Relationship between Acute Organophosphorus Pesticide Poisoning and Damages Induced by Free Radicals

ZHOU JUN-FU *, XU GEN-BO *, AND FANG WEI-JUN **

* *Second Affiliated Hospital of College of Medicine, Zhejiang University, 88 Jiefang Road, Hangzhou 310009, Zhejiang Province, China; and*

** *People's Hospital of Longquan City, Longquan 323700, Zhejiang Province, China*

Objective To study the relationship between abnormal reactions of free radicals in bodies of patients with acute organophosphorus pesticide poisoning (AOPP) and damages induced by free radicals. **Methods** 58 AOPP patients and 58 healthy adult volunteers (HAV) were enrolled in an independent samples control design, in which spectrophotometric methods were used to determine the concentrations of nitric oxide (NO) and lipoperoxides (LPO) in plasma, and LPO in erythrocytes, vitamin C (VC), vitamin E (VE) and β-carotene (β-CAR) in plasma as well as activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and acetylcholinesterase (AChE) in erythrocytes. **Results** Compared with the average values of every biochemical parameter in the HAV group, the average values of LPO in plasma and in erythrocytes, and NO in plasma in the AOPP group were significantly increased (P=0.000001), while the average values of VC, VE, β-CAR in plasma as well as SOD, CAT, GSH-Px and AChE in erythrocytes in the AOPP group were significantly decreased (P=0.000001). The findings of Pearson product-moment correlation analysis between the value of AChE in erythrocytes and the values of above biochemical parameters for 58 AOPP patients showed that there was a significant linear negative correlation between AChE in erythrocytes and LPO, NO in plasma, and LPO in erythrocytes (P=0.000001~0.001319), while there was a significant linear positive correlation between AChE in erythrocytes and VC, VE, β-CAR in plasma as well as SOD, CAT, GSH-Px in erythrocytes (P=0.000013~0.000824). The results of discriminant analysis of above chemical parameters for 58 AOPP patients and 58 HAV suggested that the correct rates of discriminant analysis were increased to 100 % when the values of AChE and LPO in plasma and in erythrocytes, or AChE and others, were jointly used for the discriminant analysis. **Conclusion** The findings of the present study suggest that a series of free radical reactions in AOPP patients' bodies are pathologically aggravated, and the discriminant analysis used the above biochemical parameters could markedly increase its correct rates for AOPP patients.

Key words: Organophosphorus pesticide poisoning; Free radicals; Lipoperoxides; Nitric oxide; Antioxidants; Antioxidases

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Biographical note of the first author: Zhou Jun-Fu, M.D., research scientist & professor, male, born in 1945, graduated from College of Medicine, Zhejiang University, having 200 papers published and having won 20 awards of science and technology advance from Governments and PLA. Research directions: preventive medicine, free radical medicine.

Correspondence should be addressed to: Prof. Zhou Jun-Fu, Second Affiliated Hospital of College of Medicine, Zhejiang University, 88 Jiefang Road, Hangzhou, 310009, Zhejiang Province, China. Tel: 86-571-87783768; Fax: 86-571-87213864; E-mail: zhuojun88@sina.com

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INTRODUCTION

Acute organophosphorus pesticide poisoning includes occupational poisoning in industrial production and agricultural application, and suicide, homicide, accidental overdose, accidental overuse and so on[1,2]. Some authors reported that reaction of reactive oxygen species, oxidative and lipoperoxidative stress, and chain reactions of a series of free radicals in patients with acute organophosphorus pesticide poisoning (AOPP) are significantly aggravated[3-1.1]. The levels of vitamins C and E as well as activity of superoxide dismutase are markedly decreased, and level of lipoperoxides is notably increased in the AOPP patients[3-11]. However, up to now, there have been neither reports on abnormal metabolism of nitric oxide and abnormal change of values of β-carotene, glutathione peroxidase, catalase and acetylcholinesterase in erythrocytes in AOPP patients' bodies, nor reports about relationship between oxidative stress, oxidative damages and AOPP. To study the relationship between abnormal reactions of a series of free radicals in the AOPP patients' bodies and damages induced by free radical abnormal reactions, 58 AOPP patients and 58 healthy adult volunteers (HAV) were enrolled in an independent samples control design, in which spectrophotometric methods were used to determine the concentrations of nitric oxide (NO) and lipoperoxides (LPO) in plasma and LPO in erythrocytes, vitamin C (VC), vitamin E (VE) and β-carotene (β-CAR) in plasma as well as activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and acetylcholinesterase (AChE) in erythrocytes. In addition, the differences in the average values of above biochemical parameters between AOPP patient group and HAV group were analyzed. Pearson product-moment correlation between the activity of AChE in erythrocytes and the above biochemical parameters for 58 AOPP patients was analyzed. The relative risk ratio (RR) of the above biochemical parameters between the AOPP group and the HAV group, and the 95 % CI of RR were estimated. In addition, the discriminant analysis was carried out for 58 AOPP patients and 58 HAV.

