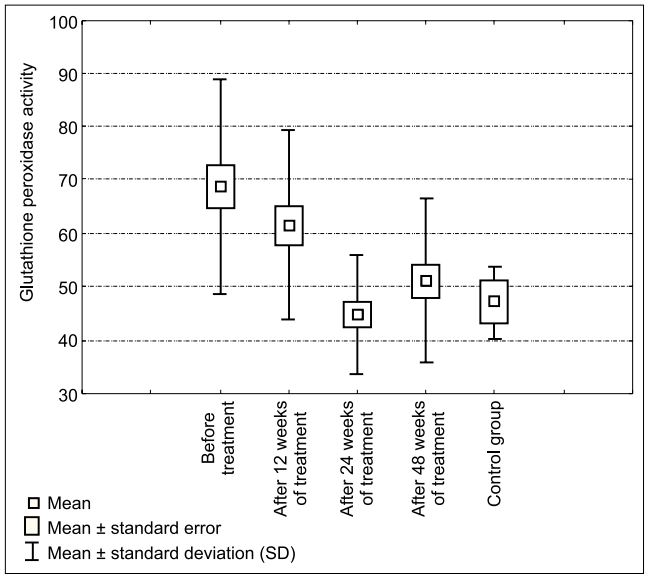

Fig. 5. Glutathione peroxidase activity in the patients with CHC treated with Peg-IFN alpha and ribavirine.

3710.875 +/- 997 U/g Hb vs 2305.375 +/- 981 U/g Hb vs 1894.833 +/- 586 U/g Hb. SOD activity after 12 weeks of treatment in comparison to the tests before treatment and to a control group was higher ($p < 0.05$).

After 24 weeks SOD activity was insignificantly elevated in comparison to a control group ($p > 0.05$), was comparable to the group before treatment ($p > 0.05$) and was significantly lower than in the 12th-week test. After 48 weeks of treatment SOD activity was significantly lower than in a control group, test before treatment and after 12 and 24 weeks of treatment ($p < 0.05$).

Superoxide dismutase activity in patients with CHC treated with Peg IFN and RBV is presented in table 9 and figure 6.

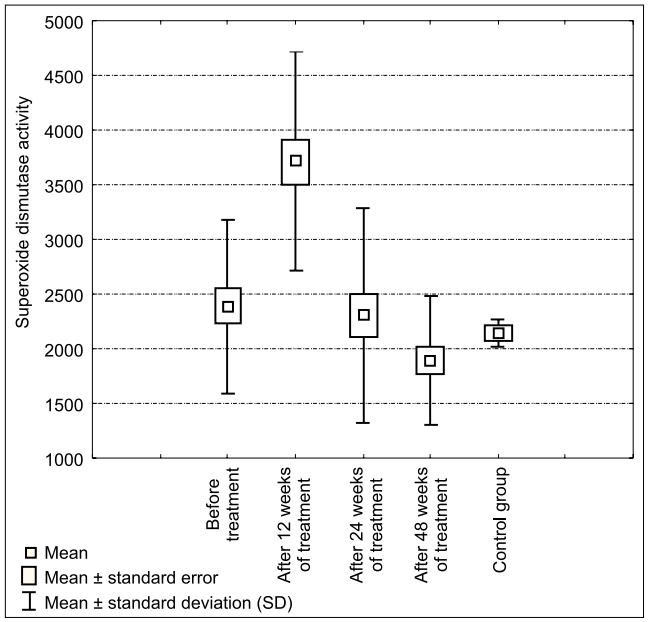

Fig. 6. Superoxide dismutase activity in the patients with CHC treated with Peg-IFN alpha and ribavirine.

Table 8. Glutathione peroxidase activity in the patients with CHC treated with Peg-IFN and ribavirine.

Characteristic	Glutathione peroxidase (U/g Hb)			
	Hiatus		X	SD
	Min.	Max.		
Control group	36.283	56.019	47.090	6.840
Before treatment	45.300	145.600	68.754	20.115
After 12 weeks of treatment	29.580	109.000	61.645	17.942
After 24 weeks of treatment	25.310	63.440	44.791	11.174
After 48 weeks of treatment	30.880	95.000	51.051	15.402

DISCUSSION

At the work herein we analysed the thesis of increased production of free oxygen radicals, which are responsible for intracellular damage in the chronic hepatitis C.

A number of observations emerge from the assessment of the results. We may divide those results into two subgroups.

