Efficacy of folic acid supplementation on DNA oxidative damage repair: a Randomized, Placebo Controlled Clinical Trial in Inner Mongolia

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Abstract

A randomized, double-blind, placebo controlled clinical trial was conducted in arsenic exposed population in Inner Mongolia to know the efficacy of folic acid supplementation on arsenic-inducted DNA d oxidative damage.

Methods: In this randomized clinical trial (RCT), a total of 450 participants were enrolled and randomly assigned to 3 groups to receive folic acid (FA) 0.4mg/day (Low FA), 0.8 mg/day (High-FA) or placebo (Control) for 8 weeks. The urine 8-hydroxy-2'-deoxyguanosine (8-OHdG) and creatinine (Cr) concentration at pre- and post-FAS were measured with enzyme-linked immunosorbent assay (ELISA) kit and high-performance liquid chromatography (HPLC), respectively. A multivariate general linear model (GLM) was applied to assess the individual effects of FA and the joint effects between FA and hypercholesterolemia on oxidative damage improvements. This clinical trial was registered with ClinicalTrials.gov, number NCT02235948.

Results: Compared with placebo, after adjusted for some potential confounding factors, the urinary 8-OHdG/Cr concentration significantly decreased after 56 days FAS in a dose-response fashion($P<0.001$). A joint effect between hypercholesterolemia and FAS on urinary 8-OHdG/Cr reduction was also observed ($P=0.001$).

Conclusions: The present study demonstrates that FAS is beneficial for DNA oxidative damage repair. People with hypercholesterolemia might have better effects on the improvement of DNA lesion.

Keywords: Arsenic, 8-OHdG/Cr, folic acid, Randomized clinical trial

1. INTRODUCTION

Oxidative damage is a major contributor to many health problems such as diabetes, atherosclerosis and cancers (Valavanidis et al, 2009). If left unrepaired, this damage can result in severely adverse cellular outcomes, including increasingly risk of mutagenesis and cell apoptosis (Sampath et al, 2014). Compelling evidences support that oxidative damage is mainly induced by reactive oxygen species (ROS), such as hydrogen peroxide, superoxide anion, singlet oxygen and hydroxyl radical, which can be produced through normal cellular metabolism or induced by a wide range of environmental factors (Wu et al, 2004; Cooke et al, 2003, Madamanchi et al, 2005; Cheng et al 2012).

Over the past several decades, it has been recognized that ROS can directly or indirectly damage cellular DNA and protein (Flora et al, 2007). The hydroxyl radical, a major

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component of ROS, is generally assumed to be the critical reactive species that directly attacks DNA. The interaction of the hydroxyl radical with the nucleases of the DNA strand, such as guanine, leads to the formation of 8-hydroxyguanine or 8-hydroxy-2'-deoxyguanosine (8-OHdG) (Kasai et al, 1997). Because 8-OHdG is the predominant form of ROS induced DNA oxidative lesions, mainly released in urine and very stable, the determination and quantitative analysis of urinary 8-OHdG has become a pivotal marker for measuring the effect of oxidative damage to DNA and widely been considered as an independent risk factor for many diseases including cancers (Qin et al, 2008; Ock et al, 2012; Fu et al, 2010).

Oxidative DNA damage, if not been reversed in time, may result in mutations generated during replication, cell death or senescence, or altered transcription of genes important to cellular function (Sampath et al, 2014). Thus, it is important to seek effective interventions to unregulate the repair mechanisms that reverse ROS mediated damages. Although specific improvement for DNA oxidative damage is limited, experimental studies suggested that some vitamins, cysteine and methionine may have ameliorative efficacy on the reversal of oxidative stress (Nandi et al, 2005). Among them, folic acid (FA) is an essential water-soluble vitamin and a key cofactor in one-carbon metabolism that can regulate many different pathways such as cell growth, differentiation, DNA-repair, apoptosis and carcinogenesis prevention (Trujillo et al, 2006). Although oxidative stress and the role of FA in preventing oxidative stress-mediated damage have been extensively studied in animals (Lee et al, 2011), limited studies are available for the influence of folic acid fortification on the oxidative stress-induced damage in human beings. In the present study, we use the data from a randomized clinical trial "Efficacy and Safety of Folic Acid Supplementation Lowering Arsenic in a Chronic, Low-level Exposed Arsenic Population: a Randomized, Double-blind, Placebo Controlled Clinical Trial (NCT02235948)" to assess the efficacy of orally administered folic acid for 56 days on DNA oxidative damage repair.

