THE EFFECTS OF CADMIUM ON THE FRAGILITY OF RED BLOOD CELL

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SUMMARY: In order to investigate the effects of cadmium induced lipid peroxidation on red blood cell membrane this experimental study was undertaken. Rats were given drinking water containing 15 ppm $CdCI_2$ for 30 days and the fragility of red blood cells obtained from cadmium exposed rats were compared with those of controls.

Despite the fact that obvious cadmium induced lipid peroxidation as indicated by increased blood TBARS, elevated phospholipids and protein contents of red blood cell membrane there was no significant changes in the fragility of erythrocytes in cadmium treated rats.

This unaltered red blood cell fragility suggested us that red blood cell membrane is more resistant to peroxidative stress than other cells and this resistance seems to be helpful in the maintenance of tissue oxygenation.

Key Words: Cadmium, red cell fragility.

INTRODUCTION

Cadmium is an extremely toxic environmental and industrial pollutant having a long half life in humans. It has been demonstrated that cadmium induces several alterations in the tissues of laboratory animals and humans (1). Mechanisms of cadmium toxicity remain incompletely understood, but elevated lipid peroxidation in tissues is observed soon after exposure to cadmium (2-4). There also is a positive correlation between cadmium intake and the cell injury. It is believed that lipid peroxidation is an early and sensitive reaction to cadmium exposure.

It is known that membrane lipids by interacting with proteins lead to changes in membrane functions which is operated mainly by proteins. These variations in the structure of membrane lipids producing on the other hand changes in cellular membrane fluidity and/or permeability (5,6). Therefore it is natural to expect that cadmium induced lipid peroxidation disturbs membrane integrity as well as cell functions. Membrane integrity is very important for the function of all cells in the body, but it must have a vital importance for red blood cells which carry 200 or 300 million molecules of hemoglobin in each one (7). For normal tissue oxygenation, iron atoms in hemoglobin molecules must be protected from oxidative stress and this protection requires a normal red blood cell membrane, a leakage of hemoglobin into plasma, a tendency of intravascular hemolysis and a deficiency in tissue oxygenation would occur.

However, despite the above mentioned evidence of cadmium induced impairment in cell membranes, the number of studies concerning its effects on red blood

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cell membrane is limited. Garty *et. al.* in their in vitro experiment showed that cadmium uptake into red blood cells was a non-saturable metabolically derived energy independent and a temperature sensitive process (8). However, during our previous experimental studies (9-12) despite the evidence of cadmium toxicity mainly in kidney, any evidence indicating a tendency to hemolysis of red blood cells in cadmium exposed rats has not been observed. Lack of sign related with a tendency to hemolysis conflicted with the cadmium induced peroxidative damage observed in the other organs and tissues.

This discrepancy led us to wander whether there is a difference between the sensitivity of red blood cell membrane and other cell membranes to cadmium induced peroxidative damage in the body. For this reason this experimental study was carried out and the effect of cadmium induced lipid peroxidation on red blood cell membrane fragility was investigated.

MATERIALS AND METHODS

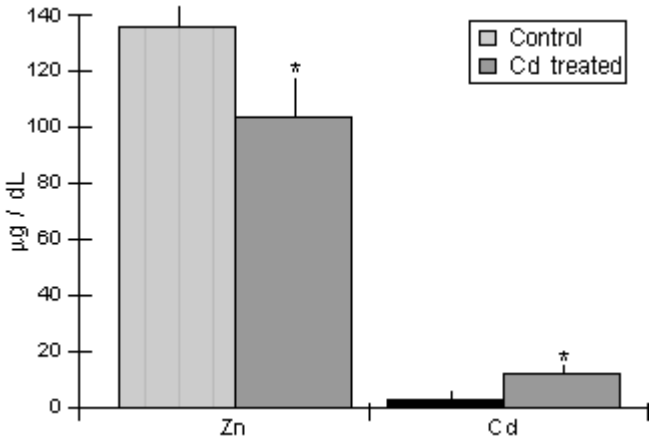
2,5 month old male Swiss albino rats were used in this experimental study. 16 animals in control group were given normal laboratory rat food containing 0.15 ± 0.03 μg Cd per gram dry weight and tap water containing 0.13 ± 0.02 μg Cd per liter, while the remaining 14 animals in the second group received 15 ppm Cadmium Chloride (Sigma C-314) in their drinking water for 30 days.

On day 30 following a 16-hour fasting period all animals were anaesthetized with urethane 1g/kg body weight i.p. and blood samples were taken by cardiac puncture using heparinized syringes.