MATERIALS AND METHODS

Study Design

The independent samples control design was used in this study. In order to obtain a comparatively objective research conclusion, the principles of random, control, replication and equilibrium, and the management factor, experimental effect and subjects were taken into full consideration[12,13].

Subjects

AOPP. 58 patients (22 males, 36 females) with acute organophosphorus pesticide poisoning were randomly selected from 103 AOPP patients who were receiving emergency treatment in the People's Hospital of Longquan City by means of "Select Cases-Random Sample of Cases" in "SPSS 10.0 for Windows". Their diagnoses were confirmed by having taken organophosphorus pesticides, such as methamidophos (n=24), dimethoate (n=19) and dichlorvos (n=15), and clinical symptoms as well as markedly decreased activity of AChE in erythrocytes. Their age was 18-39 (24.8 ± 5.4) years. Their anamnesis of disorders associated with brain, heart, lung, liver, kidney and other organs were excluded, and their anamnesis of hypertension, hyperlipoidemia, chronic bronchitis, autoimmune disease, diabetes, atherosclerosis and tumors were also excluded. They were all volunteers in this

study.

HAV. 58 healthy adult volunteers (29 males, 29 females) were randomly selected from 500 HAV by means of "Select Cases-Random Sample of Cases" in "SPSS 10.0 for Windows". Their age was 20 - 40 (25.1 ± 5.2) years. They were found to be normal in their routine blood, urine and feces examinations and radiographs, and their anamnesis and history of present illness were also excluded.

No significant difference was found between the average values for age in the AOPP group and HAV group by t test ($t = 0.2799$, $P = 0.7801$), as well as between the sexual proportions in the AOPP group and HAV group by χ^2 test ($\chi^2 = 1.7146$, $P = 0.1904$).

Within the prior month, all the subjects had not taken any antioxidant supplements such as vitamin C, vitamin E, β-carotene, ginkgo biloba, theo-polyphenols or other similar substances before they were enrolled as volunteers in this study. They, in general, had not taken fruits and greenstuff containing rich antioxidative vitamins as they all lived in remote countrysides and mountainous districts.

Methods

Collection and Pretreatment of Blood Samples. Fasting venous blood samples were collected in the morning with heparin sodium added as anticoagulant, and the separated plasma and erythrocytes were stored at –50℃.

Determination of AChE Activity in Eerythrocytes. 0.05 mL hemolytic solution in which hemoglobin concentration was measured, 0.95 mL phosphate buffer (0.067 mol/L, pH 7.20) and 1.0 mL acetylcholine chloride solution (10 μmol/L) prepared by acetate buffer (1.0 mmol/L, pH 4.50) were mixed. After 20 min in a water bath at 37℃, 4.0 mL alkalescent hydroxylamine hydrochloride solution [mixed by hydroxylamine hydrochloride solution (140 g/L) and sodium hydroxide solution (3.50 mol/L) = 1 + 1, 2.0 mL hydrochloric acid (ρ_{20}=1.19 g/mL) and 2.0 mL ferric chloride solution (0.6 mol/L)] were added. After centrifugation, the supernatant was used to determine the AChE activity with spectrophotometry at wavelength of 530 nm (1.0 cm cell). Acetylcholine chloride solution (10 μmol/L) was used as the standard, and the activity of AChE in erythrocytes was expressed as U/g.Hb.

