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World J Gastroenterol 2005;11(3):368-371
World Journal of Gastroenterology ISSN 1007-9327

• CLINICAL RESEARCH •

Potential oxidative stress in children with chronic constipation

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Received: 2003-12-10 **Accepted:** 2004-01-12

Abstract

AIM: To investigate the potential oxidative stress in children with chronic constipation and to explore its mechanisms.

METHODS: Seventy children with chronic constipation and 70 age- and sex-matched healthy children were enrolled in a randomized controlled study. Plasma levels of vitamins C and E, activities of superoxide dismutase and catalase and lipoperoxide level in erythrocytes were determined by spectrophotometry.

RESULTS: Compared with healthy children whose vitamin C, vitamin E, superoxide dismutase, catalase and lipoperoxide were 58.35±14.42 μmol/L, 27.15±6.55 μmol/L, 2 206±171 U/(g·Hb), 327.3±82.2 K/(g·Hb) and 19.18±4.27 nmol/(g·Hb) respectively, the levels of vitamin C, vitamin E, the activity of superoxide dismutase, and catalase in the children with chronic constipation significantly decreased [46.59±11.51 μmol/L, 20.65±4.80 μmol/L, 1943±147 U/(g·Hb) and 269.3±67.8 K/(g·Hb), respectively $P<0.01$], while the lipoperoxide significantly increased [25.22±5.01 nmol/(g·Hb), $P<0.01$]. With a prolonged course of disease, the levels of vitamin C, vitamin E, the activity of superoxide dismutase and catalase in the children with chronic constipation gradually decreased, while the level of lipoperoxide gradually increased.

CONCLUSION: Chronic constipation can cause potential oxidative stress in children.

Key words: Chronic constipation; Potential oxidative stress; Spectrophotometry

Zhou JF, Lou JG, Zhou SL, Wang JY. Potential oxidative stress in children with chronic constipation. *World J Gastroenterol* 2005; 11(3): 368-371
http://www.wjgnet.com/1007-9327/11/368.asp

INTRODUCTION

Constipation is a symptom rather than a specific disease. It is generally defined by patients as adefecation frequency of twice weekly or less, and the defecation frequency of patients with chronic constipation is very low[1-5]. Chronic childhood constipation is a torturing disorder, and many children with this disorder in China have physical and mental sufferings. Up to now, there have been neither reports on the abnormal oxidative stress in children with chronic constipation, nor reports about the relationship between oxidative stress and chronic childhood constipation. In order to investigate the potential oxidative stress in children with chronic constipation and to explore its mechanisms, 70 children with chronic constipation and 70 healthy child volunteers (HCVs) were enrolled in a randomized controlled study in which the levels of vitamin C (VC) and vitamin E (VE) in plasma as well as the activities of superoxide dismutase (SOD) and catalase (CAT), and the level of lipoperoxide (LPO) in erythrocytes were determined by spectrophotometry. In addition, differences between average values of the above experimental parameters in children with chronic constipation and HCVs were compared, the partial correlation including the zero order partials and the controlling for age between the course of disease and each experimental parameter in the children with chronic constipation were analyzed.

MATERIALS AND METHODS

Study design

A randomized controlled study was done for this purpose. In order to obtain an objective research conclusion, principles of random, control, replication and equilibrium, management factor, experimental effect and subjects, and inclusion and exclusion criteria of subjects were taken into consideration, and strictly executed in the research[6-8].

Subjects

Chronic childhood constipation patients (CCCPs) Seventy children were randomly sampled from 132 children with chronic constipation confirmed by the diagnostic criteria[1-5] with "Select Cases-Random Sample" in "SPSS 11.0 for Windows". Their course of disease ranged from 1 to 5 (2.2±1.1) years, and their ages were 7-14 years, systolic blood pressure and diastolic blood pressure were 60-105 mmHg and 41-66 mmHg, hemoglobin level and serum albumin level were 116-140 g/L and 31.64-42.86 g/L, and body-mass index was 20.81-24.82 respectively. They were all volunteers in this study.