After centrifugation of blood at 3000 rpm for 5 min at room temperature, the upper plasma and buffy coat were removed and the red cells in the pellet were washed three times with saline and some of these cells re-suspended in the saline to measure the fragility of red blood cells. 20 μl aliquots of the cell suspension were added dublicately into the tubes containing buffered NaCl ranging from 0.56 to 0.2%. Following 30 min incubation at 37°C all tubes were centrifuged and the Hemoglobin content in the supernatant was measured by Cyanmethemoglobin method (13).

5 ml of distilled water were added into the tube containing 1 ml of packed red blood cells and hemolized by vortexing then these hemoliysed cells were centrifuged at 3000 rpm for 30 min at 40°C using Kubota model 5800 refrigerated centrifuge. The supernatants were removed by decantation and the pellets diluted with buffered Tris HCl (pH 8.0) and centrifugation were repeated until hemoglobin was completely removed from supernatant. The protein content in the pellet, in the ghost diluted with 1 ml of saline was measured using the method of Lowry (14). Some of the pellet was extracted in 7 volume of chloroform/11 volume of isopropanol and after centrifugation, the lipid containing upper phase of extracted material was decantated into another tube and evaporated at 50°C. Then 1 ml of isopropanol was added into dry lipid extract and dissolved to use the measurement of phospholipids and cholesterol content of red blood cell membrane. The cholesterol and phospholipid content in the membrane and plasma extracts were measured

Figure 1: Reciprocol changes of Blood Cadmium and Zinc levels (M ± SD).



by using commercially available Sclavo Kits. (Cat No 81411 and 81794 respectively) and the ratio of cholesterol to phospholipid (C/PL) in the red blood cell membrane was estimated by using the values obtained by above mentioned Kits.

Cadmium induced lipid peroxidation (Thiobarbituric acid reactive substance-TBARS) was assayed by means of thiobarbituric acid reaction in fresh red blood cells using a homogeniser (Tri-R Stir-R model K-43) with a teflon pestle at 4°C for 1 min (15,16).

Acid washed glassware was used during cadmium and zinc analysis using an atomic absorption spectrophotometer (Hitachi model Z-8000 model) in heparinized blood.

Statistical analysis of the results was performed by employing Student's t test. Data are presented as Mean±SD.

RESULTS

Blood cadmium and zinc levels: In control animals the mean blood cadmium level was found to be 1.40±1.13 μg/L whereas in the rats which received 15 ppm $CdCl_2$ containing water for 30 days, blood cadmium level increased significantly to 8.42±3.02 μg/L ($p<0.01$). This increase in blood cadmium was associated with a significant decrease in plasma zinc levels in cadmium exposed rats. Plasma zinc levels in control and cadmium exposed rats were 137.16±10.87 and 105.0±22.4 μg/dL respectively ($p<0.01$) (Figure 1).

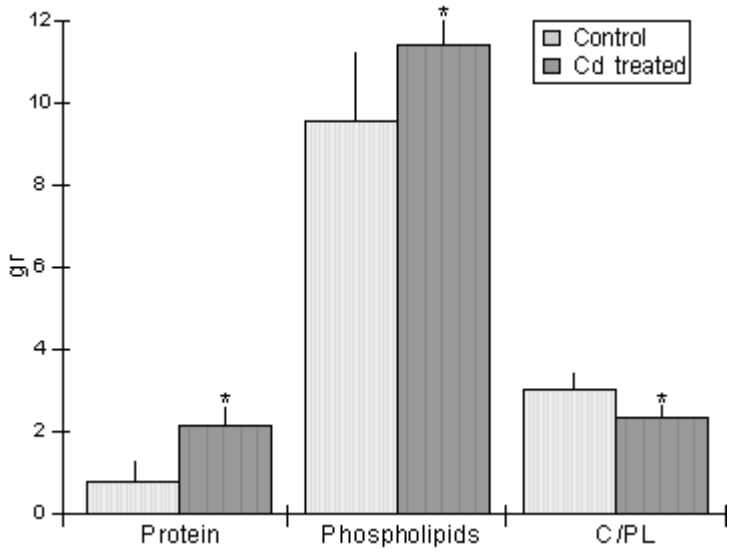
The changes in red blood cell membrane: The mean protein content in ghost of control rats was 0.810±0.255 mg/ml packed cell whereas the mean protein content of 1 ml of packed red cell membrane in cadmium treated rats was increased significantly to 2.013±0.559 mg/ml ($p<0.001$).

