Effects of Selenium, Copper and Magnesium on Antioxidant Enzymes and Lipid Peroxidation in Bovine Fluorosis

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ABSTRACT : The antioxidant enzymes, lipid peroxidation and free radicals assessment were made of the effects of selenium, copper and magnesium on bovine endemic fluorosis under high fluoride, low selenium and low copper productive conditions. Thirty-two beef cattle were selected from high fluoride area, and randomly divided into four groups with eight cattle each as follows: (1) high fluoride control group (HFC); (2) supplemented group with 0.25 mg/kg selenium (HFSe); (3) supplemented group with 15 mg/kg copper (HFCu) and (4) supplemented group with 0.25 mg/kg selenium+15 mg/kg copper+1 mg/kg magnesium (HFSeCuMg) per day for 83 days. Moreover, eight beef cattle were selected from non-high fluoride area as normal control group. Blood samples were collected from cattle on 0 d, 30 d and 83 d respectively, to analyze the enzyme activities and concentration of GSH-px, CAT, SOD, MDA and free radicals. The results showed that the contents of free radicals and MDA in HFC group were significantly higher, and the whole blood GSH-px, CAT, erythrocyte SOD activities were lower than the normal control group. Free radicals, metabolic imbalance and antioxidant disorder therefore, play an important role in fluorosis. However, GSH-px, CAT and SOD activities in HFSe group and HFSeCuMg group at 30 d and 83 d were markedly higher than the same groups at the 0 d and the HFC group at the same time. Likewise, there was a corresponding reduction in the contents of free radicals and MDA. These findings indicated that supplementation with selenium, copper and magnesium elevated high fluoride bovine antioxidant enzymes, and decreased MDA and free radicals contents. But, the activities of supplementation selenium group did not increase until day 83. These results demonstrated that fluorosis was associated with lower serum Se and Cu levels than in the control, and it was therefore concluded that fluorosis is associated with decreased serum levels of these minerals. Long-term high fluoride intake under productive condition enhances oxidative stress in the blood, thereby disturbing the antioxidant defense of cattle. Increased oxidative stress could be one of the mediating factors in the pathogenesis of toxic manifestations of fluoride. It is benefical for high fluoride cattle supplemented with proper selenium, copper and magnesium to increase fluoride excretion and obtain the protective impact of the activity of oxidative enzymes, and to decrease lipid peroxidation and free radicals contents. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 12 : 1695-1699)***

Key Words : Bovine Endemic Fluorosis, GSH-px, SOD, CAT, MDA, Free Radicals

INTRODUCTION

Fluorosis is a serious public health problem in many parts of the world, mainly including Poland, Czech Republic, Turkey, Jordan, India, Mexico and United States etc. particularly in China where it is caused not only by drinking fluoride-containing groundwater but also by misusing fluoride-containing additives (Jubb et al., 1993; Han et al., 1998; Han, et al., 2002; Wei et al., 2002). Prolonged exposure to excessive amounts of fluoride may cause osteofluorosis, fluorosed teeth and non-skeletal damage. As in the case of many degenerative diseases, increased production of reactive oxygen species and lipid peroxidation has been considered to play an important role, even in the pathogenesis of fluorosis (Mysliwiec et al., 2002; Chlubek, 2003; Reddy et al., 2003; Wang et al., 2004). However, there is inconclusive proof for an altered oxidative stress and antioxidant balance on bovine fluorosis under productive condition. Minerals are required for both physiological and biochemical functions. Many disorders of the body are associated with the altered serum mineral levels. Among of them, selenium, copper and magnesium are generally recognized as important antioxidants with numerous biological functions. The present study was therefore undertaken to evaluate the antioxidant defense system (both enzymatic and nonenzymatic) and lipid peroxidation in bovine endemic fluorosis.