Determination of the Other Biochemical Constituents. The methods used in determination of the other biochemical substances and enzymes are outlined below with additional details and references provided [14,15]. The spectrophotometry of thiobarbituric acid reactive substances (TBARS) was used to determine plasma LPO concentration expressed as μmol/L. The spectrophotometry of TBARS was used to determine the concentration of LPO in erythrocytes expressed as nmol/g.Hb. The coloration of α-naphthylamine was used in determining plasma NO concentration expressed as nmol/L. The ferrozine coloration was used to determine plasma VC and plasma VE concentrations expressed as μmol/L. The plasma β-CAR content was extracted with a mixture of ethanol and petroleum ether, and determined with spectrophotometry, and the β-CAR concentration was expressed as μmol/L. The spectrophotometry of inhibiting pyrogallol auto-oxidation was used to determine the activity of SOD in erythrocytes expressed as U/g.Hb. The spectrophotometry of coloration of hydrogen peroxide and acetic acid-potassium dichromate was used to determine the activity of CAT in erythrocytes expressed as K/g.Hb. The modified Hafeman's spectrophotometry was used to determine the activity of GSH-Px in erythrocytes expressed as U/mg.Hb.

In determination of the above biochemical substances and enzymes, the main analytical

reagents, such as vitamin C, vitamin E, β-carotene, disodium 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazinedisulfonate, Cu/Zn-superoxide dismutase, catalase, α-naphthylamine, 1,2,3-trihydroxybenzene, 1,1,3,3-tetraethoxypropane, 2-thiobarbituric acid, were all purchased from SIGMA CHEMICAL COMPANY®, USA; and the other analytical reagents were all produced in China, the fresh quadruply distilled water was prepared with a quartz glass distilling apparatus. The main analytical instruments included HEWLETT Packard 8453-Spectrophotometer, USA, and UV-754-Spectrophotometer and 721-Spectrophotometer.

In determination of the above biochemical substances, the same batch number of each reagent, the same quality control, the same lab assistant, and the identical analytical apparatus were strictly used for every experiment to control and decrease the error and bias of experiment, and to ensure the analytical quality of determinations[12,13].

Medical statistic analysis. All data were statistically analyzed with SPSS/11.0 for Windows and STATISTICA/6.0 for Windows statistic software using a Compaq Pentium III /1000 computer. The parameters in this study presented all normal distributions by Kolmogorov-Smirnov test, and were expressed as mean plus or minus standard deviation ($\bar{x} \pm s$) and 95 % confidence interval (95% CI). Hypothesis testing methods included independent-samples *t* test, χ^2 test, estimation of relative risk ratio, discriminant analysis, and so on. In the statistical analysis, the level of hypothesis testing (α) was $\leqslant$ 0.05 to avoid false positives, and the power of hypothesis testing (*power*) was $\geqslant$ 0.80 to avoid false negatives[12-15].

RESULTS

Comparison of the Average Values ($\bar{x} \pm s$) of the Above Biochemical Parameters Between the AOPP Group and HAV Group

Compared with the HAV group, the average values of LPO in plasma and erythrocytes, and NO in plasma in AOPP group were significantly increased, while those of VC, VE and β-CAR in plasma as well as those of SOD, CAT, GSH-Px and AChE in erythrocytes in AOPP group were significantly decreased (Table 1).

95 % Confidence Interval of the Average Values ($\bar{x} \pm s$) of the Above Biochemical Parameters in the AOPP Group and HAV Group

The lower limits of 95 % confidence interval (95 % CI) of the average values of LPO in plasma and in erythrocytes and the lower limit of NO in plasma in the AOPP group were greater than the upper limits of 95 % CI of the same average values in HAV group. The upper limits of 95 % CI of the average values of VC, VE and β-CAR in plasma and those of SOD, CAT, GSH-Px and AChE in erythrocytes in the AOPP group were less than the lower limits of 95 % CI of the same average values in HAV group (Table 1).

Pearson Product-moment Correlation Analysis Between the Value of AChE in Erythrocytes and the Values of the Above Biochemical Parameters for 58 AOPP Patients

The linear correlative coefficient of Pearson product-moment correlation analysis between the value of AChE in erythrocytes and the values of LPO in plasma and in erythrocytes as well as NO in plasma for 58 AOPP patients was –0.5230 (P=0.000025), –0.6224 (P=0.000001) and –0.4118 (P=0.001319) respectively, presenting a linear negative correlation. The linear correlative coefficient of Pearson product-moment correlation analysis

between the value of AChE in erythrocytes and the values of VC, VE and β-CAR in plasma as well as SOD, CAT and GSH-Px in erythrocytes for 58 AOPP patients was 0.5346 (P=0.000015), 0.4648 (P=0.000238), 0.4852 (P=0.000113), 0.4809 (P=0.000133), 0.4272 (P=0.000824) and 0.5370 (P=0.000014) respectively, presenting a linear positive correlation.