Healthy child volunteers (HCVs) Seventy volunteers were randomly sampled from 137 healthy child volunteers confirmed by the comprehensive physical examination at the Second Affiliated Hospital and the Affiliated Children's Hospital, College of Medicine, Zhejiang University, with "Select Cases-Random Sample" in "SPSS 11.0 for Windows". They had no medical history of acute or chronic constipation, and their ages were 7-14 years, systolic blood pressure and diastolic blood pressure were 60-106 mmHg and 43-66 mmHg, hemoglobin level and serum albumin level were 122-141 g/L and 31.69-42.57 g/L, and body-mass index was 21.14-24.86, respectively.

There was no significant difference between average values of age, systolic blood pressure, diastolic blood pressure, hemoglobin level, albumin level, body-mass index, and sex proportion in children with chronic constipation and HCVs.

In the above subjects, common diseases associated with

constipation, such as colon cancer, colonic neoplasm, benign stricture of colon, colonic ischemia, diverticular disease, inflammatory bowel disease, anorectal disease, inflamed hemorrhoid, anal fissure, rectal inflammation (e.g., proctitis), rectal trauma, diabetes mellitus, and hypothyroidism, were all excluded[1-5].

Demographic data and some other data of the 70 children with chronic constipation and 70 healthy child volunteers are presented in Table 1.

Table 1 Demographic data and other data in children with chronic constipation and HCVs (mean±SD)

Item	CCCPs (n = 70)	HCVs (n = 70)	Statistical analysis
Age (yr)	7-14	7-14	t = 0.473[1]
	(10.60±1.94)	(10.44±1.98)	P = 0.637
Sex	M = 38	M = 35	χ^2 = 0.258[2]
	F = 32	F = 35	P = 0.735
Systolic pressure	60-105	60-106	t = 0.050[1]
(mmHg)	(84.63±10.16)	(84.54±10.24)	P = 0.960
Diastolic pressure	41-66	43-66	t = 0.458[1]
(mmHg)	(55.85±5.95)	(55.39±5.98)	P = 0.648
Hemoglobin	116-140	122-141	t = 0.928[1]
(g/L)	(134.0±5.5)	(134.8±4.4)	P = 0.355
Albumin	31.64-42.86	31.69-42.57	t = 0.694[1]
(g/L)	(38.49±2.25)	(38.23±2.15)	P = 0.489
Body-mass	20.81-24.82	21.14-24.86	t = 0.098[1]
index	(23.27±1.08)	(23.25±1.12)	P = 0.922

[1]Independent-sample t test, [2]Pearson chi-square test (Exact Sig).

In medical histories of the children with chronic constipation and healthy child volunteers, disorders associated with the brain, heart, lungs, liver, kidneys, and other organs as well as the blood system, circulatory system, respiratory system, and other systems were all excluded by routine blood, urine and stool examinations as well as radiographs, cardiogram, and other necessary examinations. Medical histories of inflammation, hypertension, hyperlipidemia, acute or chronic bronchitis, asthma, autoimmune disease, diabetes, atherosclerosis, tumors and other diseases, and subnutrition, malnutrition and other nutritional diseases were also excluded.

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

Methods

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 promptly separated erythrocytes were stored at -50 ℃ immediately. The blood samples did not undergo any hemolysis[6-11].

Biochemical measurements

Plasma VC level Trichloroacetic acid solution was used to sedimentate proteins in plasma and to extract VC from plasma. VC in the extract solution reduced Fe^{3+} in the ferric trichloride solution to Fe^{2+}. Fe^{2+} reacted with ferrozine to form a colored end product, which was detected by the spectrophotometric analytical method at 563 nm and 10.0 mm, and its level was expressed as μmol/L[6-8].

Plasma VE level Absolute ethanol was used to sedimentate proteins in plasma and to extract VE from plasma. VE in the extract solution reduced Fe^{3+} in the ferric trichloride solution to Fe^{2+}. Fe^{2+} reacted with ferrozine to form a colored end product, which was detected by spectrophotometric analytical method at 563 nm and 10.0 mm, and its level was expressed as μmol/L[6-8].

Erythrocyte SOD activity Spectrophotometric analytical method for inhibiting pyrogallol auto-oxidation was used to determine erythrocyte SOD activity at 420 nm and 10.0 mm, and SOD activity was expressed as U/(g·Hb)[6-8].

Erythrocyte CAT activity Spectrophotometric analytical method for coloration of hydrogen peroxide and acetic acid-potassium dichromate was used to determine erythrocyte CAT activity at 570 nm and 10.0 mm, and CAT activity was expressed as K/(g·Hb)[6-8].