2. MATERIALS AND METHODS

2.1 Study Subjects

The present study was carried out between September 2010 and December 2011 in a population of 3 arsenic exposed villages stratified and randomly selected, based on the results of average arsenic concentration tests in the past two decades in Wuyuan county of Hetao Plain, Inner Mongolia, China. Of 653 total residents in the above three villages, 450 (male 169; female 281) residents, at age of 18-79 years, were recruited for this folic acid supplementation study, 203 were excluded because of not meeting the inclusion criteria (n=196) or refusing to participate (n=7). In addition, subjects who were planning to become pregnant during the study or planned to move out of the area within the study period were also excluded. The protocol was approved by the ethics committee of Wenzhou Medical University, Wenzhou, China. The purpose and procedure of the study were carefully explained to all participants. We got written informed consent from all of the participants before we began study-related procedures.

2.2 Study design

The study is designed a randomized, double-blind, placebo-controlled, parallel-group, clinical trial.

Randomization, Masking and intervention

After informed consent, we randomly assigned the eligible subjects in a 1:1:1 ratio according to the randomization schedule generated by an unmasked statistician to one of three groups: 0.4 mg (Low-FA), 0.8 mg (High-FA) folic acid per day or placebo. To achieve a good balance among the three groups, permuted block randomization with a block size randomly selected as six was performed using R version 3.1.0 (Copyright 2011 the R Foundation for Statistical Computing). This statistician came from Wenzhou Medical University and was independent of study conduct and data analysis, and supplied to an independent GMP-certified Chang-Zhou Pharmaceutical Company. Supplements were given as 2 pills to be orally taken once daily for 8 weeks and were provided free of charge. All pills including different doses of folic acid or placebo were made indistinguishable by external color and appearance, labelled the identification number of each subject and distributed to the field study site by the pharmacy. The allocation of participants was encrypted, sent to the field study site and accessible only to the staff in charge of the drug distribution. Participants and the study staffs were masked to the assignment all through the study duration. On the 1st day of the FAS period, trained stuff allocated the pills to the subjects to eat according to the identification number. No request was ever made to break the blind during the study.

Data collection

All of the eligible subjects were followed for a total of 8 weeks. Face-to-face interviews were conducted at baseline, and in the 4th and 8th week of the study. The subjects were asked not to change their daily dietary habits and not to take any B vitamins or any other medications which might affect the serum folate level during the following 8 weeks. Physical examination for all subjects at baseline and again at the end of the study (the 56th day of folic acid supplementation) was conducted by physicians from Wenzhou Medical University and Wuyuan CDC. All

subjects were asked to fast overnight for 10-12 hours before the examination. A well-organized epidemiological survey was conducted by trained research staff according to the standard operating procedure (SOP) on the first morning of the study. Each subject was interviewed with a standardized questionnaire designed specifically for the study. Chronic arsenic exposure information was obtained from the subjects' self-reporting including history and type of drinking water. Body mass index (BMI) was calculated as weight (kilograms)/height (meters) squared. Seated blood pressure (BP) in all subjects was measured with the same type of mercury column sphygmomanometer between 8:00-10:00 a.m. All of the examinations such as weight, height, seated BP were measured three times by the same research staff at each time point and the mean values were used in the data analysis. Measures were taken to perform quality assurance (QA) and quality control (QC) during the study. All investigators and physicians had been trained for two weeks before the study commenced. All data collected from field studies, physical examinations and laboratory assessments had to strictly follow the project SOP.

