THE EFFECT OF ANTIOXIDANT AGENTS ON CADMIUM INDUCED IMPAIRMENT IN GASTRIC MUCOSA OF RATS

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SUMMARY : The balance between antioxidative and oxidative capacity of gastric mucosal epithelium plays an important role in maintenance of gastric mucosal integrity. High cadmium intake, disturbs this balance by increasing lipid peroxidation.

In this experimental study, lipid peroxidation induced mucosal damage and its prevention due to antioxidant agents were investigated in rats exposed to high cadmium instake. It was found that gastric mucin decreased from 188.06 ± 10.76 µg/g wet wt to 158.41 ± 22.74 µg/g wet wt ($p<0.01$) and PGE2 levels declined from 1321.92 ± 271.65 µg/g wet wt to 1030.30 ± 278.51 µg/g wet wt ($p<0.05$) and an increment of hemoglobin leakage into luminal fluid was observed in rats received 1.5 µg/ml of cadmium containing water for 30 days in these exposed animals. TBARS levels in blood and gastric mucosa increased from 13.33 ± 3.30 nmol/g Hb to 19.54 ± 6.92 nmol/g Hb ($p<0.05$) and from 685.0 ± 123.70 nmol/g protein to 1090 ± 154.40 nmol/g protein, respectively. Co-administration of cadmium with antioxidant agents (1.42 µg/kg Se, 0.21 U/kg Vit. E, 14.3 U/kg Vit. A, 0.86 mg/kg Vit. C) did not prevent the lipid peroxidative effect of cadmium in gastric mucosa of rats.

Key Words : Cadmium, gastric mucin, lipid peroxidation, PGE_2, SOD.

INTRODUCTION

Experimental studies have shown that despite continuous exposure to the oxidant agents gastric mucosa has a very strong self-defense mechanism against endogenous and exogenous noxious stimuli. The reason of this resistance is attributed to the presence of a well developed self-defense mechanism of the gastric mucosa (1, 2). It has been reported that antioxidant capacity, which is composed of several metalloenzymes such as superoxide dismutase, glutathion peroxidase, catalase, is involved in the protection of gastric mucosa against oxidative stress (3, 4). Lipid peroxidation, a consequence of free radical formation is implicated as a molecular mechasm in the pathogenesis of several chronic diseases. Any agent inducing lipid peroxidation disturbs cell membrane integrity and leads to functional and morphological alterations.

Among these agents, environmental factors play an important role from the public health point of view. People who are subjected to lipid peroxidative agents such as cadmium in the in environments are therefore expected to be susceptible to oxidative stress induced disorders. The results of Manca *et al.* (5) and Hussain *et al.* (6) indicating increased lipid peroxidation of liver and kidney in animals exposed to high intake of oral

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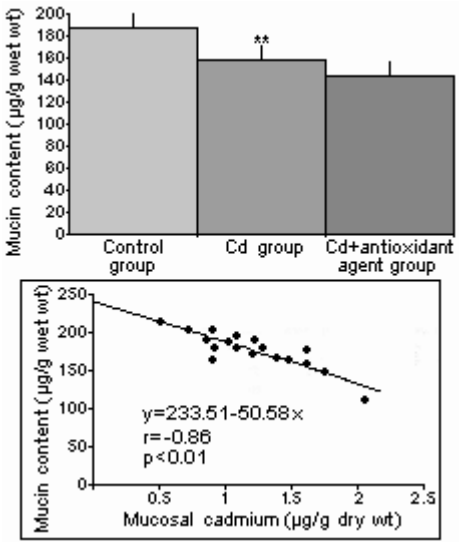
Figure 1: The effect of cadmium and antioxidant agent on mucin content of gastric mucosa, ** p < 0.01.



cadmium support this expectation. Among the others, gastric mucosa being the tissue exposed to cadmium before the other organs it could be under the high risk of cadmium induced lipid peroxidation. However there is presently no evidence that the incidence of disorders related to injured gastric mucosa in polluted areas are higher than those encountered in the other areas.

