·Original Article·

Increased oxidative stress and oxidative damage associated with chronic bacterial prostatitis

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Abstract

Aim: To investigate whether chronic bacterial prostatitis might increase oxidative stress and oxidative damage in chronic bacterial prostatitis patients (CBPP), and to explore its possible mechanism. **Methods:** Enrolled in a case-control study were 70 randomly sampled CBPP and 70 randomly sampled healthy adult volunteers (HAV), on whom plasma nitric oxide (NO), vitamin C (VC), vitamin E (VE) and β-carotene (β-CAR) level, erythrocyte malondialdehyde (MDA) level, as well as erythrocyte superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activities were determined by spectrophotometry. **Results:** Compared with the HAV group, values of plasma NO and erythrocyte MDA in the CBPP group were significantly increased ($P < 0.001$); those of plasma VC, VE and β-CAR as well as erythrocyte SOD, CAT and GPX activities in the CBPP group were significantly decreased ($P < 0.001$). Findings from partial correlation for the 70 CBPP showed that with prolonged course of disease, values of NO and MDA were gradually increased ($P < 0.001$), and those of VC, VE, β-CAR, SOD, CAT and GPX were gradually decreased ($P < 0.05$–0.001). The findings from stepwise regression for the 70 CBPP suggested that the model was $Y = -13.2077 + 0.1894MDA + 0.0415NO - 0.1999GPX$, $F = 18.2047$, $P < 0.001$, $r = 0.6729$, $P < 0.001$. **Conclusion:** The findings suggest that there exist increased oxidative stress and oxidative damage induced by chronic bacterial prostatitis in the patients, and such phenomenon was closely related to the course of disease. *(Asian J Androl 2006 May; 8: 317–323)*

1   Introduction

In human boday, Vitamin C (VC), vitamin E (VE) and β-carotene (β-CAR) are the most important antioxidants, and superoxide dismutase (SOD), catalase (CAT) and

glutathione peroxidase (GPX) are the most important antioxidative enzymes. They play very important roles in scavenging excessive superoxide anion radicals (O_2^-), hydroxyl radicals (·OH), nitric oxide radicals (NO·) and other free radicals, as well as excessive reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) in human body [1–10]. Nitric oxide (NO) is one of the very important neurotransmitter molecules, and NO can directly modify enzymes that produce second messengers [1, 6, 8, 9]. Malondialdehyde (MDA) is a metabolic product of peroxidative

reactions (auto-oxidation) of lipids exposed to oxygen [1, 6, 8, 9]. Therefore, VC, VE, β-CAR, SOD, CAT, GPX, NO and MDA play very important roles in the metabolism in humans [1–10]. Both significantly decreased VC, VE, β-CAR, SOD, CAT and GPX, and markedly increased NO and MDA can cause metabolic disorders and increase oxidative stress and oxidative damage in humans, therefore inducing a variety of diseases related to abnormal oxidative stress and oxidative damage in human body [1–10]. The present study aims to investigate whether chronic bacterial prostatitis might increase oxidative stress and oxidative damage in chronic bacterial prostatitis patients (CBPP), and to explore its possible mechanism.

2 Materials and methods

2.1 Study design

According to the diagnostic criteria and the inclusion criteria [11], 70 randomly sampled chronic bacterial prostatitis patients (CBPP) and 70 randomly sampled healthy adult volunteers (HAV) were enrolled in a case-control study, in which the levels of NO, VC, VE and β-CAR in plasma, and the level of MDA in erythrocytes, as well as the activities of SOD, CAT and GPX in erythrocytes were determined by spectrophotometry. In addition, the differences between the values of the above-mentioned parameters in the two groups, the partial correlation and a stepwise regression model for 70 CBPP were computed. This research was approved by the Ethics Committee of Second Affiliated Hospital, College of Medicine, Zhejiang University.

2.2 Subjects
2.2.1 Chronic bacterial prostatitis patients (CBPP)

From 102 CBPP confirmed using the diagnostic criteria and the inclusion criteria [11] in our hospital (Second Affiliated Hospital, College of Medicine, Zhejiang University, China), 70 patients were randomly sampled using "select cases-random sample of cases" in SPSS 11.0 for Windows (SPSS, Chicago, USA). Their age, systolic blood pressure (SBP), diastolic blood pressure (DBP), hemoglobin, serum albumin and body-mass index (BMI) were from 21 to 30 years, from 101 to 139 mmHg, from 70 to 88 mmHg, from 128.5 to 146.0 g/L, from 36.64 to 47.86 g/L, and from 20.81 to 24.82, respectively. The course of disease ranged from 1 to 12 years. All subjects were volunteers in this research.

