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Original Research Paper

The Effect of Selenium on the Blood Radioimmunological Indexes Induced by High Dose of Fluorine

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ABSTRACT

Endemic fluorosis is prevalent in China, which can seriously impair the bones, teeth and cardiovascular system. The study was intended to explore antagonism of selenium (Se) on high dose fluorine (F) inducing plasma 6-Keto-prostaglandin $F_{1\alpha}$,Thromboxane B_2 and Endothelin-1. Twenty male rabbits were randomly divided into 4 groups: High F group (NaF,100mg/L), Se group (Na_2SeO_3,1mg/L), High F + Se group (NaF,100mg/L; Na_2SeO_3,1mg/L) and Control group (without fluorine and selenium). The ear vein blood was collected for measurement of serum selenium and fluorine at the end of 0, 3rd and 6th months. At the end of 6th months, plasma 6-Keto-prostaglandin$F_{1\alpha}$ (6-K-P), Thromboxane B_2 (TXB_2) and Endothelin-1 (ET-1) were detected. Serum fluorine was increased in high F group and serum Se was increased in Se group. Plasma 6-K-P was decreased, while the plasma TXB_2 and ET-1 increased with the development of fluorosis. Compared with the high F group, the plasma 6-K-P was higher ($p<0.01$), whereas plasma TXB_2 and ET-1 were lower ($p<0.01$) in high F + Se group. The interaction showed that there was a significant antagonistic action of Se on decreased 6-K-P and increased TXB_2 induced by high dose fluorine. Our study showed that the Se may antagonize the adverse effects of high F.

INTRODUCTION

Endemic fluorosis is serious in China. Some epidemiological studies indicated that the morbidity of atherosclerosis (AS) was related to the concentration of fluorine in water, and it was higher among fluorosis patients than others, in addition the morbidity increases with increasing of fluorosis degree (Wang 1991, Sun 2010). There were many factors, including fluorine, that could cause dysfunction and injury of vascular endothelial cells which correlated with AS (Zhao & Yang 2006). Selenium, on the other hand, could prevent AS due to its antioxygenation (Guo & Lin 2005). In this study, we observed the changes of 6-K-P, TXB_2, ET-1 induced by high dose fluorine and suitable amount of Se, which may help AS prevention in fluorosis areas.

MATERIALS AND METHODS

Animal treatment: Twenty healthy pure lines New Zealand white rabbits weighing 2.0±0.5kg were procured from Academy of Agricultural Sciences of Shandong Province. After 7 days of acclimatization, these rabbits were randomly divided into 4 groups (n=5 per group). Control group was given distilled-deionized drinking water. High F group was administered aqueous sodium fluoride (NaF100mg/L). Se group was administered sodium selenium ($Na_2SeO_3$1mg/L). High F + Se group received NaF (100mg/L) + Na_2SeO_3 (1mg/L). All rabbits were fed with normal diet (50g per day) and given water *ad libitum* for 6 months, each rabbit was breed in a single cage. The temperature and humidity are appropriate. Ear vein blood was collected for measurement of serum selenium and fluorine at the end of 0, 3rd and 6th months during the experiment. And plasma 6-K-P, TXB_2, ET-1 were measured at the end of 6th month.

Chemicals and reagents: NaF solid was obtained from Tianjin Kemiou Chemical Reagent Development Center, sodium selenite solid was produced from Tianjin Damao Chemical Reagent Factory. ^{125}I-TXB_2 radioimmuno assay kit, ^{125}I-6-K-P and ^{125}I-ET radioimmuno assay were purchased from Beijing Puerweiye Biological Technology Co. Ltd.

Measurements of fluoride, selenium, 6-K-P, TXB_2, ET-1: The serum fluoride was detected by using fluoride ion-selective electrode method. The serum Se level was measured by 2,3-diaminonaphthalene fluorescence spectrometry method. Plasma 6-K-P, TXB_2 and ET-1 were measured by radioimmunology.

Statistical analysis: The values were expressed as means ± standard deviation (SD) ($\bar{x} \pm s$) and analysed using SPSS17.0 software. Comparison of means were conducted using one-way analysis of variance with subsequent Dunnett2 t-test between groups. $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

General condition: During the experiment, all animals were in good condition. Their body weights remained stable during the experiment.

Serum fluoride: As seen in Table 1, there was no statistical difference in serum fluoride among groups at 0 month. At the end of 3rd and 6th months, the serum fluoride was significantly higher ($P<0.01$) in High F group and High F + Se group compared to it was in Control group, and there was no statistical difference in serum fluorine between Se group and Control group. The serum fluoride in High F group was significantly increased ($P<0.01$) at the end of 3rd and 6th months compared with it was in 0 month, and it was higher ($P<0.05$) at the end of 6th month than it was at the end of 3rd month.

