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CHANGES OF OXIDATIVE AND LIPOPEROXIDATIVE METABOLISM IN PATIENTS WITH ACUTE VIRAL MYOCARDITIS

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Abstract: Objective: To study the changes of oxidative metabolism and lipoperoxidative metabolism in patients with acute viral myocarditis (AVM), and to research pathological chain reactions of a series of free radicals, and oxidative damage and lipoperoxidative damage in AVM patients' bodies. Methods: A random paired control design was used to investigate 70 cases of AVM patients and 70 cases of healthy adult volunteers (HAV) on the basis of contents of nitric oxide (NO), lipoperoxide (LPO) in plasma, and LPO in red blood cell (RBC) as well as activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in RBC as revealed by spectrophotometric assays. Results: Compared with the average values (AV) of the above biochemical parameters (BP) in the HAV group, the AV of NO in plasma, and LPO in plasma and in RBC in the AVM group were significantly increased ($P = 0.0001$), while the AV of SOD, CAT and GSH-Px in the AVM group were significantly decreased ($P = 0.0001$). Conclusion: The findings in this study suggested that in the AVM patients bodies the metabolism of NO was disturbed, and the pathological chain reactions of a series of free radicals were so severely aggravated, as to cause the oxidative damage and lipoperoxidative damage in the AVM patients' bodies.

Key words: oxidation, lipoperoxidation, myocarditis, metabolism
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INTRODUCTION

Myocarditis is a common cardiac disease. There are reports that activities of superoxide dismutase and catalase in blood of viral myocarditis patients and animals produced some changes, and aggravated reactions of free radicals in their bodies (Hiraoka et al., 1992, 1993, 1995; Suzuki et al., 1994). But there are up to now no reports on changes of nitric oxide and other free radicals in acute viral myocarditis patients' bodies, nor the reports about the relationship between oxidative and lipoperoxidative damage, and acute viral myocarditis. We used random paired control design to study oxidative stress and lipoperoxidative stress in AVM patients' bodies, and determined the concentration of some oxidative biochemical constituents such as nitric oxide (NO), lipoperoxide (LPO) in plasma and lipoperoxide (LPO) in red blood cells (RBC) of 70 acute viral myocarditis (AVM) patients and 70 healthy adult volunteers (HAV). At the same time we determined the activities of some antioxidases such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in the RBC of AVM patients and HAV. We compared the differences between the average values (AV) of the above biochemical parameters (BP) in the AVM group and in the HAV group.

MATERIALS AND METHODS

Subjects

AVM: Seventy cases of patients with acute viral myocarditis were randomly sampled from 150 cases of the AVM patients with "Select Cases-Random Sample of Cases" of "SPSS 10.0 for Windows", and the confirmed diagnosis of the above 150 cases of AVM patients were made on the basis of "the reference diagnosis criteria for adult acute viral myocarditis" (Yang, 1995) and the blood serum virology immunoassay in the

Second Affiliated Hospital of the Medical College of Zhejiang University in 1999 to 2000 was based on inclusion and exclusion criteria (Wynne et al., 1997; O'Connell et al., 1998; Peters et al., 1991a, 1991b; Dec et al., 1990). Their ages were 16 to 52 (35.8 ± 8.1); 41 cases were males, 29 cases were females. These patients were all confirmed to have Coxsackievirus B infections, and displayed ST-segment and T-wave abnormalities and arrhythmias originated from ventricular, and atrioventricular conduction disturbances as shown in their electrocardiogram. Their myocardial involvement was associated with symptoms of fatigue, dyspnea, palpitations, precordial discomfort, tachycardia, temperature elevation. Their first heart sound was muffled, but they had no specific complaints referable to the cardiovascular system, such as serious cardiac function abnormality and so on (Wynne et al., 1997; O'Connellet al., 1998; Peters et al., 1991a, 1991b; Dec et al., 1990). All the above patients were within normal ranges in their routine blood, urine and feces tests and radiographs; had no disorders associated with brain, lung, liver, kidney and other organs; and or other medical problems such as hypertension, chronic bronchitis, autoimmune disease, diabetes, atherosclerosis, and tumors.