2.2.2 Healthy adult volunteers (HAV)

From 100 HAV in the same hospital, 70 men were randomly sampled by the above-mentioned program in SPSS 11.0 for Windows (SPSS, Chicago, USA). Their age, SBP, DBP, hemoglobin, serum albumin and BMI were from 21 to 30 years, from 98 to 138 mmHg, from 68 to 88 mmHg, from 128.3 to 147.0 g/L, from 36.47 to 47.57 g/L, and from 18.91 to 24.50, respectively. All subjects were volunteers in this research.

There were no significant differences on the average values of age, SBP, DBP, hemoglobin, serum albumin and BMI between the CBPP group and the HAV group by independent sample t test. There were also no significant differences on nutritional condition, annual earnings, education level, profession or occupation, residence region, daily diet (food and drink) and mental status between the two groups by independent sample t-test, or Pearson χ^2-test.

The demographics and some other data for the 70 CBPP and 70 HAV are presented in Table 1.

By routine blood, urine and stool examinations, radiographs, cardiograms and other necessary examinations, it was determined that the above subjects have no history of disorders associated with the brain, heart, lungs, liver, kidneys, and blood system, circulatory system or respiratory system. They have no history of hypertension, hyperlipidemia, acute or chronic bronchitis, asthma, autoimmune disease, diabetes, atherosclerosis, tumors and cancers, subnutrition, malnutrition or other nutritional diseases.

In the previous month, none of the subjects had taken any antioxidant supplements, such as VC, VE, β-CAR, ginkgo biloba, tea polyphenols or other similar substances.

2.3 Methods
2.3.1 Collection and pretreatment of blood samples

Fasting venous blood samples were collected from all the subjects in the morning. Heparin sodium was added as an anticoagulant. Plasma and erythrocytes were separated promptly, and stored at −50ºC immediately, and the blood samples did not undergo any hemolysis [6].

2.3.2 Measurement of NO, VC, VE, β-CAR, MDA, SOD, CAT and GPX

Spectrophotometry for α-naphthylamine coloration was used to determine plasma NO level expressed as nmol/L [6]. Trichloroacetic acid solution was used to precipitate proteins in plasma and to extract VC from plasma. The VC in the extract solution reduced Fe^{3+} in the ferric trichloride solution to Fe^{2+}, and Fe^{2+} reacted

Table 1. The demographic data and some other data in the CBPP group and the HAV group. CBPP: chronic bacterial prostatitis patients; HAV: healthy adult volunteers.

Item	CBPPs (n = 70)	HAVs (n = 70)	Statistic analysis	
			Levene's test for equality of variances	Independent samples t-test
Age (year)	21 – 30	21 – 30	F = 0.011	t = 0.171
	(24.35 ± 2.77)	(24.43 ± 2.78)	P = 0.918	P = 0.864
Systolic blood	101–139	98 – 138	F = 0.065	t = 0.293
pressure (mmHg)	(121.2 ± 9.1)	(120.8 ± 8.8)	P = 0.799	P = 0.770
Diastolic blood	70 – 88	68 – 88	F = 0.243	t = 0.659
pressure (mmHg)	(79.3 ± 5.3)	(78.6 ± 5.6)	P = 0.622	P = 0.511
Hemoglobin (g/L)	128.5 – 146.0	128.3 – 147.0	F = 0.200	t = 0.118
	(140.6 ± 4.7)	(140.7 ± 4.4)	P = 0.655	P = 0.906
Albumin (g/L)	36.64 – 47.86	36.47 – 47.57	F = 0.141	t = 0.030
	(43.45 ± 2.29)	(43.46 ± 2.18)	P = 0.708	P = 0.976
Body-mass index	20.81 – 24.82	18.91 – 24.50	F = 3.775	t = 1.479
	(23.21 ± 1.02)	(22.92 ± 1.27)	P = 0.054	P = 0.141
The course of disease (year)	1 – 12	—	—	—
	(6.0 ± 3.2)			
Smoking history	No	No	—	—
Abusing alcohol history	No	No	—	—

