The Effects of Cobalt Chloride on the Formation of Blood Lipid Peroxide related to Glutathione Peroxidase in the Erythrocytes of Rabbits

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INTRODUCTION

Heavy metals, such as zinc, have been shown to inhibit lipid peroxidation in rat liver tissue and red blood cells, whereas injections of large doses of iron has been shown to increase the formation of lipid peroxides in mice liver microsomes. Levin et al. have demonstrated that trace metals such as cobalt can inhibit the lipid peroxidation in vitro systems, preventing the loss of cytochrome p- 450 heme.

The major site of lipid peroxidative damage within cells are at the membranes. Mitochondrial and microsomal membranes as well as red blood cell membranes contain relatively large amounts of polyunsaturated fatty acids susceptible to lipid peroxidation in their phospholipids.

Glutathione peroxidase (GSH-Px), a selenium-containing enzyme, has been shown to decompose lipid peroxides, and to reduce them to the corresponding hydroxy acids. To reduce fatty acid hydroperoxides to hydroxy-fatty acids, it is necessary for GSH to be converted to oxidized glutathione (GSSG) which is catalyzed by GSH-Px. The GSH peroxidase-reductase system may act as an in vivo mechanism for the detoxification of lipid hydroperoxides.

The present study demonstrates that cobalt chloride acts to accelerate the in vivo formation of lipid peroxides in the blood of rabbits along with a slight tendency to hemolysis, but fails to alter GSH peroxidase activity and GSH concentrations in red blood cells.

MATERIALS AND METHODS

Male albino rabbits weighing 3~4 kg were used for all experiments. Eleven rabbits were injected subcutaneously with cobalt chloride at a level of 25 mg/kg/day for 3 days and were deprived of food after the first injection. Another eleven animals were injected with the same dose of saline for 3 days under the same deprivation condition.

Erythrocyte malondialdehyde (MDA) was measured by the method developed by Stocks et al. Serum MDA was measured according to a modification of the Yagi method. GSH-Px activity in erythrocytes was assayed by the coupled assay procedure of Paglia and Valentine. Blood glutathione was measured with the method of Beutler et al.

Slight hemolysis was represented by the measurement of hemoglobin concentration in plasma.

RESULTS

There was no significant difference in red blood cell, count, hematocrit and concentration of blood hemoglobin between cobalt-treated and control rabbits except for the plasma hemoglobin concentration, which increased by the treatment, suggesting the occurrence of
Table 1 The effects of CoCl₂ treated on the formation of MDA in plasma and erythrocytes, plasma lipid concentration, activity of erythrocytes GSH-Px and blood glutathione content

<table>
<thead>
<tr>
<th></th>
<th>Starved Control</th>
<th>CoCl₂-treated + Starved</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Erythrocyte MDA n mole/gHb</td>
<td>(11) 80.9±12.5</td>
<td>81.5±13.2</td>
</tr>
<tr>
<td>Plasma MDA n mole/ml</td>
<td>(11) 1.07±0.53</td>
<td>1.10±0.56</td>
</tr>
<tr>
<td>GSH-Px activity μmole NADPH/min/10⁹ cells</td>
<td>(11) 5.2±2.1</td>
<td>4.7±2.3</td>
</tr>
<tr>
<td>GSH mg % in the red cells</td>
<td>(8) 94.9±18.1</td>
<td>99.3±11.0</td>
</tr>
<tr>
<td>Plasma Hb mg/dl</td>
<td>(11) 28.8±10.6</td>
<td>22.1±9.4</td>
</tr>
<tr>
<td>Plasma triglyceride mg/dl</td>
<td>(11) 25±8</td>
<td>39±19*</td>
</tr>
<tr>
<td>Plasma cholesterol mg/dl</td>
<td>(11) 26±9</td>
<td>53±20**</td>
</tr>
</tbody>
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The values are the means±S.D. Values in parentheses are animal numbers used in the calculation
* p<0.05 compared with before treatment ** p<0.01 compared with before treatment