HVM: Seventy cases of the healthy adult volunteers (HAV) without any myocarditis (as confirmed by comprehensive physical examination at the Second Affiliated Hospital of the Medical College of Zhejiang University) were randomly sampled from 300 cases of HAV with "Select Cases-Random Sample of Cases" of "SPSS 10.0 for Windows". Their ages were 16 to 52 (35.8 ± 8.1), 41 were males, 29 were females.

All the above subjects were never exposed to radiation, nor engaged in work exposing them to intoxicating materials or pesticides. Within one month before recruitment they had not taken ginkgo biloba, theo-polyphenols or other agents.

The above 70 AVM patients were randomly paired up with the 70 HAV according to "the random numbers table".

Methods

Collection and pretreatment of the blood samples

Heparin sodium was added as anticoagulant to the fasting venous blood samples collected in the morning from all the subjects; and the separated plasma and RBC were stored at −50 ℃ immediately.

Measurement methods

The coloration of α-naphthylamine was used to determine plasma NO content (nmol/L); the colorimetry of thiobarbituric acid reactive substances (TBARS) was used to determine plasma LPO content (μmol/L); the colorimetry of the TBARS was used to determine RBC LPO content (nmol/g·Hb); the spectrophotometry of inhibiting pyrogallol auto-oxidation was used to determine SOD activity (U/g·Hb); the spectrophotometry of coloration of hydrogen peroxide and acetic acid-potassium bichromate was used to determine CAT activity (K/g·Hb); and the improved Hafeman's spectrophotometry was used to determine GSH-Px activity (U/mg·Hb) (Zhou et al., 2000a, 2000b).

In the determination of the above biochemical substances, major analytical reagents, such as Cu/Zn-Superoxide dismutase, Catalase, α-Naphthylamine, 1, 2, 3-Trihydroxybenzene, 1, 1, 3, 3-Tetraethoxypropane, 2-Thiobarbituric acid, all were from SIGMA CHEMICAL COMPANY®, USA; and the other analytical-grade reagents were all produced in China. Fresh quadruply distilled water was prepared with a quartz glass distilling apparatus. The main analytical instruments were HP 8453-Spectrophotometer, USA, and UV-754-Spectrophotometer, 721-Spectrophotometer. In the determination of the above biochemical substances, the standardization of experiment, e.g. the same lot number of each reagent, the same quality control, the same lab assistant, and identical analytical apparatus, were strictly used for every experiment in order to control and decrease the error and bias of the experiment, and to insure the veracity of the measurements.

Medical Statistic Analysis

All data were statistically analyzed with SPSS/10.0 for Windows and STATISTICA/6.0 for Windows statistic software using a Compaq Pentium Ⅲ/1000 computer. The parameters in this study were expressed as mean plus or minus standard deviation ($\bar{\chi} \pm s$) and 95% confidence interval (95% CI). Hypothesis testing method used was paired-samples t test. In the statistical analysis of this study, the level of hypothesis testing (α) was $\leqslant 0.05$ in order to avoid false

positives, and the power of hypothesis testing (power) was $\geqslant 0.80$ to avoid false negatives (Lang and Secic, 1997).

RESULTS

Comparison between the AV ($\bar{\chi} \pm s$) of the BP in the AVM group and in the HAV group

Compared with the AV of the above BP in the HAV group, the AV of NO in plasma, LPO in plasma and LPO in RBC in the AVM group were significantly increased ($P = 0.0001$), and the AV of SOD, CAT and GSH-Px in RBC in the AVM group were significantly decreased ($P = 0.0001$) (Table 1).

The 95% CI of the AV of the BP in the AVM group and in the HAV group

The lower limits of the 95% CI of the AV of NO in plasma, LPO in plasma and in RBC in the AVM group were greater than the upper limits of the 95% CI in the corresponding AV in the HAV group. The upper limits of the 95% CI in the AV of SOD, CAT and GSH-Px in RBC in the AVM group were less than the lower limits of the 95% CI in the corresponding AV in the HAV group (Table 2).