Despite the lack of clinical information, our previous results showed that due to decrease in the components of gastric barrier such as mucin and PGE_2 (7,8), the mucosal injury in response to stress was exaggerated in rats exposed to high cadmium. The mechanism by which cadmium induced this impairment is uncertain, but the disturbing effect of cadmium in the balance between oxidant and antioxidant defenses of gastric mucosa could be involved in the breakdown of mucosal cell integrity. If this hypothesis is true, the treatment of antioxidative drugs may prevent this cadmium induced mucosal damage in rats. This experimental study was carried out to test this possibility.

MATERIALS AND METHODS

In this experimental study, 45 days old male albino rats were used. 10 of these animals were fed as a control group and received normal rat food and tap water. 10 rats received 15 ppm of cadmium as $CdCl_2$ in their drinking water while other 10 animals received 15 ppm cadmium in drinking water simultaneously with antioxidant agents (1.42 µg/g/kg selenium, 0.21 U/kg Vit. E, 14.3 U/kg Vit. A, 0.86 mg/kg Vit. C) by gastric gavage for 30 days.

At the end of this feeding period, following 24 h fasting, the animals were anesthetized with 1 g/kg of urethane. Abdomen was opened by a midline incision. The lowermost level of the oesophagus was tied by preserving vagal nerves and vessels. A catheter placed into their stomach through the duodenum was used for gastric irrigation and sampling.

After irrigating the stomach with 37°C saline three times, 2 ml of distilled water was given into the stomach and kept there for 30 min, it was then taken back and its acid concentration was measured by titration with 0.01 N NaOH and its hemoglobin concentration by the method of cyanmethemoglobin.

The blood and mucosal scrapings were used for the analysis of PGE_2 (9), mucin (10) TBARS (MDA) (11,12), SOD (13) levels as well as cadmium and zinc contents. The PGE_2 content of the gastric mucosa was measured by using HPLC (Varian 5020) and graphite furnace of Atomic Absorption Spectrophotometer (Hitachi 8000) was used for determination of cadmium in the gastric mucosa.

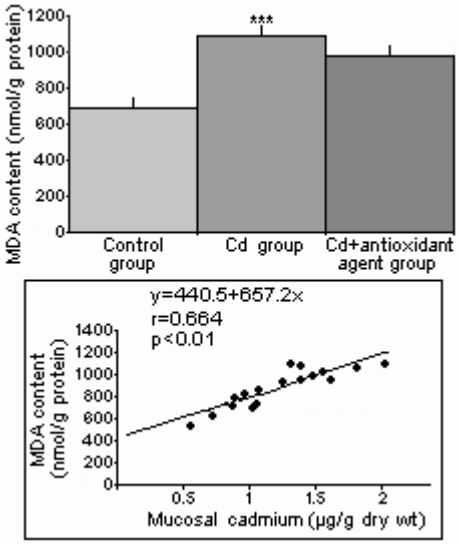
The results were expressed as mean±SD and Student ' t' test was used for statistical analysis.

RESULTS

Cadmium levels of blood and mucosa

The mean blood cadmium level was found to be 1.40±1.13 µg/L in control rats. Addition of 15 µg/ml cadmium into drinking water for 30 days caused a sig-

Figure 2: The effect of cadmium and antioxidant agent on MDA (TBARS) content of gastric mucosa, ***p < 0.001.



nificant increase in blood cadmium levels of animals (8.42±3.02 μg/L, p<0.01). The cadmium content of gastric mucosa rose from 0.070±0.047 μg/g dry wt to 0.255±0.149 μg/g dry wt in cadmium exposed rats. Treatment antioxidant combination did not cause any change in blood and gastric mucosa (6.74±3.50 μg/L and 0.140±0.045 μg/g dry wt, respectively).

Gastric acid secretion and hemoglobin content in luminal fluid

Cadmium treatment for 30 days decreased gastric acid output from 37.84±1.42 mEq/h to 25.64±6.93 mEq/h (p<0.01). However the acid secretion in gastric mucosa was found to be unchanged in rats treated with antioxidant agents (29.40±8.89 mEq/h).