Erythrocyte LPO level Spectrophotometric analytical method for thiobarbituric acid reactive substances (TBARS) was used to determine erythrocyte LPO level at 532 nm and 10.0 mm, and erythrocyte LPO level was expressed as nmol/(g·Hb)[6-8].

Analytical reagents in determining the above biochemical substances and enzymes, such as vitamin C, vitamin E, 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazinedisulfonic acid disodium salt (ferrozine), Cu·Zn-superoxide dismutase, 1,2,3-trihydroxybenzene (pyrogallol), catalase, and 1,1,3,3-tetraethoxypropane, 2-thiobarbituric acid (TBA), were purchased from SIGMA® Chemical Company, USA. Other analytical reagents were produced in China. Fresh quadruply distilled water was prepared with a quartz glass distilling apparatus. In determination of the above biochemical substances and enzymes, main analytical instruments included Hewlett Packard 8453-spectrophotometer, USA, and others.

In determination of the above biochemical substances and enzymes, the same batch number of each reagent, same quality control, same laboratory assistant, and same analytical apparatus, were strictly used for each experiment in order to decrease errors and ensure the analytical quality of determinations[6-8].

Statistical analysis

All experimental data were statistically analyzed with SPSS 11.0 for Windows using a Compaq Pentium IV/2.4 GHz computer. Experimental parameters in this study presented normal distributions by Kolmogorov-Smirnov Z test, and were expressed as mean±SD and 95% confidence interval (95% CI). Hypothesis testing methods included independent-sample t test, Pearson chi-square test (χ^2 test), and partial correlation analysis including the zero order partials and the controlling for age. In the statistical analysis, the level of hypothesis testing (α) was $\leqslant$0.05 in order to avoid false positives (type I error), and the power of hypothesis testing (*power*) was $\geqslant$0.85 to avoid false negatives (type II error)[6-8].

RESULTS

Compared with the average values of experimental parameters in HCVs, the average values of VC and VE in plasma as well as those of SOD and CAT in erythrocytes in children with chronic constipation significantly decreased (P<0.01), while the average value of LPO in erythrocytes in children with chronic constipation significantly increased (P<0.01) (Table 2). The upper limits of 95% CI of the average values of VC and VE in plasma as well as those of SOD and CAT in erythrocytes in children with chronic constipation were less than the lower limits of 95% CI of the same values in HCVs, while the lower limit of 95% CI of the average value of LPO in erythrocytes in children with chronic constipation was greater than the upper limit of 95% CI of the same value in HCVs (Table 2).

The findings from both the zero order partials and the controlling for age in partial correlation analysis between the

Table 2 Comparison between average values of biochemical parameters in children with chronic constipation and HCVs (mean±SD)

Group	*n*	Plasma		Erythrocyte		
		VC (μmol/L)	VE (μmol/L)	SOD (U/g·Hb)	CAT (K/g·Hb)	LPO (nmol/g·Hb)
CCCPs	70	46.59±11.51	20.65±4.80	1 943±147	269.3±67.8	25.22±5.01
		(43.84-49.33)	(19.51-21.80)	(1908-1978)	(253.0-285.6)	(24.03-26.42)
HCVs	70	58.35±14.42	27.15±6.55	2 206±171	327.3±82.2	19.18±4.27
		(54.91-61.79)	(25.59-28.71)	(2165-2247)	(307.7-346.9)	(18.17-20.20)
t^1		5.535	6.695	9.796	4.543	7.677
P		<0.01	<0.01	<0.01	<0.01	<0.01

[1]Independent-samples *t* test. The figures in parentheses are 95% confidence interval (95% CI).

course of disease and each experimental parameter for the 70 children with chronic constipation showed that with the prolonged course of the disease, the levels of VC and VE in plasma as well as the activities of SOD and CAT in erythrocytes gradually decreased ($P<0.01$), while the level of LPO in erythrocytes gradually increased ($P<0.01$) (Table 3).