Table 1 Comparison between the AV ($\bar{\chi} \pm s$) of the BP in the AVM group and in the HAV group

Group	n	Oxidative substances			Antioxidative substances		
		Plasma		RBC	RBC		
		NO (nmol/L)	LPO (μmol/L)	LPO (nmol/g·Hb)	SOD (U/g·Hb)	CAT (K/g·Hb)	GSH-Px (U/mg·Hb)
AVM	70	520.3 ± 140.8	13.24 ± 1.87	34.13 ± 6.81	1892 ± 198	248.2 ± 56.5	24.12 ± 4.50
HAV	70	351.7 ± 125.5	10.95 ± 1.76	28.57 ± 5.72	2118 ± 214	302.7 ± 60.6	28.37 ± 5.19
t^*		6.7892	8.6203	7.3715	7.1153	7.2674	8.3517
P		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

* paired-samples t test

Table 2 The 95% CI of the AV of the BP in the AVM group and in the HAV group

Group	n	Oxidative substances			Antioxidative substances		
		Plasma		RBC	RBC		
		NO (nmol/L)	LPO (μmol/L)	LPO (nmol/g·Hb)	SOD (U/g·Hb)	CAT (K/g·Hb)	GSH-Px (U/mg·Hb)
AVM	70	406.7 ~ 553.9	12.79 ~ 13.68	32.51 ~ 35.75	1845 ~ 1939	234.7 ~ 261.7	23.05 ~ 25.19
HAV	70	321.8 ~ 381.6	10.53 ~ 11.37	27.21 ~ 29.93	2067 ~ 2169	288.3 ~ 317.1	27.13 ~ 29.61

DISCUSSION

Nitric oxide is a neurotransmitter playing a very important role in the human body metabolism (Murray, 2000; Zhou et al., 2000a, 2000b). The metabolic state and functional state of nitric oxide in the human body closely relate with human health (Murray, 2000; Zhou et al., 2000a, 2000b). If the metabolism of nitric oxide is abnormal, the dynamic balance between the oxidative system and antioxidative system in the human body may be affected or destroyed; and as a consequence, the concentration of free radicals in the human body may increase abnormally and a series of free radical chain reactions in the human body may be aggravated pathologically; which may lead to abnormal vital signs, and accelerate senility of human cells, thus inducing various diseases (Murray, 2000; Zhou et al., 2000a, 2000b). Superoxide dismutase, catalase and glutathione peroxidase are the most important antioxidases in the human body, and play an important role in scavenging oxygen free radicals (such as superoxide anion radical,

hydxoyl radical, hydroperoxyl radical and other free radicals as well as singlet oxygen, hydrogen peroxide and other reactive oxygen species which are excessive in the human body) and in preventing physiological and pathological aggravation of a series of free radical chain reactions induced by excessive superoxide anion radical, and thereby protect biological membranes of cells against oxidative damage and lipoperoxidative damage. Marked decrease of the activities of the above antioxidases in the human body may cause metabolic disorders and pathological aggravation of a series of free radical chain reactions, resulting in oxidative damage and lipoperoxidative damage of DNA, proteins, enzymes and biological membranes, and inducing a variety of diseases related to the abnormal reactions of free radicals (Ginsberg and Fietrich, 1989; Zhou et al., 2000a, 2000b). Lipoperoxide is a product of peroxidation (auto-oxidation) of lipids exposed to oxygen, and the peroxidation of erythrocyte lipids containing polyunsaturated fatty acids can lead to generation of a large number of free radicals (Murray, 2000; Zhou et al., 2000a, 2000b). Lipoperoxidative reaction's metabolic products, such as malondialdehyde, conjugated diene, etc., are important poisonous residual products in the human body, and may strongly attack the body's DNA, proteins, enzymes, biological membranes, polyunsaturated fatty acids, etc., leading to lipoperoxidative damage of the biological membranes, cells and tissues in the human body (Murray, 2000; Zhou et al., 2000a, 2000b).