Hemoglobin content of gastric fluid increased above the control value of 3.39±1.42 μg/ml in animals receiving high cadmium (4.47±1.33 μg/ml).

The components of gastric mucosal barrier

The mean alcian blue binding capacity which is indicator of acidic mucopolysaccharide content of gastric mucin was 188.06±10.76 μg/g wet wt in control rats and 158.41±22.74 mg/g wet wt in cadmium receiving group (p<0.01). There was a significant negative correlation between gastric mucin and cadmium content (r=0.835, p<0.01) (Figure 1).

PGE_2 level of gastric mucosa declined to 1030.50±278.51 μg/g wet wt from 1321.92±271.5 μg/g wet wt (p<0.05) due to high cadmium intake.

The combination of antioxidant agents did not change the components of gastric mucosal barrier.

TBARS level and SOD activity

TBARS, a product of lipid peroxidation, significantly increased in blood and mucosa while a member of antioxidant capacity, SOD remained unchanged. A significant correlation was found between cadmium level and MDA level of mucosa (r=0.664, p<0.01) (Figure 2). MDA content remained unchanged while SOD activity increased significantly (p<0.05).

DISCUSSION

The present results indicate that exposure to oral cadmium intake for 30 days caused a significant cadmium accumulation in the gastric mucosa as well as blood of rats. This accumulation is accompanied by an impaired mucosal barrier of the stomach. The reduced PGE_2 and mucin levels in mucosa and increased leakage of hemoglobin into the luminal fluid in cadmium exposed rats are the overt evidences of injury in mucosal barrier. Close and negative correlation

Table 1: * p<0.05, ** p<0.01, *** p<0.001, Comparison of control and Cd groups + p<0.05, ++ p<0.01, Comparison of Cd and Cd+ Antioxidant agent groups.

	Control	Cd	Cd+Antioxi-dant agent
Blood			
Cd (μg/L)	1.40±1.13	8.42±3.02	6.74±3.5
MDA (nmol/g HB)	13.33±3.33	19.50±6.92	18.67±2.86
SOD (Ug/HB)	2816.10±906.40	2701.70±710.40	3855±869.20
Gastric acid secretion (mEq/h/2 ml)	37.84±6.02	25.64±6.93	29.40±8.89
Hb leakage (μg/ml)	3.39±1.42	4.47±1.33	14.99±6.74
PGE_2 content of gastric mucosa (μg/g wet wt)	1321.92 ±271.65	1030.50 ±278.51	1282.60 ±210.60

between cadmium content and mucin levels in the mucosa has pointed out that cadmium is one of the causative factors of disruption of mucosal integrity. There also was a positive and significant relationship between mucosal and blood (MDA) and cadmium levels in cadmium exposed animals. This relation supports that cadmium increases the products of lipid peroxidation in these animals. The unchanging of SOD levels despite elevated TBARS is a reliable sign of imbalance between oxidative and antioxidative capacity of blood (Table 1).

Due to lack of knowledge about the cadmium induced breakdown in gastric barrier and increase in lipid peroxidation of gastric mucosal cells, our observations can not be compared with the finding of literature but they are in well accord with our previous data (14). However the studies showing deleterious effects of cadmium on kidney and liver functions (15 -17) support indirectly our data related gastric mucosal integrity and functions.

Despite many reports about the beneficial effects of Selenium and Vitamins A, C, E as reducing agents (18- 20) neither the increase in TBARS levels of blood and mucosa nor the decrease in mucin content in rats were prevented by the treatment with this antioxidant combination. The dose of this antioxidant combination used in our study is equivalent to the dose prescribed for daily intake of human. Slight increase in mucosal PGE_2 levels and significant elevation in blood SOD levels due to antioxidant therapy did not overcome the progress of mucosal breakdown and elevation of TBARS.

As a result we conclude that either insufficiency of the treatment dose or the severity of cadmium induced toxicity used in our experiments are responsible for the failure of the antioxidant treatment of cadmium induced changes of gastric mucosal barrier.

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