The first one are reactive oxygen forms – superoxide anion radical, production at rest and on stimulation and lipids peroxidation product – malonic dialdehyde – which is a halfway effect of free radicals activity.

The second group are defensive mechanisms of an organism – antioxidant barrier rates – Gpx, Cat, SOD.

Superoxide anion radical activity in healthy people in comparison to a CHC group, at rest and on stimulation, is lower. Results after 24 and 48 weeks of treatment are significantly lower than before the treatment ($p < 0.05$). At the tests performed to asses an at rest production after 48 weeks the levels were similar to those observed in a control group, nevertheless they were higher ($p < 0.05$). In case of stimulated production, in a tested group after 48 weeks we obtained results lower than in a control group ($p < 0.05$).

Reduction in superoxide anion radical production suggests oxidative stress reduction in the patients with CHC during the treatment with IFN. Because there is not much information in the literature to compare with, the results I got I may only compare with two works, in which similar results were achieved *in vitro* or in the patients with acute hepatitis C.

Malonic dialdehyde, which is the next tested parameter, is a product of lipids' peroxidation. Products of peroxidation are less reactive than ROF. Therby they can diffuse further through the cells. They play a role of secondary transmitters of damages caused by ROF.

It is suggested that this process may be initiated by a number of free radicals – superoxide anion radical, hydrogen peroxide, superoxide radical, aloxy radical. Among the final products of lipids' peroxidation are: aldehydes, hydroxyaldehydes, alkenals and malonic dialdehyde (MDA). MDA is the best known biochemical product of the abovementioned process.

It was proved that MDA modifies cell membranes' properties, as they consist of lipids. Their structure is changed, as well as their permeability for ions and membrane potential. The final effect can be losing of the intracellular and plasmatic membranes' integrity.

The results from the tested group before treatment showed lower concentration in comparison to control group. Possibly because enhanced activity of antioxidant barrier enzymes, which is an effect of an increased free radicals production. In herein work we showed an increased production of superoxide anion radical at rest, as well as on stimulation.

Subsequent tests showed decreasing in MDA concentration through the course of treatment. Results obtained after 12, 24, 48 weeks of treatment were significantly different (0.300 vs 0.216 vs 0.312; $p < 0.05$). The lowest concentration was observed in 24th week of treatment. In 48th week MDA concentration was significantly lower in the tested group than in a control group (0.312 vs 0.400; $p < 0.05$).

The results confirm results of Higueras et al. and Serejo et al. from 2004. Also Kageyama et al. in 2000 compared MDA concentrations at the liver samples obtained at liver biopsy before and after IFN treatment. Their observations are unanimous with mine results (13-15).

Observations of antioxidative barrier enzymes' activity confirm the theses of the free radical character of CHC from herein work.

In a tested group before treatment Cat and Gpx activity was higher than in a control group. SOD activity was higher in a tested group than in a control group but the difference was not statistically significant ($p > 0.05$).

In case of all three enzymes – catalasys, glutathione peroxidasis and superoxide dismutase – the tests showed decrease of activity after 48 weeks in comparison to activity levels before treatment and control group.

Catalasys activity after 12 weeks is still significantly elevated in comparison to control group. Decrease lower than in control group was seen after 24 weeks ($p < 0.05$). Activity in 24 week is also significantly lower than in 12 week. After 48 weeks Cat activity is significantly lower than in a control group and in a group after 12 weeks ($p < 0.05$). It is also lower than in the tests after 24 weeks but with no statistical significance ($p > 0.05$).

Glutathion peroxidase activity after 12 weeks is still significantly increased in comparison to a control group. We observe significantly lower activity than before treatment. After 24 weeks Gpx activity decrease in comparison to a control group, results obtained before treatment and after 12 weeks. After 48 weeks Gpx activity was comparable to the activity in a control group ($p > 0.05$) and results obtained after 24 weeks. It is significantly lower than before treatment and after 12 weeks of treatment ($p < 0.05$).

Superoxide dismutase activity in a tested group was higher than in a control group but with no statistical significance ($p > 0.05$). After 12 weeks in a tested group activity was still higher than in a control group ($p < 0.05$).

After 24 weeks of treatment the activity was lower than in 12 week ($p < 0.05$). After 48 weeks SOD activity was lower than in a control group, lower than before treatment and after 12 and 24 weeks ($p < 0.05$).