MATERIALS AND METHODS

Eight 6-7 year-old non-high fluoride beef cattle from Yongning city, Ningxia, China, and foodstuff was produced in non-high fluoride area. Thirty-two 6-7 year-old beef cattle, which were raised by farmers in Guangwu county Qin Tongxia city, Ningxia, China, were selected at natural high fluoride area, and randomly divided into four groups with eight cows being fed with local fluoride foodstuff. The groups were as follows: (1) high fluoride control group (HFC), (2) supplemented with 0.25 mg/kg selenium (sodium selenite)(HFSe), (3) supplemented with 15 mg/kg copper (copper sulfate)(HFCu), (4) supplemented with 0.25 mg/kg selenium+15 mg/kg copper +1 mg/kg magnesium (magnesium sulfate) per day for 83 days, respectively, and

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Table 1. Fluoride contents of drinking water, feedstuff, soil, air, serum, bone and tooth (mg/L)

Groups	Drinking water	Feedstuff	Soil	Air (g/m^3)	Serum	Bone	Tooth
High fluoride	3.27±0.23** (n=6)	51.97±11.96** (n=10)	8.00±0.87** (n=6)	2.44±0.53** (n=6)	0.62±0.12** (n=8)	8,854.66±1,723.98** (n=5)	9,393.46±48.99** (n=5)
Control	0.58±0.21 (n=5)	23.75±1.77 (n=2)	3.75±1.77 (n=3)	0.12±0.09 (n=8)	0.24±0.01 (n=8)	300-1200 (n=2)	240-625 (n=2)
GB	1.0-1.5	40.0	/	1.8	0.2	/	/

** Compared with control group p<0.01.

Table 2. Contents of selenium and copper in soil, drinking water, feedstuff and blood (mg/L)

Groups	Selenium				Copper			
	Feedstuff	Soil	Drinking water	Blood	Feedstuff	Soil	Drinking water	Blood
High fluoride	0.072±0.058 (n=6)	0.108±0.08* (n=5)	0.00	0.064±0.048** (n=7)	6.32±1.27** (n=7)	1.87±0.68** (n=5)	0.00	0.56±0.05** (n=7)
Control	0.087±0.073 (n=5)	0.194±0.02 (n=2)	0.00	0.114±0.056 (n=6)	8.73±2.64 (n=6)	3.62±0.28 (n=2)	0.00	0.95±0.20 (n=8)

* Compared with control group p<0.05, ** Compared with control group p<0.01.

each group drank local high fluoride water. The fluoride content of bovine internal and external environment were measured, the whole blood was taken for baseline value, then trace elements additives were exposed to corresponding groups daily for 83 days, and the blood was tested for corresponding index on day 30 and 83, respectively.

The fluorine was measured by fluoride ion selective electrode, selenium was measured by Shimadzu RF-540 (Japan) spectrofluorophotometer, copper was measured by AA-370MC atomic absorption spectrometer (Shanghai, China), whole blood GSH-px was tested by method of 5,5-dithiobi (2-nitro benzoic acid)(DTNB); erythrocyte SOD by xanthine oxidase; the whole blood CAT by CATase substrate analysis; MDA of erythrocyte lipid peroxides by thiobarbituric acid chromometry (TBA). Blood free radicals content was tested by JEOL JES-FE1XG electron spinning resonant (ESR) spectrometer, Japan; blood sample was transferred swiftly from fluid nitrogen into co-consussing chamber with temperature -190°C±5°C, regulated central magnetism 3,300±500 Gauss, regulating magnetism 100 kHz 6.3 Gauss, microwave power 2 mW, magnified times 1×1,000, scanning time 4 s, and answer time 0.1 s. From ESR spectrum obtained from above condition, it was sure that 0 top value represents reactive free radicals content, and its aptitude based on baseline represents relative content of reactive free radicals.

Statistical analysis

The results were presented as the least squares means value±standard error of the mean (SEM) and one-way analysis of variance was performed using the General Linear Models Procedures of the SAS software (1994). Differences among means were tested using Duncan's multiple range tests. A significant level of 0.05 was used.

RESULTS

Investigation of fluoride, selenium and copper condition between the internal and external environment

The examined beef cattle in Qing Tongxia city, Ningxia appeared fleshy mandible, and egg-shaped osteoma broken-through could be cured for a long time. The cattle showed osteoproliferation in body surface, fluoride dotted dentals and un-neat, grinded dentals, poor utilization of feed and unthriftiness, dystrophy, and emaciation. Fluoride contents (Table 1) of drinking water, foodstuff, air, serum, bone and teeth indicated that bovine fluoride contents between internal and external environment in Qing Tongxia Ningxia were significantly higher than the control group.