with ferrozine to form a colored end product that was detected by spectrophotometry at 563 nm and 10.0 mm cell, and its level was expressed as μmol/L [6]. Absolute ethanol was used to precipitate proteins in plasma and to extract VE from plasma, and other procedures were the same as those of VC, with its level expressed as μmol/L [6]. The plasma β-CAR was extracted with a mixture of ethanol and petroleum ether, and was determined by spectrophotometry, and plasma β-CAR level was expressed as μmol/L [6]. Spectrophotometry for thiobarbituric acid reactive substances was used to determine erythrocyte MDA level expressed as nmol/g·Hb [6]. Spectrophotometry for inhibiting pyrogallol auto-oxidation was used to determine erythrocyte SOD activity expressed as U/g·Hb [6]. Spectrophotometry for coloration of hydrogen peroxide and acetic acid-potassium dichromate was used to determine erythrocyte CAT activity expressed as K/g·Hb [6]. The improved Hafeman's spectrophotometry was used to determine erythrocyte GPX activity expressed as U/mg·Hb [6].

Main analytical reagents for the determination of above biochemical substances and enzymes, including α-naphthylamine, VC, VE, 5,6-diphenyl-3-(2-pyridyl)-1,2, 4-triazinedisulfonic acid disodium salt (ferrozine), β-CAR, 1,1,3,3-tetraethoxypropane, 2-thiobarbituric acid, Cu·Zn-

SOD, 1,2,3-trihydroxybenzene (pyrogallol) and CAT, were purchased from SIGMA Chemical (St. Louis, USA), and the other analytical reagents were obtained in China (Shanghai Chemical Company, Shanghai, China). The fresh quadruply distilled water was prepared with a quartz glass distilling apparatus. The main analytical instrument was the Hewlett Packard 8453-Spectrophotometer (Hewlett Packard Company, Boise, ID, USA).

In the above assays, standardization (e.g. same batch number of each reagent, same quality control, same lab assistant and identical analytical apparatus) was strictly used for every experiment to decrease errors and bias, and to ensure the analytical quality of determinations [6].

2.4 Medical statistical analysis

All data in this research were statistically analyzed with SPSS 11.0 for Windows statistics software (SPSS, Chicago, USA). The biochemical parameters in this research presented normal distributions using the Kolmogorov-Smirnov Z test, and were expressed as mean ± SD with 95% confidence intervals (95% CI). Hypothesis testing methods included the independent samples t-test, the Pearson χ^2-test, partial correlation analysis, stepwise regression analysis, and reliability analysis In the statistical analysis in this research, the level of hy-

pothesis testing (α) was ≤ 0.05 to avoid false positives (type I error), and the power of hypothesis testing (*power*) was ≥ 0.85 to avoid false negatives (type II error)) [6].

3 Results

Compared with the average values of NO, VC, VE, β-CAR, MDA, SOD, CAT and GPX in the HAV group, the average values of VC, VE, β-CAR in plasma as well as those of SOD, CAT and GPX in erythrocytes in the CBPP group were significantly decreased ($P < 0.001$), while those of NO in plasma and MDA in erythrocytes were significantly increased ($P < 0.001$) (Table 2). The upper limits of the 95% CI of average values of VC, VE, β-CAR, SOD, CAT and GPX in the CBPP group were less than the lower limits of the 95% CI of those in the HAV group, and the lower limits of the 95% CI of average values of NO and MDA in the CBPP group were greater than the upper limits of the 95% CI of those in the HAV group (Table 2). The findings from partial correlation analysis between the course of disease and NO, VC, VE, β-CAR, MDA, SOD, CAT and GPX for 70 CBPP controlling for age suggested that with prolonged course of disease, the values of VC, VE, β-CAR, SOD, CAT and GPX were gradually decreased ($P < 0.02 – 0.005$), and those of NO and MDA were gradually increased ($P < 0.001$) (Table 3). The findings from stepwise regression among the course of disease and NO, VC, VE, β-CAR, MDA, SOD, CAT and GPX for 70 CBPP suggested that the model of stepwise regression was $Y = -13.2077 + 0.1894MDA + 0.0415NO - 0.1999GPX$, $F = 18.2047$, $P < 0.001$, $r = 0.6729$, $P < 0.001$ (Table 4). The findings from a reliability analysis for the levels of VC, VE, β-CAR, SOD, CAT, GPX, NO and MDA used to reflect increased oxidative stress and oxidative damage in the CBPP were that the reliability coefficients' alpha (8 items) was 0.7109 ($P < 0.0001$), and that the standardized item alpha was 0.9214 ($P < 0.0001$).