Treatment compliance evaluation (the percentage of participants following the study procedure) was performed in week 4 and 8. "Lost to follow-up" was defined as subjects who had not completed the entire study procedure. Finally, we excluded 12 participants with missing values on any of the covariates and, thus, obtained a final study sample of 438 subjects (96.9%), and we analyzed all available data on baseline and 56 days later.

Covariates

After 10–12 hours of fasting, 8 ml venous blood sample was obtained from each subject with tubes containing ethylene-diaminetetra-acetic acid (EDTA) for plasma, or tubes without EDTA for serum at baseline and on the 56th day of the study. They were separated within 30 minutes and analyzed or stored at -86°C in a freezer. Blood samples were collected for the assessment of serum lipids including total cholesterol (TC), high-density lipoprotein (HDL) and triacylglycerols (TG), creatinine (Cr), serum urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), plasma total homocysteine (t-Hcy) and serum folic acid (FA) concentration. Fasting plasma glucose (FPG) was measured within 30 minutes. All of these tests were performed in the laboratory of Wuyuan CDC, using standard reagents and an automatic biochemistry analyzer. Low-density lipoprotein (LDL) was calculated by Friedewald's equation (LDL=TC-HDL-TG/2.2).

2.3 Urine 8-OHdG/Cr analyses

A total of 20 ml urine samples from each subject were also collected twice, at baseline (pre-supplementation) and on the morning of the 56th day (post-supplementation) at the same time-point as blood sample collection. These were put in a 0℃ ice box promptly, separated within 30 minutes and stored at -86℃ in a freezer until they were sent to the central laboratory of Wenzhou Medical University packed in dry ice. An enzyme-linked immunosorbent assay (ELISA) kit was used (Immuno-Biological Laboratories Co., Ltd., Germanny) for the determination of 8-OHdG in urine by well-trained technicians in the central laboratory. Urine creatinine was determined with high-performance liquid chromatography (HPLC) by the same technician in the same lab. Each sample was parallel measured three times at the same time point. Urine creatinine was applied to adjust for the bias induced by other factors in the urine. The level of 8-OHdG in urine was expressed as nanograms per milligram creatinine and as the final outcome. The intra- and inter-assay coefficients of variation were 3.4% and 4.1%, respectively.

2.4 Statistical analysis

Epidata (version 3.1, Denmark) was used to set up a dataset and conduct the double data entry. A Kolmogorov-Smirnov test was applied to assess the normality of data distribution before statistical analysis. Because urine 8-OHdG/Cr level at baseline and the end of the study were positively skewed, natural log-transformation was selected to increase the normalization of the distribution. One-way analysis of variance for continuous variables and a chi-square test for categorical variables were applied to compare the subjects characteristics in these three groups. Generalized linear mixed models were applied to compare changes in 8-OHdG/Cr in response to folic acid supplementation, with the adjustment of corresponding baseline levels, age, gender, BMI, duration of arsenic exposure, and dietary habits. All tests were two-sided and $P \leq 0.05$ was set as the significant level. Data management and all statistical analyses were performed using R version 3.10 (Copyright 2014 the R Foundation for Statistical Computing) and figures was drawn by Sigmaplot 12.5 for Windows (SYSTAT Software Inc., Richmond, CA, USA).

We calculated the sample size of 132 participants per group with the assumption of a mean change of Urinary 8-OHdG/Cr among the three groups for 0.00, 1.00 and 2.50 ng/mg after 8 weeks intervention with placebo or placebo, Low-FA or High-FA and standard deviation (SD) 3.00 ng/mg, which provided 90% power to detect the differences with one-way analysis of variance with a two-sided type I error of 0.05. We added additional 10% more participants for each group in view of the possible lost during the follow-up. Finally, we recruited 450 subjects (150 per group) to conduct this study. This trial was registered with ClinicalTrials.gov, number NCT02235948.