Table 3 Partial correlation analysis between the course of disease and each parameter for children with chronic constipation

Correlative item	*n*	Partial correlation coefficient			
		Zero order partials		Controlling for age	
		r	*P*	*r*	*P*
Course and VC	70	–0.4999	<0.01	–0.4823	<0.01
Course and VE	70	–0.4868	<0.01	–0.4658	<0.01
Course and SOD	70	–0.5856	<0.01	–0.5129	<0.01
Course and CAT	70	–0.5848	<0.01	–0.4933	<0.01
Course and LPO	70	0.7491	<0.01	0.5027	<0.01

DISCUSSION

It is well known that VC and VE are important antioxidants, SOD and CAT are important antioxidases in human bodies. They play important roles in scavenging superoxide anion radical (O_2^-), hydroxyl radical (·OH), 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 excessively generated in human bodies. They also play important roles in preventing physiological and pathological aggravation of a series of FRs chain reactions induced by excessive O_2^-, thereby protecting biological membranes of the cells against oxidative stress and oxidative damage[26-28]. LPO is a product of peroxidation (auto-oxidation) of lipids exposed to oxygen, and lipoperoxidation *per se* is a source of FRs and, in all likelihood, a potential cause of cancer, inflammatory disease, atherosclerosis, aging, *etc*[6-11,20]. LPO and its metabolites, such as malondialdehyde, conjugated diene and others, are poisonous residual products, and may strongly attack DNA, proteins, enzymes, cellular membranes, polyunsaturated fatty acids (PUFAs), other lipids, and lipid-contained organic compounds, leading to lipoperoxidative damages to cellular membranes and cells, and cytoclasis[12-19].

These findings suggest that chronic constipation can induce potential oxidative stress in children. There might be several interpretations.

The pathophysiologic mechanisms of constipation often involve a poor colonic propulsive activity, colonic dysfunction or colonic motor disorders[1-5]. Therefore, besides psychological and physiologic factors, disordered colonic transit and anorectal function may play important roles in these disorders[1-5]. These abnormalities would gradually lead to increased water absorption and solid consistency of stools. At the same time, toxicants in stools, such as ammonia, hydrogen sulfide, and indole, are absorbed largely by intestinal tract in children with chronic constipation, and enter into their blood circulation[1-5]. In addition, these abnormalities would cause intestinal flora imbalance in children with chronic constipation, thereby drying up their feces and aggravating their constipation[29,30].

A lot of FRs and ROS can be generated both by excessive ammonia in intestinal tract and blood, and by intestinal flora imbalance in children with chronic constipation[29-33], which can directly interact with DNA, thus causing DNA damage, inhibiting or depressing DNA replication, producing oxidative decomposition and peroxidative modification of many organic compounds, and can also strongly attack active sites and groups in the molecular structures of VC, VE, SOD, and CAT, thereby deactivating them[26-28]. As a result, the levels of VC and VE as well as the activities of SOD and CAT in chronic childhood constipation patients significantly decreased[26-28]. Additionally, superfluous FRs, ROS and decreased plasma VE level could accelerate lipoperoxidative reactions, leading to a markedly increased LPO level in children with chronic constipation[14-17].

According to the data from this study, most CCCPs (51/70, 72.86%) like fried foods, such as fried chicken, meat, pie, pork chop, roll, and French potato, and do not like foods rich in vitamins and celluloses. Such phenomena not only increase the solid and hard consistency of stools and disorder the colonic transit and anorectal function and thus easily causing constipation[34,35], but also decrease the intake of VC, VE, and other antioxidant-vitamins, thereby decreasing the levels of VC and VE in chronic childhood constipation patients[20-25].

Bivariate correlation analysis, *viz* a simple linear correlation analysis, can not really reflect the relationship between age and above experimental parameters because of a close correlation between age and each parameter in human bodies[20-22,24,25]. In this study, therefore, partial correlation analysis was used to analyze the correlation between the course of disease and each parameter for the 70 children with chronic constipation in order to eliminate the effect of age on the parameters[36]. The findings from both the zero order partials and the controlling for age in the partial correlation analysis showed that the above parameters were closely related to the course of disease, and that when the course of disease was prolonged, the values of VC, VE, SOD, and CAT in children with chronic constipation gradually decreased, while the value of LPO gradually increased. In other words, the longer the course of disease, the severer the potential oxidative stress in the body. In addition, the findings of partial correlation analysis suggest that chronic constipation might be a risk factor doing harm to children's

physical and mental health.

In conclusion, chronic childhood constipation can cause potential oxidative stress in children.

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Edited by Wang XL and Chen ZR **Proofread by** Chen WW