In the available literature there is no unambiguous opinion how does the activity of antioxidant barrier changes in patients with CHC. In case of each of the three parameters I checked (Cat, SOD, Gpx) we can find data being a sign of an increase, as well as a decrease of their activity. Some scientists suggest increased activity of antioxidant barrier to be a reaction to an increased free radicals production. Others suggest ROF activity to be responsible for a decreased barrier activity as a result of their ability to suppress an organisms defense mechanisms.

Unambiguous results and contradictory conclusions drawn at their basis by different authors, show a necessity of further research. Lengthy review of different researchers results was published in a separate work (16).

Results from the herein work can be confirmed on the basis of the available literature. There is a number of dissertations covering IFN treatment in patients with HCV infection, in which different rates of aerobic metabolism were researched. Among the authors Higueras et al., Serejo et al., Levent et al., Vendemiale et al., Romero et al. can be mentioned (13-19). They suggest the IFN therapy has positive effects on an aerobic metabolism, suppressing "oxidative stress". What should be emphasized is a fact that I have not found a work assessing aerobic metabolism parameters during the treatment but only before and after the treatment completion.

Despite the HCV research of many years duration (the virus was discovered by Choo et al., the disease etiopathogenesis and the course of an infection still has not become clear (20). In the available literature a number of dissertations suggest free radicals foundation of CHC (21-23). It is implied that some antiviral therapies may reduce oxidative stress and at the same time reduce risk of liver cirrhosis and HCC.

Influence of free radicals on the basic cellular components (deoxyribonucleic acid DNA) mentioned at the introduction may be one of the explanations of anomalies in the genetic material of a cell. Those impairments being a result of improper pairing of alkali or DNA replication, may cause mutations or be carcinogenic. Research carried out on hamster's ovaries proved a superoxide anion radical mutagenic effect. Analogical effect showed research with singlet oxygen.

Superoxide anion radical and hydrogen peroxide are unavoidable products of intracellular processes. Physiologically there is a balance between their production and decomposition. Concentration of superoxide anion radical in a hepatocyte is 10-11 mol 1-1, and concentration of H_2O_2 10-8 mol x 1-1 (24).

In an available research it was stated that HCV core protein expression in a hepatocyte stimulate oxidative stress. Direct evidence of HCV proteins influence to the cell metabolism is confirmed by the presence of HCV proteins and non-structural NS3 protein in a site, where the most of ROF are produced – inside mitochondrium. It is also assumed that oxidative stress causes a decrease in hepatic, serum and lymphocytic glutathione levels, which is one of the major endogenous antioxidants (7, 25).

In the available literature we can also find authors stating that ROF production is one of a defense mechanisms, which inhibits HCV-RNA replication (26).

As for Peg-IFN and ribavirine therapy in the patients with CHC our results were comparable to the results of large randomized multicentre researches. In treatment of genotype 1 38-56% of the patients achieved ETR (Imazeki, Fried, Wiegand). In case of herein work treatment ETR was achieved by a group of 21 patients of the 31 at the beginning, which makes 67%. SVR achieved 17 patients of 31 that started the treatment (54%). SVR for genotype 1 HCV according to the literature is 50% (12).

All the patients were infected with genotype 1: 28 patients with genotype 1b, 2 – with genotype 1a. During the research the treatment was discontinued in 3 patients due to the lack of satisfactory results. No 2 log decrease of viral load after 12 weeks was an indicator for the treatment cessation. Probability of therapeutic success in this group is up to 2%. The other 3 patients were disqualified because of side effects (leucopaenia).

Table 9. Superoxide dismutase activity in the patients with CHC treated with Peg-IFN alpha and ribavirine.

Characteristic	Superoxide dismutase (U/g Hb)			
	Hiatus		X	SD
	Min.	Max.		
Control group	1172.0	2872.0	2145.0	127.0
Before treatment	1031.0	4052.0	2386.958	790.553
After 12 weeks of treatment	2315.0	6618.0	3710.875	997.156
After 24 weeks of treatment	783.0	5686.0	2305.375	981.414
After 48 weeks of treatment	273.0	3352.0	1894.833	586.885

CONCLUSIONS

The obtained results allow to draw following conclusions:

1. Chronic hepatitis C should be rated among free radical diseases.
2. Oxidative stress in chronic hepatitis C stimulates antioxidative barrier to increased production of antioxidative enzymes.
3. Combined therapy with interferon alpha 2 and ribavirine has a positive effect on aerobic metabolism in patients with chronic hepatitis C, causing a reduction in ROF production and reducing the activity of antioxidative barrier.

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