3. RESULTS

Characteristics of the study subjects

A total of 438 of 450 participants completed the follow-up (Table 1), 12 people lost because of not strictly following the protocol (n=4) or lost to follow up (n=8). The mean (±SD) age was 49.71±12.36 years, and men constituted 37.56% (169/450) of the sample. More than half of them had no history of smoking or alcohol consumption and 80% of the subjects normally cooked using animal oil which is a quite common practice in farming families in Inner Mongolia, and people with high blood cholesterol levels were also identified.

Table 1. Characteristics of study subjects

Characteristics	Placebo N=150	Low-FA N=145	High-FA N=143	P
Age in years	51.58±11.53	48.90±12.48	48.66±12.90	0.075
Gender				0.576
Male	58(38.7)	58(40.0)	49(34.3)	
Female	92(61.3)	87(60.0)	94(65.7)	
Body mass index, kg/m^2	23.80(22.00, 26.20)	24.50(22.30,26.40)	24.60(22.30, 26.90)	0.207
Smoking				0.142
Never	85(56.7)	95(65.5)	95(66.4)	
Yes	62(41.3)	49(33.8)	43(30.1)	
Missing	3(2.0)	1(0.7)	5(3.5)	
Alcohol consumption				0.169
Never	93(62.0)	101(69.7)	101(70.6)	
Yes	54(36.0)	42(29.0)	36(25.2)	
Missing	3(2.0)	2(1.4)	6(4.2)	
Occupation				0.559
Farmers	140(93.3)	130(89.7)	125(87.4)	
Others	7(4.7)	11(7.6)	13(9.1)	
Missing	3(2.0)	4(2.8)	5(3.5)	
Education level				0.368
Illiteracy	30(20.0)	31(21.4)	35(24.5)	
Primary school	53(35.3)	52(35.9)	37(25.9)	
Middle school or above	63(42.0)	61(42.1)	66(46.2)	
Missing	4(2.7)	1(0.7)	5(3.5)	
Race/ethnicity				0.153
Han	147(98.0)	139(95.9)	133(93.0)	
Others	1(0.7)	5(3.4)	5(3.5)	
Missing	2(1.3)	1(0.7)	5(3.5)	
Marriage				0.596
Others [a]	8(5.3)	7(4.8)	7(4.9)	
Married	139(92.7)	135(93.1)	129(90.2)	
Missing	3(2.0)	3(2.1)	7(4.9)	
Arsenic exposure, years	46.63±12.48	44.39±12.95	44.37±12.80	0.218
full animal oil intake [b]				0.392
No	25(16.7)	21(14.5)	22(15.4)	
Yes	119(79.3)	122(84.1)	113(79.0)	
Missing	6(4.0)	2(1.4)	8(5.6)	
Vegetable consumption, g/week				0.635
<500	33(22.0)	27(18.6)	29(20.3)	
500~	76(50.7)	78(53.8)	64(44.8)	
1500+	36(24.0)	35(24.1)	41(28.7)	
Missing	5(3.3)	5(3.4)	9(6.3)	
Hypertension				0.353
No	77(51.3)	71(49.0)	76(53.1)	
Yes	71(47.3)	73(50.3)	62(43.4)	
Missing	2(1.3)	1(0.7)	5(3.5)	
Fasting plasma glucose, μmol/L	5.01(4.73,5.49)	5.07(4.55,5.39)	5.09(4.67,5.50)	0.863
Total Cholesterol, μmol/L	4.62(4.09,5.44)	4.52(3.76,5.46)	4.51(3.82,5.37)	0.514
Triglycerides, μmol/L	1.44(1.04,2.22)	1.48(1.05,2.13)	1.45(1.09,2.12)	0.765
High-density lipoprotein, μmol/L	1.17(0.97,1.38)	1.08(0.90,1.25)	1.14(0.95,1.31)	0.055
Low-density lipoprotein, μmol/L	3.08(2.64,3.54)	3.02(2.60,3.46)	3.02(2.49,3.59)	0.772
Blood Urea nitrogen, μmol/L	6.47(5.02,7.91)	5.98(4.90,7.50)	6.42(4.96,8.10)	0.321
Creatinine, μmol/L	90.14(77.00, 107.48)	90.76(79.77,104.82)	89.38(79.40, 102.20)	0.970
Hypercholesterolemia				0.135
No	121(80.7)	129(89.0)	118(82.5)	
Yes	19(12.7)	9(6.2)	11(7.7)	
Missing	10(6.7)	7(4.8)	14(9.8)	
Skin damage				0.252
No	62(41.3)	63(43.4)	55(38.5)	
Yes	86(57.3)	81(55.9)	82(57.3)	
Missing	2(1.3)	1(0.7)	6(4.2)	