The findings of the present study showed that there were serious disturbance of nitric oxide metabolism and grave imbalance between oxidation and antioxidation, and also pathological aggravation of oxidative stress and lipoperoxidative stress in the AVM patients. There might be several interpretations. The cytokines, particularly interleukin-I (IL-1), released by inflammatory cells, such as phagocytes like lymphocytes, neutrophilic granulocytes and macrophagocytes in the inflammatory reaction in the cardiac muscle tissues and blood, might activate immediately inducible nitric oxide synthase (iNOS), and stimulate the synthesis and /or release of nitric oxide, thus producing a large amount of nitric oxide (Zhou et al., 2000a, 2000b). Excessive nitric oxide might inactivate antioxidases, such as superoxide dismutase, catalase and glutathione peroxidase, by combining with the hydrosulfide group (-SH); and nitric oxide might combine with superoxide anion radical to produce superoxide nitroso radical ($ONOO^-$), damaging cell functions and deactivating the above antioxidases with its extra-strong oxidative ability (Zhou et al., 2000a, 2000b). Moreover, excessive nitric oxide might be rapidly oxidated into nitrogen dioxide, as a strong active catalyst in lipoperoxidation, nitrogen dioxide might aggravate the lipoperoxidation of polyunsaturated fatty acids in cell membranes and tissues in the human body (Murray, 2000; Zhou et al., 2000a, 2000b). Excessive oxygen free radicals and reactive oxygen species might also directly attack polyunsaturated fatty acids, leading to lipoperoxidation of a large number of polyunsaturated fatty acids, and subsequent production of massive amounts of lipoperoxide which damage cell functions. Additionally, the significant decrease in the synthesis or regeneration of glutathione peroxidase decomposing lipoperoxide and the marked weakness or loss of glutathione peroxidase activity, which condition might also result in significantly increased level of lipoperoxide in the AVM patients' bodies (Murray, 2000; Zhou et al., 2000a, 2000b). The inflammatory cardiac muscle cells and other organic substances in the cardiac muscle tissues also release a large number of oxygen free radicals, reactive oxygen species and other free radicals; thus causing pathological aggravation of a series of free radical chain reactions (Ginsberg and Fietrich, 1989; Zhou et al., 2000a, 2000b).

In addition, the AVM patients appeared to have poor appetite because of their temperature elevation, so antioxidants (such as vitamin C, vitamin E and beta-carotene) absorbed by their bodies were decreased in quantity, resulting in a significant decrease of the antioxidants levels in their bodies, so that the antioxidative vitamins failed to sufficiently scavenge oxygen free radicals, reactive oxygen species and other free radicals so as to keep the dynamic balance between oxidation and antioxidation, thus leading to pathological aggravation of a series of free radical chain reactions in the patients' bodies (Zhou et al., 2000a, 2000b). Under such circumstance the AVM patients had to make use of a great quantity of the antioxidases like superoxide dismutase, catalase and glutathione peroxidase in

their bodies to scavenge the excessive oxygen free radicals, reactive oxygen species and other free radicals, so that the dynamic balance between oxidation and antioxidation might be restored and maintained, and the oxidative damage and lipoperoxidative damage in their bodies might be lessened (Ginsberg and Fietrich, 1989; Zhou et al., 2000a, 2000b).

The findings in this study suggested that the balance between oxidation and antioxidation in the AVM patients' bodies was seriously destroyed, and the oxidative stress and lipoperoxidative stress in their bodies were severely aggravated, and the pathological reaction of a series of free radicals was gravely accelerated, thereby leading to oxidative damage and lipoperoxidative damage of the the AVM patients' bodies. We therefore recommend treatment of AVM patients with daily doses of antioxidants such as vitamin C (300 mg, tid), vitamin E (100 mg, bid) in order to alleviate potential oxidative damage and lipoperoxidative damage in their bodies (O'Connell and Renlund, 1998; Dec et al., 1990; Zhou et al., 2000a, 2000b). Vitamin C and vitamin E are the most important antioxidants in the human body, and play an important role in scavenging excessive oxygen free radicals, reactive oxygen species and other free radicals in AVM patients' bodies, and so, prevent physiological and pathological aggravation of a series of free radical chain reactions in their bodies, maintaining the balance between oxidation and antioxidation, protecting cardiac muscle cells and cardiac muscle tissues in the AVM patients against oxidative damage and lipoperoxidative damages (O'Connell and Renlund, 1998; Dec et al., 1990; Zhou et al., 2000a, 2000b).

In summary, the study results showed that the metabolism of nitric oxide in the AVM patients' bodies caused serious disorders, destroyed the balance between oxidation and antioxidation; severely aggravated the oxidative stress and lipoperoxidative stress; and gravely worsened the pathological reaction of a series of free radicals, the net result of which was oxidative damage and lipoperoxidative damage of the the AVM patients' bodies.

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