The contents of selenium and copper of the above same samples were as follows (Table 2): selenium and copper contents of foodstuff, soil and whole blood in high fluoride area were significantly lower than the control area, this finding indicated that high fluorine was accompanied by low selenium, and low copper in internal and external environment in Qing Tongxia area, China. The subclinical copper and selenium deficient syndrome therefore accompanied by the endemic fluorosis was the essence of the disease.

The changes of GSH-px activity in serum

From Table 3, it can be obtained that the serum GSH-px activity in HFC was markedly lower than the normal control group (p<0.05). They were no significant difference at different times (p>0.05). The GSH-px activity of HFSe on day 30 was higher than that on day 0 by 7.78 U/ml (p<0.05), also higher than that of HFC at the same time by 5.77 U/ml (p<0.05). The GSH-px activity in HFSe on day 83 was higher than day 0 by 14.79 U/ml (p<0.01), and by 15.24 U/ml (p<0.01) in HFC at the same time, it was higher

Table 3. The whole blood glutathione peroxidase (GSH-px) activities in cattle (U/ml blood)

Groups	0 d	30 d	83 d
Normal control beef cattle	/	24.78±6.43[a]	25.63±3.16[a]
High F control beef cattle	14.62±5.90	16.40±2.97	13.94±5.52
High F suppl.Se beef cattle	14.39±3.43	22.17±4.89*. [a]	29.18±4.83**
High F suppl.Cu beef cattle	17.59±7.48	18.91±7.05	17.47±1.94
High F suppl.SeCuMg beef cattle	19.95±2.95	27.10±4.19*,[a]	28.54±6.24**, [aa]

N=8. * Compared with the same group base line $p<0.05$, ** compared with the same group base line $p<0.01$.
[a] Compared with high fluoride control group at the same time $p<0.05$.
[aa] Compared with high fluoride control group at the same time $p<0.01$. Same as the following.

Table 4. The erythrocyte superoxide dismutase (SOD) activities in cattle (NU·ml blood^{-1})

Group	0 d	30 d	83 d
Normal control beef cattle	/	1,873.66±445.93[a]	1,824.17±250.68
High F control beef cattle	1,747.52±462.90	1,535.81±142.78	1,728.31±318.24
High F suppl.Se beef cattle	1,948.04±332.37	2,034.31±66.85**	1,933.86±315.24
High F suppl.Cu beef cattle	1,944.69±521.48	1,803.04±189.71**	1,838.53±477.62
High F suppl. SeCuMg beef cattle	1,522.10±249.20	1,957.66±289.40*	2,104.09±245.39**,[aa]

Table 5. The catalase activities of blood in cattle (U/gHb)

Groups	0 d	30 d	83 d
Normal control beef cattle	/	132.57±54.12	123.95±40.74
High F control beef cattle	111.20±39.38	120.08±26.24	135.21±13.35*
High F suppl.Se beef cattle	147.34±19.98	152.45±31.61*	174.95±17.62**,[aa]
High F supple.Cu beef cattle	139.80±58.52	136.68±49.90	97.67±40.54
High F suppl.SeCuMg beef cattle	116.40±26.02	136.85±27.75**	144.03±33.69**

Table 6. The erythrocyte mononaldehyde (MDA) concentrations in cattle (nmol/mg Hb)

Groups	0 d	30 d	83 d
Normal control beef cattle	/	0.41±0.14	0.39±0.06
High F control beef cattle	0.37±0.09	0.41±0.08	0.41±0.06
High F suppl.Se beef cattle	0.43±0.21	0.28±0.04**,[aa]	0.31±0.05[aa]
High F suppl.Cu beef cattle	0.37±0.10	0.26±0.01**,[aa]	0.36±0.09[a]
High F suppl.SeCuMg beef cattle	0.33±0.08	0.22±0.06**,[aa]	0.32±0.01[aa]

7.01 U/ml ($p<0.05$) on day 30 in the same group. The GSH-px activity of HFSeCuMg was almost similar to HFSe, but the value on day 30 was higher ($p<0.05$) than that of HFC at the same time, and the difference between day 83 and 30 was not apparent ($p>0.05$). The GSH-px activity of HFCu did not change apparently ($p>0.05$).