Estimation of the Relative Risk Ratio (RR) of the Above Biochemical Parameters Inducing Oxidative Damage of the AOPP Patients, and 95 % CI of RR

Supposing the values of LPO in plasma and in erythrocytes, and NO in plasma in AOPP patients $>$ the upper limits of 95 % CI of the values of same biochemical parameters in HAV group to be the reference criteria of oxidative damage positive number of the AOPP patients, and supposing the values of VC, VE and β-CAR in plasma as well as SOD, CAT and GSH-Px in erythrocytes in AOPP patients $<$ the lower limits of 95 % CI of the values of same biochemical parameters in HAV group to be the reference criteria of oxidative damage positive number of the AOPP patients, the relative risk ratio (RR) of the above biochemical parameters inducing oxidative damage of the AOPP patients ranged from 6.502 to 48.125, and 95 % CI of the RR ranged from 2.803 to 191.985 (Table 2).

Discriminant Analysis for 58 AOPP Patients and 58 HAV

In the study, the discriminatory correct rates were only 65.5 % and 81.0 % when the value of AChE in erythrocytes was used to make the discriminant analysis for 58 AOPP patients and 58 HAV. However, the discriminatory correct rates were increased to 100 % when the values of AChE in erythrocytes and LPO in plasma and in erythrocytes were used to make the discriminant analysis for 58 AOPP patients and 58 HAV. In the same way, the discriminatory correct rates were increased to 100 % when the values of AChE in erythrocytes and LPO in plasma and VC in plasma, or AChE and LPO and VE in plasma, or AChE and LPO and β-CAR in plasma, or AChE and LPO and SOD in erythrocytes, or AChE and LPO and CAT in erythrocytes, or AChE and LPO and GSH-Px in erythrocytes, or AChE and LPO in erythrocytes and NO in plasma, or AChE and SOD and CAT in erythrocytes, or AChE and SOD and GSH-Px in erythrocytes, or AChE and CAT and GSH-Px in erythrocytes, were jointly used to make the discriminant analysis for 58 AOPP patients and 58 HAV.

DISCUSSION

Lipoperoxide (LPO) is a product of peroxidation (auto-oxidation) of lipids exposed to oxygen, and lipoperoxidation is a source of free radicals and may be a cause of cancer, inflammatory diseases, atherosclerosis, aging, etc[13-23]. LPO and its metabolic products, such as malondialdehyde (MDA), conjugated diene (CD) and others, are important poisonous residual products, and significantly increased LPO, MDA and CD in human body may strongly attack DNA, proteins, enzymes, biological membranes, polyunsaturated fatty acids (PUFAs) and others, leading to lipoperoxidative damages of biological membranes, and loss of the functions of biological membranes and cells[10,11,14-18]. Nitric oxide (NO) is a very important neurotransmitter molecules, and NO can directly modify enzymes that produce second messengers. Therefore, NO plays a very important role in the metabolism in human body[14-22,24]. Vitamin C (VC), vitamin E (VE) and β-carotene (β-CAR) as well as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) are the most

TABLE 1

Comparison of Biochemical Parameters ($\bar{x} \pm s$) Between the AOPP Group and the HAV Group, and Their 95 % CI

Group	n	Oxidative Biochemical Substances			Antioxidative Biochemical Substances						Enzyme
		Plasma		Erythrocyte	Plasma			Erythrocyte			Erythrocyte
		LPO (μmol/L)	NO (nmol/L)	LPO (nmol/gHb)	VC (μmol/L)	VE (μmol/L)	β-CAR (μmol/L)	SOD (U/gHb)	CAT (K/gHb)	GSH-Px (U/mgHb)	AChE (U/gHb)
AOPP	58	14.59 ± 2.12	587.4 ± 135.6	39.39 ± 5.53	34.36 ± 8.49	15.35 ± 3.66	1.24 ± 0.32	1674 ± 129	217.9 ± 54.4	18.44 ± 4.88	207.2 ± 61.7
		(14.03 15.15)	(551.7 623.0)	(37.94 40.85)	(32.11 36.58)	(14.38 16.31)	(1.15 1.32)	(1640 1708)	(203.6 232.2)	(17.16 19.72)	(191.0 223.4)
HAV	58	11.04 ± 1.60	374.7 ± 86.5	28.72 ± 4.13	54.36 ± 13.44	24.46 ± 5.83	1.72 ± 0.44	2069 ± 159	291.3 ± 72.7	28.73 ± 7.61	298.5 ± 88.8
		(10.62–11.46)	(351.9–397.4)	(27.63–29.81)	(50.83–57.90)	(22.93–25.99)	(1.60–1.84)	(2027–2111)	(272.2–310.4)	(26.73–30.73)	(275.2–321.9)
t^a		10.1926	10.0706	11.7740	9.5847	10.0821	6.7835	14.7162	6.1629	8.6662	6.4322
P		0.000001	0.000001	0.000001	0.000001	0.000001	0.000001	0.000001	0.000001	0.000001	0.000001