The cholesterol content of red cell membrane was measured as 27.8±1.4 mg/ml in control and 26.9±1.8 μg/ml in rats exposed to high cadmium containing drinking water for 30 days. Membrane phospholipids were also measured and found to be 9.73±1.47 and 11.48±1.33 μg/ml in control and cadmium treated rats respectively ($p<0.05$). Because of a significant increase in membrane phospholipids cholesterol to phospholipid ratio in the red blood cell membrane was estimated as 2.92±0.45 in control animals and 2.37±0.37 in cadmium exposed rats ($p<0.05$) (Figure 2).

TBARS content in the blood

The mean TBARS levels in the blood of control animals was 13.3±3.3 nmol/g hemoglobin. In cadmium exposed animals this value increased significantly to 19.5±6.9 nmol/g Hb ($p<0.05$).

Figure 2: Cadmium induced changes in the components of RBC membrane (M ± SD).



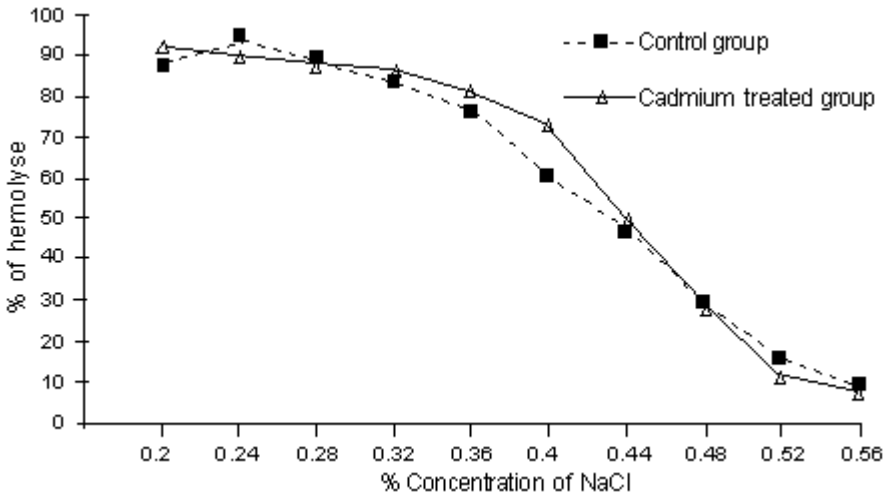
Fragility of red blood cells

As seen in Figure 3 there was no significant difference between the fragility curve of red blood cell obtained from control and cadmium exposed animals. 30 minutes incubation of red blood cells from control animals in a buffered solution containing 0.36% of NaCl caused a 76.97±10.90% of hemolysis while during the incubation of cell of rats exposed to cadmium for 30 days resulted in 81.33±13.69% of hemolysis. Difference between these two values were not statistically significant.

DISCUSSION

Previous studies from our laboratory showed that exposure to 15 ppm cadmium Chloride containing drinking water for 30 days is sufficient to provide a reliable chronic cadmium toxicity in rats (9-12). Lack of significant changes in either daily food intake or water consumption of rats receiving 15 ppm Cd are comparable with the results of our previous studies (12) as well as to the mean body weight gain which displayed no significant difference in their groups. Numerous in vitro studies showed that cadmium has deleterious effects on cell function. The study of Nelson *et. al.* (6) who studied the effect of cadmium on phospholipid metabolism in cultured bovine pulmonary artery endothelial cells which are accepted as vulnerable to the toxic effect of this metallic ion showed that cadmium causes a profound time and dose dependent depletion of adenosine triphosphate levels in these cells as well as inhibits the activity of arachidonyl specific acyl coenzyme A synthesis. It is also reported that cadmium exposure results in disturbances in ATP driven Ca transport systems in intestinal epithelium. On the other hand the data of the present study showed that despite the exposure of rats to 15 ppm cadmium containing drinking water for 30 days which is sufficient to produce Cd toxicity and hypertension as shown previously, the same exposure time and dose of cadmium had no effect on the fragility of red blood cell membrane. However cadmium induced alterations in the phospholipids and protein content of the red blood cell membrane which are accepted normally as evidence of disturbed membrane fluidity were associated in this case with unaltered membrane fragility. A supportive finding for our results comes from the study of Garty *et. al.* (8) who exposed rat red blood cells to cadmium in vitro and found that cadmium uptake by the red blood cells occurs by passive transport and accumulation of this metal is not dependent on meta-

Figure 3: The effect of cadmium on red blood cell fragility.



bolically derived energy. Garty *et. al.* also could not observe any tendency to hemolysis or changes in K permeability in the membrane of cadmium exposed cells in their study. According to above mentioned evidence it was thought that the dissimilar cadmium transport mechanism between nucleated and red blood cell membranes can explain the different affect of cadmium toxicity on erythrocytes and the other nucleated cells of the body.