Urinary 8-OHdG/Cr level in three groups

Table 2 show the median and geometric mean value of urinary 8-OHdG levels of the study population before and after the folic acid supplement. The concentration of urinary 8-OHdG/Cr at baseline were not significant different among 3 groups (p-value = 0.190), but after 56 days the urinary 8-OHdG/Cr was decreased in the three groups. The geometric mean (±anti-log SD) at baseline for the above three groups were 60.89±1.62 ng/mg, 55.48±1.74 ng/mg and 55.81±1.72 ng/mg, respectively. While at the 56th day, The geometric mean (±anti-log SD) were 60.82±1.63 ng/mg for the placebo group, 54.27±1.77 ng/mg for the Low-FA group and 52.40±1.78 ng/mg for the High-FA group, respectively. There is a significant difference of the change of 8-OHdG/Cr among the placebo, low-FA and high-FA groups, which were 0.00±3.28 ng/mg, -1.01±2.96 ng/mg and -2.80±3.10 ng/mg, respectively (p-value <0.001).

The change in urinary 8-OHdG levels with folic acid supplementation

A multivariate generalized linear regression model is undergone to assess the relationship between the change of urinary 8-OHdG/Cr and folic acid. After adjusting the confounding factors including the baseline urinary 8-OHdG/Cr, either supplementation with 0.4 or 0.8 mg/day folic acid produced a significant decrease in 8-OHdG levels (p-value = 0.020 for 0.4mg and p-value < 0.001 for 0.8mg group), compared with placebo group (Table 3). Moreover, a dose-response relationship between the dose of folic acid and the decrease of urinary 8-OHdG/Cr was shown by multivariate generalized linear regression model (trend test p-value < 0.001). In addition, the average reduction of urinary 8-OHdG/Cr in subjects with hypercholesterolemia fortified with folic acid indicate 0.96ng/mg that is more than those with normal blood cholesterol, but small sample size induced a low power to identify the difference (p-value =

0.175).

Table 2. Comparison of 8-OHdG/Cr and the change during folic acid supplementation among the three treatment groups

8-OHdG/Cr (ng/mg)	Placebo N=150	Low-FA N=145	High-FA N=143	*P*
Baseline				
Median(Q1-Q3)	58.19(43.90,82.26)	53.51(38.97,72.74)	54.73(39.58,76.63)	0.190
Geometric mean ξ 56th day	60.89±1.62	55.48±1.74	55.81±1.72	0.237
Median (Q1-Q3)	57.77(44.35,81.33)	51.73(38.20,71.30) *	50.65(37.64,76.17) *	0.041
Geometric mean ξ	60.82±1.63	54.27±1.77*	52.40±1.78*	0.049
8-OHdG/Cr change	-0.00±3.28	-1.01±2.96**	-2.80±3.10**▲▲	<0.001

Table 3. The change of urinary 8-OHdG/Cr with folic acid supplementation and blood cholesterol levels