4 Discussion

The findings in this research suggest that VC, VE, β-CAR, SOD, CAT and GPX were significantly decreased, and NO and MDA were significantly increased in the patients with chronic bacterial prostatitis, and that there existed increased oxidative stress and oxidative damage in the patients. There might be several interpretations.

During inflammatory reactions induced by chronic bacterial prostatitis, a large number of inflammatory cells might generate and release a number of inflammatory mediators: for example, proinflammatory cytokines and inflammatory cytokines, such as interleukins [6, 12–14], tumor necrosis factor-alpha [6, 14], p53 [6, 15], cytochrome P-450 [6, 16] and NADPH-cytochrome P-450 [6, 16], causing abnormal metabolism of the hypoxanthine/xanthine oxidase system and the xanthine/xanthine oxidase system, producing many abnormal metabolites [6, 17]. These inflammatory cells and reactions might also activate and release a large amount of cycloox ygenase-2 [6, 13], transcription factor nuclear factor-kappa B [6, 13], inducible nitric oxide synthase, and an amount of inflammatory oxidants and other chemokines [1, 6, 18]. Without question, they might induce, generate and release a large number of O_2^-, $\cdot OH$, $NO^\bullet$ and other free radicals, as well as $^1O_2$, H_2O_2, and other ROS [1, 6, 8, 9, 12–15, 17]. Furthermore, in the process of chronic bacterial prostatitis, prostatodynia, perineal and/or suprapubic pain (chronic pelvic pain syndrome), prostatic hyperaemia and/or hemorrhage, and other prostatic disorders might induce, generate and release a large amount of free radicals and ROS [1, 6, 8–10, 19].

Excessive free radicals and ROS, as strong oxidants, might interact directly with DNA in human, therefore damaging DNA, inhibiting and/or depressing DNA replication, and might destroy the active sites and active groups in molecular structures of VC, VE, β-CAR, SOD, CAT, GPX and others, thereby inactivating and deactivating them [1–10]. Excessive free radicals and ROS might also cause oxidative decomposition and peroxidative modification of many organic compounds in human body, further deactivating antioxidants and antioxidative enzymes [1–10]. As a consequence, the levels of VC, VE and β-CAR, as well as the activities of SOD, CAT, and GPX in the CBPP were significantly decreased, and the level of NO was significantly increased. In addition, excessive peroxynitrite anion ($ONOO^-$), a very strong oxidant species generated and released by a combination of $NO^\bullet$ and O_2^-, might damage DNA and its functions, and inactivate and deactivate antioxidants and antioxidative enzymes [1, 6–8], further leading to significantly decreased VC, VE, β-CAR, SOD, CAT and GPX in the CBPP. At the same time, excessive free radicals, $ONOO^-$ and ROS, accelerate and aggravate the lipid peroxidative reaction of polyunsaturated fatty acids, unsaturated phospholipids, glycolipids, cholesterol, other lipids, other organic compounds containing lipids in blood, tissues, and cellular membranes in humans, resulting in signifi-

Table 2. Comparison of the average values (mean ± SD) of the biochemical parameters between the CBPP group and the HAV group and 95% CI. *Levene's test for equality of variances. **Independent-samples *t*-test. Figures in parentheses are 95% CI. CBPP: chronic bacterial prostatitis patients; HAV: healthy adult volunteers; NO: nitric oxide; VC: vitamin C; VE: vitamin E; β-CAR: β-carotene; MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase.