Zinc deficiency has been shown to inhibit Cd accumulation into hepatocyte (5) and addition of zinc reduces cadmium induced endothelial cell impairment (17). These effects of zinc were found to be concentration dependent (8). On the other hand it has been shown that zinc deficiency is also associated with increased hydroxyl production as well as decreased zinc and copper dependent SOD activity. In contrast to hepatocyte and endothelial cells, zinc has been found to enhance cadmium accumulation into red blood cells (8). In our study there was no obvious changes in blood SOD activity (data were not shown), but increased blood cadmium levels were associated with decreased zinc levels. Reciprocal changes of blood zinc with cadmium in cadmium exposed rats suggest the presence of disbalance between oxidative and antioxidative capacities in these animals and increased blood TBARS which is an indicator of lipid peroxidation due to increased free radicals was well in accordance with this defective antioxidant capacity.

As conclusion, our data suggested that unlike the other nucleated cells of the body, such as endothelial and hepatocytes, red blood cells seem less sensitive to cadmium induced lipid peroxidation despite a significant increase in TBARS levels.

When taken into consideration the vital importance of red blood cells in oxygen carrying capacity it is thought that this diminished sensitivity of red blood cell membrane against cadmium toxicity is a kind of protection and it explains why there is no obvious disturbance in red blood cell fragility and hemolysis during our studies.

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REFERENCES

1. Foulkes CE : Handbook of Experimental Pharmacology. Ed by CE Foulkes, Chapter 3, Springer Verlag, Berlin, Heidelberg, New York, Tokyo, Vol 80, 1986.

2. Hudecava A and Ginter E : The influence of ascorbic acid on lipid peroxidation in Guinea pigs intoxicated with cadmium. Food and Chemical Toxicology, 30:1011-1013, 1992.

3. Hussain T, Shukla GS and Chandra SV : Effects of cadmium on superoxide dismutase and lipid peroxidation in liver and kidney of growing rats: In vivo and in vitro studies. Pharmacology and Toxicology, 60: 355-358, 1987.

4. Manca D, Richard AC, Trottier B and Chevalier G : Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. Toxicology, 67: 303-323, 1991.

5. Meshitsuka SM, Ishizawa and Nose T : Uptake and toxic effects of heavy metal ions: Interactions among cadmium copper and zinc in culture cells. Experientia, 43: 151-156, 1987.

6. Nelson JM, Duane PG, Rice KL and Niewoehner DE : Cadmium ion induced alteration of Phospholipid Metabolism in Endothelial Cell Am J Respr Biol, 5: 328-36, 1991

7. Human Physiology : The mechanisms of body functions. Ed by AJ Vander, JH Sherman, DS Luciano, Mc Graw-Hill Inc, New York, St Louis, San Francisco, p 396, 1994.

8. Garty M, Bracken WM and Klaasen D : Cadmium Uptake by Rat Red Blood Cells. Toxicol, 42: 11-119.

9. Öner G, Izgüt-Uysal VN and Sentürk ÜK : Role of lipid peroxidation in cadmium induced impairment of the gastric mucosal barrier. Food and Chemical Toxicology, 32:799-804, 1994.

10. Öner G, Izgüt-Uysal VN and Sentürk ÜK : The susceptibility to stress induced gastric injury of rats exposed to cadmium. Biol Trace Elem Res, 47: 219-223, 1995.

11. Öner G, Izgüt-Uysal VN and Sentürk ÜK : The role of atrial natriuretic peptide in cadmium induced hypertension. J Trace Elm in Experiment Med, 8: 147-153, 1995.

12. Öner G, Izgüt-Uysal VN and Sentürk ÜK : Role of cadmium induced lipid peroxidation in kidney response to atrial natriuretic hormone. Nephron, 72: 257-262, 1996.

13. Drabkin DL and Austin JH : Spectrophotometric studies II. preparations from washed blood cells sulfhemoglobin and nitric oxide hemoglobin. J Biol Chem, pp 112-51, 1935.

14 Ann M, Markwell K and Haas SM : Protein determination by Lowry method. Methods in Enzymology, 72: 296-303, 1981.

15. Stocks J and Dormandy TL : The autoxidation of human red cell lipids induced by hydrogen peroxide. British Journal of Hematology, 20: 95-111, 1971.

16. Stocks J, Offerman EL, Modell CB and Dormandy TL : The susceptibility to autoxidation of human red cell lipids in health and disease. British Journal of Hematology, 23: 713-724, 1972.

17. Nocentini S : Inhibition of DNA replication and repair by cadmium mammalian cells protective interaction of zinc. Nucleic Acids, Res, 15: 4211-25, 1987.

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