Variable	N	8-OHdG/Cr Change ξ	*Crude*			*Adjusted*		
			β(95% CI)	*SE*	*P*	*β(95% CI)*	*SE*	*P*
Group								
Placebo	150	0.00±3.28	Ref	Ref	----	Ref	Ref	----
Low_FA	145	-1.01±2.96	-1.00(-1.71,-0.29)	0.36	0.006	-0.88(-1.62,-0.14)	0.38	0.020
High_FA	143	-2.80±3.10	-2.80(-3.51,-2.09)	0.36	<0.001	-2.68(-3.42,-1.94)	0.38	<0.001
Trend test					<0.001			<0.001
Group								
Placebo	150	0.00±3.28	Ref	Ref	----	Ref	Ref	----
Folic acid supplementation	288	-1.90±3.16	-1.90(-2.53,-1.27)	0.32	<0.001	-1.76(-2.43,-1.09)	0.34	<0.001
Hypercholesterolemia ζ								
No	247	-1.71±3.14	Ref	Ref	----	Ref	Ref	----
Yes	20	-2.54±3.36	-0.83(-2.26,0.60)	0.73	0.258	-0.96(-2.35,0.43)	0.71	0.175

Joint effect between folic acid and blood cholesterol level on the reduction of urinary 8-OHdG/Cr

To clear the impact of blood cholesterol level on the efficacy of FAS in urinary 8-OHdG/Cr reduction, sub-analysis was conducted stratified by subjects' blood cholesterol level. Subjects with either normal blood cholesterol level or hyper-cholesterol both showed significant reduction of 8-OHdG/Cr after folic acid supplementation and a dose-response relationship between intake folic acid and urinary 8-OHdG/Cr has been shown (Figure 1).

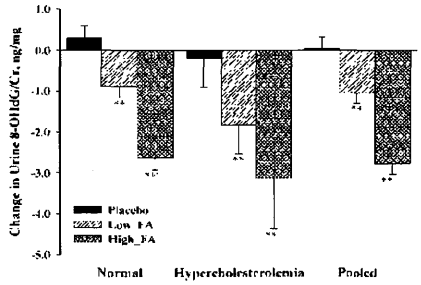

Figure 1. Comparison of 8-OHdG/Cr reduction among the three groups stratified by blood cholesterol level.

Furthermore, compared to the subjects with no folic acid supplementation and with normal blood cholesterol levels, the reduction in urinary 8-OHdG/Cr levels was 0.14±0.79, 1.67±0.36 and 2.66±0.77 ng/mg in subjects who did not take folic acid but had Hypercholesterolemia, and subjects who took folic acid but had normal cholesterol levels, as well as subjects with hypercholesterolemia and folic acid supplementation, respectively (Table 4). The interest finding is that there is jointly effect to reduction of urinary 8-OHdG/Cr between folic acid supplementation and blood cholesterol in the present study (Figure 4).

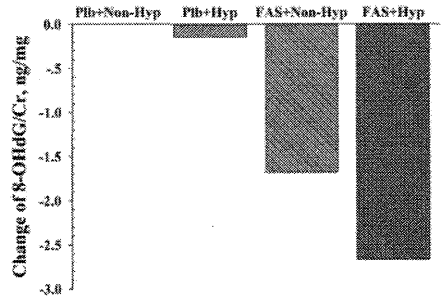

Figure 4. Joint effect of folic acid supplementation and Hypercholesterolemia on reduction of urinary 8-OHdG/Cr.

Table 4. The effect of folic acid and blood cholesterol levels on the reduction of urinary 8-OHdG/Cr.

Intake Folic acid	Hyper-cholesterol	n	8-OHdG/Cr Change$^{\xi}$	*Crude*			*Adjusted*		
				β	*SE*	*P*	*β*	*SE*	*P*
No	No	121	0.03±3.30	Ref	Ref	----	Ref	Ref	----
No	Yes	19	-0.02±3.03	-0.06(-1.59,1.47)	0.78	0.943	-0.14(-1.69,1.41)	0.79	0.863
Yes	No	247	-1.71±3.14	-1.75(-2.44,-1.06)	0.35	<0.001	-1.67(-2.38,-0.96)	0.36	<0.001
Yes	Yes	20	-2.54±3.36	-2.57(-4.08,-1.06)	0.77	0.001	-2.66(-4.17,-1.15)	0.77	0.001

4. DISCUSSION

The present study demonstrates that folic acid is efficacious for decreased urinary 8-OHdG/Cr in population with long-term exposure to low levels of arsenic, and high cholesterol might also influence the efficacy of FAS.