| Group | n | Plasma | | | |
		NO (nmol/L)	VC (μmol/L)	VE (μmol/L)	β-CAR (μmol/L)
CBPPs	70	425.5 ± 31.1	47.36 ± 11.65	18.14 ± 4.75	1.38 ± 0.45
		(418.1 – 432.9)	(44.58 – 50.14)	(17.01 – 19.27)	(1.27 – 1.49)
HAVs	70	378.2 ± 32.7	55.11 ± 13.45	25.74 ± 4.48	1.71 ± 0.53
		(370.4 – 386.0)	(51.90 – 58.31)	(24.67 – 26.80)	(1.58 – 1.84)
F^*		0.115	0.476	0.148	0.034
P		0.735	0.491	0.704	0.854
t^{**}		8.773	3.642	9.737	3.922
P		< 0.001	< 0.001	< 0.001	< 0.001

| Group | Erythrocytes | | | |
	MDA (nmol/g·Hb)	SOD (U/g·Hb)	CAT (K/g·Hb)	GPX (U/mg·Hb)
CBPPs	34.69 ± 4.67	1912 ± 221	253.8 ± 67.1	24.97 ± 4.95
	(33.58 – 35.80)	(1860 – 1965)	(237.8 – 269.8)	(23.79 – 26.15)
HAVs	28.58 ± 4.40	2081 ± 222	293.5 ± 76.9	28.78 ± 5.11
	(27.53 – 29.63)	(2028 – 2134)	(275.1 – 311.8)	(27.56 – 29.99)
F^*	1.327	1.137	1.076	0.153
P	0.251	0.288	0.301	0.696
t^{**}	7.972	4.506	3.251	4.479
P	< 0.001	< 0.001	0.001	< 0.001

Table 3. Partial correlation between the course of disease and the each experimental parameter for 70 CBPP. NO: nitric oxide; VC: vitamin C; VE: vitamin E; β-CAR: β-carotene; MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase.

Variables correlated	n	Variables controlled	r	P
Course and NO	70	Age	0.4801	< 0.001
Course and VC	70	Age	− 0.3437	0.004
Course and VE	70	Age	− 0.3712	0.002
Course and β-CAR	70	Age	− 0.3192	0.008
Course and MDA	70	Age	0.4364	< 0.001
Course and SOD	70	Age	− 0.3490	0.003
Course and CAT	70	Age	− 0.2805	0.020
Course and GPX	70	Age	− 0.2868	0.017

cantly increased lipid peroxides and MDA levels in the CBPP [1, 6–9].

Because of the close correlation between age and the investigated biochemical parameters, and that between age and the alterations of O_2^- and $NO^·$ in inflammatory response in humans [6], in this research, partial correlation analysis was used to compute the correlations between the course of disease and NO, VC, VE, β-CAR, MDA, SOD, CAT and GPX for 70 CBPP to eliminate disturbance from age [6]. The findings from the partial correlation suggested that the values of the above biochemical substances and enzymes were closely related

Table 4. The model of stepwise regression among the course of disease and the experimental parameters for 70 CBPP. Dependent variable: the course of disease. NO: nitric oxide; MDA: malondialdehyde; GPX: glutathione peroxidase.

Model	Coefficients	t	P	Partial correlation		ANOVA		R value	
				r	P	F	P	r	P
Constant	−13.2077	2.8520	0.006	—	—	18.2047	< 0.001	0.6729	< 0.001
MDA	0.1894	2.5952	0.012	0.3043	0.002				
NO	0.0415	4.2745	< 0.001	0.4656	< 0.001				
GPX	−0.1999	2.9629	0.004	−0.3426	< 0.001				

to the course of disease in the CBPP, and that with prolonged course of disease, the values of VC, VE, β-CAR, SOD, CAT and GPX in the CBPP were gradually decreased, and those of NO and MDA were gradually increased. In other words, the longer the course of disease was, the severer might be the oxidative stress and potential oxidative damage in the patients. In addition, the findings suggest that chronic bacterial prostatitis, in all likelihood, affects such patients' physical and mental health.

In this research, the model of stepwise regression among the course of disease and the parameters for the 70 CBPP suggested that the closest correlation existed between the course of disease and MDA, NO and GPX in the CBPP. This suggests that the increased oxidative stress which induced by increased MDA and NO and by decreased GPX, might be risk factors causing the potential oxidative damage in the patients [20].

In conclusion, the findings in the present research suggested that chronic bacterial prostatitis likely induces increased nitric oxide and malondialdehyde, and decreased VC, VE, β-CAR, SOD, CAT and GPX, and that there is increased oxidative stress and oxidative damage induced by chronic bacterial prostatitis in the patients, and such phenomenon is closely related to the course of disease in the patients.

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