Note. [a] Independent-samples t test. Figures in parentheses are 95 % CI.

TABLE 2

Estimation of the Relative Risk Ratio (RR) of Biochemical Parameters Inducing Oxidative Damage of the AOPP Patients, and 95 % CI of RR

Group	n	Plasma				Erythrocyte		Plasma						Erythrocyte					
		NO		LPO		LPO		VC		VE		β-CAR		SOD		CAT		GSH-Px	
		+	−	+	−	+		+	−	+	−	+	−	+		+	−	+	−
AOPP	58	50	8	54	4	55	3	56	2	55	3	49	9	50	8	47	11	54	4
HAV	58	18	40	18	40	16	42	23	35	21	37	23	35	22	36	23	35	20	38
Relative Risk Ratio (95% CI)		13.889 (5.475–35.231)		30.000 (9.423 95.506)		48.125 (13.157–176.034)		42.609 (9.456–191.985)		32.302 (8.986–116.117)		8.285 (3.422 20.061)		10.227 (4.094–25.551)		6.502 (2.803–15.080)		25.650 (8.114–81.084)	
χ^2		36.392		47.455		55.223		43.217		44.111		24.753		28.707		20.750		43.145	
P		0.0001		0.0001		0.0001		0.0001		0.0001		0.0001		0.0001		0.0001		0.0001	

Note "+" Indicates "number cases" that the values of the NO and LPO in plasma, and the LPO in erythrocytes in AOPP group $>$ the upper limits of 95 % CI of the values of the same biochemical parameters in HAV group, and that the values of the VC, VE and β-CAR in plasma as well as SOD, CAT and GSH-Px in erythrocytes in AOPP group $<$ the lower limits of 95% CI of the values of the same biochemical parameters in HAV group, while " " expresses opposite those.

important antioxidants and antioxidases in human body[3,4,7,9-11,14-26]. They play important roles in scavenging oxygen free radicals (OFRs), such as superoxide anion radical (O_2^-), hydroxyl radical (·OH), hydroperoxyl radical (HO_2) and other free radicals (FRs) as well as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and other reactive oxygen species (ROS) which are excessive in the human body, and they prevent physiological and pathological aggravation of a series of FRs chain reactions induced by excessive O_2^-, thereby protecting biological membranes of cells against oxidative and lipoperoxidative damages[14-26]. The metabolic state and functional status of LPO, NO, antioxidants and antioxidases in the human body are closely related with human health[14-26]. If their metabolic states are abnormal, the dynamic balance between the oxidative system and antioxidative system in human body is affected or destroyed[14-26]. As a consequence, concentration of FRs may unusually increase, and a series of FRs chain reactions may be pathologically aggravated in human body. This situation may lead to abnormal vital signs, and accelerate senility of human cells, thus inducing various diseases[14-26].