8-OHdG is excreted through urine without metabolizing any further and its urinary appearance reflects oxidative DNA lesion and its repair (Wu et al, 2004; Loft et al, 1998). Due to easy to be detected, 8-OHdG has been widely accepted as the specific biomarker of the "whole body" oxidative DNA damage and the cellular oxidative stress (Rebelo et al, 2004).

As an antioxidant and a key cofactor in one-carbon metabolism, folate is the major contributor of S-adenosylmethionine (SAM), which is the primary intracellular methyl donor (Hall et al, 2009). Folate binding protein mediated intracellular folate uptake is one possible route by which cells harvest folate cofactors and supplementation with folic acid, the synthetic form of folate, as a major source of methyl groups for SAM synthesis (Rogers et al, 2007). Meanwhile, according to extensive studies in animal models, DNA methylation also plays a key role in repairing oxidative stress mediated DNA lesions. As the major methyl donor, SAM is critical in DNA methylation. It is well known that homocysteine (Hcy) and 5-Methyltetrahydrofolate are two major substrates to produce SAM, and folate can maintain its normal concentration because of its role in methionine metabolism (Kolling et al, 2011). Increasing evidences suggests that folate deficiency will lead to hyper-homocysteinemia (HHcy) and largely influence DNA synthesis and repair (Rogers et al, 2007; Devi et al, 2012; Kolling et al, 2011). It may decrease DNA stability and increase the risk of malignant transformation, either by disrupting DNA methylation or perturbing the nucleotide pool, negatively altering DNA synthesis and repair, leading to altered gene transcription and proto-oncogene expression (Bae et al, 2014). Furthermore, independent of Hcy lowering effects, other studies find that folate may have protective effects through free-radical scavenging activity (Nakano et al, 2001) and endothelial dysfunction improving (Verhaar et al, 1998). Lee (Lee et al, 2011) firstly reports that exogenous administration of folic acid is beneficial to reduce oxidative stress in ethanol-fed rats, an animal model of alcoholic liver disease.

Arsenic is a well-known potent environmental oxidative stressor in a number of countries (Fu et al, 2010; Rodriguez et al, 2013). Inner Mongolia is one of the most serious water-caused arsenic exposure regions in China, with approximately 400,000 people obtaining their drinking water from private wells exposed to arsenic levels above 50μg/L, and suffering various adverse health effects (Guo et al, 2007). Arsenic is metabolized in human beings mainly via methylation reactions, associated with one-carbon metabolism and SAM as the methyl donor (Brauner et al, 2014). Methylation of inorganic arsenic consumes a large amount of SAM, leads to HHcy, folate deficiency and oxidative stress induced DNA lesion. Long-term exposure to arsenic associated with a lot of major chronic diseases such as diabetes and cancers. Our previous studies have also demonstrated that urinary 8-OHdG concentration significantly positively correlates with chronic arsenic exposure in Inner Mongolia (Fujino et al, 2005; Mao et al, 2010). DNA oxidative damage may be one of the most important mechanisms in the pathogenesis of arsenic induced diseases.

High cholesterol levels and oxidative stress play an important role in the pathologies of atherosclerosis. Dimitrova reports that high fat high cholesterol atherogenic (HFHC) diet leads to increased oxidative stress in the liver and aorta (Dimitrova et al, 2010). Another study finds that high intake of saturated fatty acids (SFAs) is associated with high plasma concentrations of total Hcy (Berstad et al, 2007). In our study, there is high fat diet habit in subjects in Inner Mongolia, and 9.51% of them have been found with high blood cholesterol by medical check. Base on the cholesterol levels on homeostatic responses to folate perturbation (Kitami et al, 2008) we consider that cholesterol metabolism might influence urinary 8-OHdG/Cr levels by the effects of folate on cholesterol metabolism, and find the effect between folic acid and higher blood cholesterol for reduced urinary 8-OHdG/Cr levels in the study. The findings imply that cholesterol metabolism might join an impact of folic acid on DNA oxidative damage repair.