Serum selenium: As seen in Table 2, there was no difference in serum selenium among groups at 0 month. At the end of 3rd and 6th months, the serum Se was significantly higher ($P<0.01$) in Se group and High F+Se group compared with the Control group, and serum Se did not differ between High F ($P<0.01$) at the end of 3rd and 6th months than 0 month. It was higher ($P<0.05$) at the end of 6th month than it was at the end of 3rd month group and Control group. The serum Se in Se group was significantly increased.

The effect of high dose fluorine and selenium on plasma 6-K-P, TXB_2 and ET-1: As seen in Table 3, plasma 6-K-P in High F group significantly decreased ($P<0.01$) than Control group, but TXB_2 and ET-1 were significantly raised ($P<0.01$). There were no differences of the three indexes between Se group and Control group. 6-K-P in High F+Se group was significantly higher than the High F group ($P<0.01$). TXB_2 and ET-1 in High F+Se group were significantly lower than the High F group ($P<0.01$, $P<0.05$).

The interaction of fluorine and selenium: As seen in Table 4, compared with non-fluorinated groups, the 6-K-P in fluorine-containing groups was lower ($P<0.05$) and TXB_2 in fluorine-containing groups was higher ($P<0.05$).

Fluorine is an essential trace element for human being which will generate deleterious effects when it is lacking or excess *in vivo*. Endemic fluorosis is prevalent in China because of high fluoride water (Luo & Feng 2008), brick tea (Cao et al. 2004) and indoor combustion of high fluorine coal (Ando et al. 2001). Endemic fluorosis can seriously impair the bones and teeth. In recent years, exploring the damage on the non-bone tissue and its mechanism induced by high dose fluorine and fluorosis have become a hot research. One of the emphases was the harm to cardiovascular system. In an epidemiological study (Liang 1986), the researchers investigated the endemic fluorosis areas in Hebei province, and they found that the morbidity of AS was higher in these areas than in non-fluorosis area, and the morbidity was positive correlated with the fluoride level in water. In the 24th World Conference of the International Society for Fluorine Research, the American and Polish scholars presented that the high dose fluorine can promote the formation of AS (Sun & Wang 2002). However, Se was considered to be a component of GSH-px which played an important role in antioxidation. Se deficiency was accompanied by a decreased activity in GSH-px. Simultaneously, Se could exert the antioxidant effects by scavenging free radicals and repairing the damage of membrane, and then alleviated fluoride toxicity (Zhang et al. 2009, Hayshi et al. 1997, Chen et al.2005). Another epidemiological study proved that the morbidity of AS was negatively correlation with the Se level in the environment (Shanberger 1975). On the basis of experiments and related literature, our team explored a suitable dose of Se (Se 1mg/L) in antagonizing detrimental effects caused by high dose fluorine (Bian & Wang 2004).

In recent years, some scholars found that the occurrence of AS was due to the dysfunction and damage of vascular endothelial cells (Qiu 2007). Abnormal secretion of vasoactive substances was considered to be the principal manifestation. As we all know, the PGI_2, TXA_2 and ET family can reflect the vascular endothelial function preferably. PGI_2 has the function of diastolic blood vessels and anti-platelet aggregation. TXA_2 possess the function of promoting vasoconstriction and platelet aggregation. PGI_2 and TXA_2 can metabolize to 6-K-P and TXB_2 quickly, so it can well reflect the concentration of PGI_2 and TXA_2 *in vivo* by measuring plasma 6-K-P and TXB_2. Rossi et al. (1997) found that the TXA_2/PGI_2 balance plays an important role in the maintenance of vascular homeostasis. TXA_2/PGI_2 value raised was one of reasons to platelet aggregation and vasoconstriction, so it was an important determinant to AS. The ET were a family of endothelium-derived peptides that possess characteristically sustained vasoconstrictor properties, ET-1 appeared to be the predominant member of the family, which is generated by vascular endothelial cells (Haynes & Webb 1998). So it was important for this study. Therefore, we observed the effects of Se on the plasma 6-K-P, TXB_2, ET-1 exposed to high dose fluoride. In addition, we wanted to explore the role and mechanism of high dose fluorine on AS, and the intervention effect of Se.