The changes of erythrocyte SOD activity

The erythrocyte SOD activity of HFC at different time was lower than that of the normal control group at the same time ($p<0.05$, $p<0.01$) (Table 4). It decreased on day 30 compared with the baseline value ($p<0.05$), it increased to the level of day 0 on day 83. The SOD activity of HFSe on day 30 was clearly higher than that of on day 0 ($p<0.01$), but the SOD activity on day 83 was lower than that of day 30, and lower than the baseline value. The erythrocyte SOD activity of HFSeCuMg was higher on day 30 than that of on day 0 by 435.56 NU/ml of blood ($p<0.05$), on day 83 than day 0 by 582.01 NU/ml of blood ($p<0.01$), and at the same time by 375.78 NU/ml of blood ($p<0.01$) in HFC.

The changes of serum CAT activity

From Table 5, CAT activities of HFSe and HFSeCuMg were increased markedly ($p<0.05$, $p<0.01$) on day 30 and day 83, respectively. But the effect time of HFSeCuMg was earlier than that of HFSe. CAT activity of HFCu was significantly lower on day 83 than that of the day 0 ($p<0.01$).

The changes of erythrocyte MDA

From Table 6, erythrocyte MDA of HFC had no significant changes ($p>0.05$). The value of MDA of HFSe, HFCu and HFSeCuMg on day 30 were totally lower markedly than the baseline value ($p<0.05$), and had less changes on day 83 compared with the baseline value of the same group ($p>0.05$), but it was apparently lower than that of HFC at the same time ($p<0.05$). This indicated that supplemented selenium, copper or selenium+copper+ magnesium under high fluoride condition can reduce the MDA content and inhibit lipid peroxides.

Table 7. The blood free radical contents in cattle (mm)

Groups	0 d	83 d
Normal control beef cattle	/	42.20±3.35^{aa}
High F control beef cattle	51.17±8.13	60.40±8.65
High F suppl.Se beef cattle	46.83±11.05	33.67±8.94**
High F supple.Cu beef cattle	47.00±12.63	40.00±6.75*,^{aa}
High F suppl.SeCuMg beef cattle	43.33±9.85	31.67±14.79**,^{aa}

The changes of serum free radicals

From Table 7, the free radicals content of HFSe, HFCu and HFSeCuMg on day 83 were lower than baseline value of each group, also lower than that of HFC ($p<0.01$) at the same time. A decrease in the free radicals content of HFSeCuMg was most significant ($p<0.01$) compared with HFC group, the HFSe was better ($p<0.01$), and HFCu was significant ($p<0.05$). This indicated that combined supplementation of Se, Cu and Mg can markedly reduce the production of free radicals under high fluoride condition.

DISCUSSION

There is no doubt that antioxidant enzymes of which clear free radicals in body include GSH-px, SOD and CAT. The importance of these factors is unquestionable. However, to understand and explain the various differing results concerning oxidative stress in fluoride intoxication. Actually, H_2O_2 is reduced into H_2O by GSH-px, enzymolyzed by CAT, both GSH-px and CAT therefore can clear H_2O_2, and prevent the damage of H_2O_2. GSH-px also decompose lipid peroxide into non-toxic substance to protect the damage of cells. $O^-_2\cdot$is dismutated into H_2O_2 by SOD, and H_2O_2 enzymolyzed into H_2O by CAT.

The present study showed that selenium, copper contents in the internal and external environment are lower, moreover fluoride is markedly higher than in the case of normal control group under productive condition. The similar proposals have been reported by some authors (Fujihara et al., 1995; Naresh et al., 2001; Meral et al., 2004; Gowda et al., 2004). Earlier studies by Han et al. (1998) observed exerted fluoride excretion by supplemented selenium, and lightened fluoride-induced damage of free radicals. On the other hand, selenium deficiency deteriorates the fluorosis, high fluoride and low selenium do more hazard to body than high fluoride alone, and if accompanied by low copper, the affected animals thereby will collapse (Han et al., 2004).