The findings of the present study showed that in the AOPP patients' bodies the metabolism of nitric oxide presented serious disorders, the dynamic balance between oxidation and antioxidation produced grave imbalance, and the oxidative stress came forth pathological aggravation, which led to serious oxidative and lipoperoxidative damages of the AOPP patients' bodies. There might be several interpretations. After organophosphorus pesticides come into alimentary tract and stomach, which combine rapidly with hydrochloric acid and other organic acid in the stomach to produce vehement chemical reactions, thus producing a large amount of deadly toxins[25-30]. At the same time, the chemical reactions would produce and release a large number of OFRs, such as O_2^-, ·OH and HO_2 and other FRs as well as ROS like $^1O_2$ and H_2O_2, thereby promoting violent aggravation of a series of FRs chain reactions, attacking strongly mucous membranes of the alimentary tract, and important organs and tissues with blood circulation, resulting in chemical poisoning inflammatory reaction[25-30]. Subsequently, the organophosphorus pesticides, and the deadly toxins produced in the chemical reactions, are absorbed largely by mucous membranes of the alimentary tract, which would present a series of metabolic disturbance, result in producing toxic symptoms of extensive bleeding in heart, lung, liver, kidney, intestines and others[25-30]. Cytokines, especially interleukin-1 (IL-1) released by inflammatory cells such as phagocytes like lymphocytes, neutrophilic granulocytes and macrophagocytes in the inflammatory reaction in the organs, tissues and blood in the AOPP patients' bodies, might activate immediately inducible nitric oxide synthase (INOS), and stimulate synthesis and /or release of NO, thus producing a large amount of NO[10,11,14,15]. Excessive NO might inactivate antioxidases by combining with hydrosulfide group (-SH), and excessive NO might combine with O_2^- to produce superoxide nitroso radical ($ONOO^-$), damaging cell functions and deactivating antioxidases with its extra-strong oxidative ability[14,15,17,19,20]. Moreover, excessive NO might be rapidly oxidized into nitrogen dioxide (NO_2), as a strong active catalyst in lipoperoxidation, NO_2 might aggravate the lipoperoxidation of PUFAs[14,15,17,19,20]. Excessive OFRs and ROS might also directly attack PUFAs, leading to lipoperoxidation of a large number of PUFAs, and subsequent production of a mass of LPO, MDA and CD, which would damage cell functions[14,15,17,19,20]. Additionally, abnormal metabolism of cytochromes P-450, especially the abnormal metabolism of cytochrome P-450 2E1 (CYP 2E1) in the AOPP patient body, would produce and release a large number of OFRs, such as O_2^-, ·OH, HO_2 and other FRs, and ROS like $^1O_2$ and H_2O_2, thus resulting in antioxidants and antioxidases being attacked strongly, destroyed and inactivated rapidly[9,25,26].

The findings of Pearson product-moment correlation analysis between the AChE activity in erythrocyte able to reflect sensitively and accurately toxic degree of

organophosphorus pesticides and the above biochemical parameters suggested that the active groups of AChE are attacked and destroyed by not only the covalent binding of pesticide's phosphate radicals, but also a large number of FRs and ROS, which would lead to decrease and/or loss of the AChE activity in erythrocytes, thereby further reflect the toxic degrees of AOPP patients[2,14,15,17,19,20,25,26]. The findings of the relative risk ratio (RR) estimation in this study showed that the RR of oxidative and lipoperoxidative damages in human body might increase 5.5～47.1 times after acute organophosphorus pesticide poisoning, further suggesting that the changes of oxidative stress in human body play important roles in the toxic mechanism of acute organophosphorus pesticide poisoning.

Stepwise discriminant analysis in this study showed that the discriminatory correct rates were only 65.5 % and 81.0 % respectively when the value of AChE in erythrocytes was singly used to make the discriminant analysis for 58 AOPP patients and 58 HAV. However, the discriminatory correct rates were all increased to 100 % when the values of AChE in erythrocytes and LPO in plasma and in erythrocytes were jointly used to make the discriminant analysis for 58 AOPP patients and 58 HAV. In the same way, the discriminatory correct rates were all increased to 100 % when the values of AChE in erythrocytes and LPO in plasma and VC in plasma, or AChE and LPO and VE in plasma, and so on. These results further suggested that although the marked decrease of erythrocyte AChE (true cholinesterase) activity is a more specific indicator of organophosphorus pesticide poisoning than plasma cholinesterase (pseudocholinesterase) level[12], but its' discriminatory correct rate is dissatisfactory when erythrocyte AChE activity is singly used to make the discriminant analysis for AOPP. Therefore, it is considered that the satisfactory discriminatory correct rate for AOPP can be obtained so long as erythrocyte AChE activity is properly combined with the above biochemical parameters. This combination would play an important role in the differential diagnosis of acute organophosphorus pesticide poisoning.

In conclusion, the findings in this study suggested that the serious abnormality of oxidative stress and the severe oxidative damage and lipoperoxidative damage were presented in the AOPP patients' bodies. Therefore, the authors recommend that in the emergency treatment of acute organophosphorous pesticide poisoning patients, the antioxidants at suitable doses, such as vitamin C (3～5 g, q.d.), vitamin E (0.2～0.5 g, q.d.) and others, should be given in order to reduce oxidative damage and lipoperoxidative damage in their bodies, which would be effective in speedy recovery of AOPP patients[14,15,17,19,20,25,26].

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