Our results showed that compared with Control group, plasma 6-K-P in High F group was significantly lower ($P<0.01$) and plasma TXB_2 and ET-1 in High F group were significantly higher ($P<0.01$). Demonstrating that PGI_2 was lower and TXA_2 was higher in high F group, and the TXA_2/PGI_2 value increased. It was consistent with the mechanism of AS generation. When a person has the chronic fluorosis,

Table 1: Serum fluorine in different groups ($\bar{x}$ ± s, mg/L).

Group	0 month	3rd month	6th month
Control	0.153±0.017	0.174±0.002	0.206±0.032
High F	0.156±0.010	0.589±0.113^{pp}	0.779±0.199^{pp}
Se	0.152±0.016	0.175±0.020	0.194±0.018
High F+Se	0.156±0.017	0.502±0.057^{pp}	0.695±0.153^{pp}

$^{p}P<0.05$, $^{pp}P<0.01$ compared with Control group
$^{\text{è}}P<0.05$, $^{\text{è è}}P<0.01$ compared with High F group at 0 month
$^{Ý}P<0.05$, $^{ÝÝ}P<0.01$ compared with High F group at 3 months

Table 2: Serum selenium in four groups ($\bar{x}$ ± s, mg/L).

Group	0 month	3rd month	6th month
Control	0.135±0.007	0.136±0.069	0.166±0.013
High F	0.131±0.013	0.124±0.026	0.161±0.005
Se	0.136±0.012	0.256±0.020^{pp}	0.319±0.041^{pp}
High F+Se	0.137±0.007	0.239±0.022^{pp}	0.294±0.016^{pp}

$^{p}P<0.05$, $^{pp}P<0.01$ compared with Control group
$^{\text{è}}P<0.05$, $^{\text{è è}}P<0.01$ compared with Se group at 0 month
$^{Ý}P<0.05$, $^{ÝÝ}P<0.01$ compared with Se group at 3 months

Table 3: The concentration of 6-K-P, TXB2, ET-1 at 6th month (pg/mL).

Group	6-K-P	TXB_2	ET-1
Control	823.13±82.2	113.77±34.5	208.±14.6
High F	623.17±94.8^{pp}	248.35±53.4^{pp}	242.7±14.1^{pp}
Se	816.89±71.8	107.50±29.7	208.2±10.3
High F+Se	768.96±57.6$^{\text{è è}}$	151.78±25.7$^{\text{è è}}$	216.4±13.4$^{p\text{è}}$

$^{p}P<0.05$, $^{pp}P<0.01$ compared with Control group
$^{\text{è}}P<0.05$, $^{\text{è è}}P<0.01$ compared with High F group

Table 4: The ANOVA about the effect of fluorine and selenium and their interaction on 6-K-P, TXB2, ET-1 (pg/mL).

	MS	F	P
6-K-P			
F	78717.37	12.51	0.003
Se	23260.97	3.7	0.072
F+Se	28680.47	4.56	0.049
TXB2			
F	43328.74	19.1	<0.001
Se	13204.66	5.82	0.028
F+Se	10664.81	4.7	0.046
ET-1			
F	1983.43	6.57	0.021
Se	844.09	2.79	0.114
F+Se	775.64	2.57	0.129

fluoride ions can deposit in blood vessels with large number, they can injure the vascular endothelial cells, and reduce the activity of PGIs. In this study, the secretion of ET-1 was raised. They may work together in constricting blood vessels, even blocking blood vessels, which can aggravate the process of AS.

The antagonistic effects of Se on fluorine were found by Polish scholars, they indicated that Se can increase the excretion of urinary fluorine in children (Wasowics 1985). The study prompted that increasing intake of Se can reduce the deleterious effects caused by high dose fluorine. In this study, we can see that there were significant differences in the 6-K-P, TXB_2, ET-1 between High F+Se group and High F group. The interaction showed that there was an antagonistic effect of Se on fluorine in the 6-K-P and TXB_2. In addition, our team confirmed Se, in appropriate level, was able to antagonize the adverse effects of fluorine within a certain range (Hou 2008). A moderate amount of selenium intake can effectively improve lipid metabolic disorder and the whole blood rheology abnormality, as well as the lipid peroxidation disorder *in vivo* induced by high dose fluorine (Zhu et al. 2008). Se can also inhibit abnormal expression of iNOS and eNOS induced by high dose fluorine (Hou et al. 2008).

CONCLUSIONS

High dose fluorine can lower plasma 6-K-P, while increase TXB_2 and ET-1 in blood. However, intake of Se adequately can obviously antagonize these changes, so it can play a beneficial role in inhibiting vasoconstriction and platelet aggregation, and slow down the formation and development of AS. Our experiment prompted that it is important to add the intake of Se moderately, together with reducing the fluorine content in drinking water, in prevention and treatment of AS in the fluorosis areas.

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