Results of the present study revealed that the activities of blood GSH-px have decreased levels, as well as the activities of blood CAT and erythrocyte SOD, but the products of erythrocyte MDA and blood free radicals have increased levels in high fluoride control group, these effects correlated with increased levels of lipid peroxides, this investigation has been supported earlier (Chinoy et al., 2000; Kapoor et al., 2001; Reddy et al., 2003; Shivarajashankara et al., 2003). Even stronger evidence has been presented by Wang et al. (2004) and Chinoy (2003), thereby chain reaction of free radicals is out-numbered. Once one kind of antioxidant enzymes is damaged, the whole antioxidant enzymes will collapse, and the body tissue damaged (Chlubek, 2003). In their valuable study on fluoride and the earthworm *Eisenia fetida*, Lawson et al. (2003) paid attention to fluoride as a competitive inhibitor of SOD. Their proposed mechanism for inhibition of SOD by fluoride involves its binding to the active site of Cu on SOD, thus displacing water. In their view the binding of fluoride to the active site of SOD is not readily, or spontaneously, reversible, and that the reaction rate for fluoride binding is fairly constant, reaching an equilibrium within a very short period of time. This could explain why the activity of SOD may be decreased in fluoride toxication. As is known, When rats have fluorosis, lipid peroxidation is increased and deteriorated with time extending, the antioxidant ability is decreased, the reactive oxygen is over-produced, causing cell damage based on the level of cell, sub-cell and molecule, thus, resulting in pathological course of fluorosis (Myśliwiec et al., 2002; Han et al., 2004). A increase in blood free radicals content under high fluoride, low selenium and low copper condition was observed in our study, which is undoubtedly associated with the presence of regressive changes occurring more intensively in those experimental groups. A number of studies on oxidative stress in fluorotic humans and fluoride-intoxicated animals indicate that generation of free radicals and lipid peroxidation (MDA formation) can be directly induced by fluoride (Patel et al., 1998). Moreover, there is evidence that both free radicals and lipid peroxides play an important role in fluorosis (Chlubek, 2003).

Our results also indicated that selenium in HFSe group, in sufficient doses, may exert a certain protective impact on the activity of oxidative enzymes GSH-px and SOD, and decrease the contents of MDA and free radicals. These observation are accord with those of Pang et al. (1996) on *in vivo* and *in vitro* studies of skeletal muscles of rats treated with fluoride and selenium. These authors associate the protective effect of selenium with improved stability of mitochondrial membranes as a result of decreased lipid peroxidation. Supplemented selenium can correct high fluoride-induced metabolic disorder of free radicals (Choi et al., 1995). However, the activity of GSH-px of HFCu group is unaltered, moreover, the changes in SOD and CAT activities, and the contents of MDA and free radicals reduced. These finding indicates that supplemented copper of 15 mg/kg does not satisfy with the body under the high fluoride, low selenium and low copper condition. It is lower than the standard in the blood copper until to day 83 (Han et al., 1998). The influence of fluoride together with copper on antioxidant defense enzymes and lipid peroxidation will be

continued. In the present study, an attempt was made, therefore, to investigate the effect on certain blood antioxidant defense enzymes and lipid peroxidation of fluoric cattle supplement with selenium copper and magnesium in feedstuff. Data on blood indicated that antioxidant enzymes significantly increased their activities, in the same way the MDA and free radicals significantly decreased their contents, In agreement with this view, these factors can influence one another and must also be considered in fluoride intoxication. It has been suggested that magnesium interferes with F (Machoy-Mokrzynska, 1995). The radium of Mg^{2+} is smaller than that of Ca^{2+}, Mg^{2+} can substitute Ca^{2+} to combine with fluoride, the blood calcium thereby is increased, decalcification of target organ is decreased, at the same time magnesium combines with fluoride, inhibiting the fluoride continuous damage. Magnesium is a kind of fluoric scavenger. Supplemented Se, Cu and Mg not only exert antioxidant defense, but also de-fluoric function of magnesium, and lighten the peroxidation damages and fluoric toxification.

It can be concluded therefore, under the experimental condition of this study, that we find that fluoride acts differently on the enzyme activities and lipid peroxidation of the blood, depending upon the mineral status of the animals. Se, Cu and Mg supplemented with the proportion of 0.25 mg/kg, 15 mg/kg and 1 mg/kg may stem from bovine fluorosis. There are many high fluoride, low selenium and low copper areas, application and popularization of the findings can exert great benefits.

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