hemolysis (Table 1). Not only erythrocyte MDA, but also plasma MDA was elevated in cobalt-treated rabbits. The significant correlation (p<0.05) between erythrocytes and plasma MDA concentration signifies a common mechanism is involved in eliciting lipid peroxides. An eightfold increase in plasma triglyceride and a doubling or greater increase in plasma cholesterol were observed in cobalt-treated rabbits. The high correlation between concentrations of plasma MDA and plasma cholesterol (r=0.69, n=22), plasma MDA and plasma triglyceride (r=0.51, n=22) suggest that cobalt does promote the formation of both MDA and lipids, and releases them from the liver.

Cobalt chloride did not affect GSH-Px activity in red blood cells or blood glutathione content.

DISCUSSION

Chvapil et al.¹ found that high concentrations of dietary zinc (1,000 ppm) produced a significant decrease in lipid peroxide-linked hemolysis when compared to low concentrations (40 ppm). Cobalt ions (Co²⁺) have been shown to inhibit microsomal lipid peroxidation²-³. No experiments, however, have yet been made concerning the erythrocyte lipid peroxide formation in vivo relating to cobalt chloride treatment. The present study shows that cobalt chloride causes an increased formation of lipid peroxides in erythrocytes of rabbits associated with a slight increase in hemolysis.

The erythrocyte membrane is highly susceptible to oxidation owing to their polyunsaturated fatty acid content and to the fact that they are directly to exposed molecular oxygen⁴-⁵. To confirm whether cobalt treatment induces hemolysis or not, the following research is offered.

An increase of MDA concentration in plasma erythrocytes of rabbits treated with cobalt chloride suggests that cobalt acted directly on red blood cells or on liver cells to facilitate the formation of lipid peroxides. Plasma MDA is, like other plasma lipids, released from the liver⁶. An eightfold increase in the plasma concentration of triglyceride and a doubled cholesterol level were found in cobalt-treated rabbits. High correlations between plasma cholesterol and plasma MDA or plasma triglyceride and plasma MDA, suggest that cobalt chloride enhanced the synthesis of lipoprotein in the endoplasmic
reticulum in hepatocytes and accelerated its release with lipid peroxides from the liver.

Since Co$^{2+}$ acts to inhibit the MDA formation by liver microsomes from normal rats\textsuperscript{83}, such action conflicts with the results obtained that cobalt chloride accelerated the formation and consequently the release of lipid peroxides from the liver. Differences between in vitro and in vivo study may explain the discrepancy between our findings and those of former investigators.

The mechanism by which cobalt chloride promotes MDA formation in the erythrocytes was unclear and so, to attempt to clarify it, the enzyme GSH-Px, a selenium-containing enzyme, and GSH content in the red blood cells were examined.

GSH-Px catalyzes the conversion of GSH to GSSG while simultaneously reducing hydroperoxides of unsaturated fatty acids into hydroxy-fatty acids, suggesting that this enzyme is involved in the prevention of peroxidation in cells. GSH-Px is responsible for the detoxification of most lipid peroxides that may be formed in vivo, and it protects tissue components from the deleterious effects of peroxides. GSH-Px was shown to increase significantly in lungs of ozone-exposed rats\textsuperscript{14}, and in tissues of vitamin E-deficient rats, at which time lipid peroxides may appear\textsuperscript{15}. We assumed cobalt chloride would accelerate GSH-Px activity in the red blood cells of rabbits, because cobalt has been shown to increase MDA formation there.

Cohen and Hochstein\textsuperscript{16} evaluated the competition between erythrocyte catalase and glutathione peroxidase for their common substrate, H$_2$O$_2$, and concluded that catalase does not play an important role in protecting erythrocytes against endogenous H$_2$O$_2$, whereas GSH-Px under physiologic conditions linked to hexose shunt activity represents the major pathway of H$_2$O$_2$ metabolism in intact erythrocytes.

Cells genetically lacking this enzyme can be expected to allow increased susceptibility to lipid peroxidation damage. Necheles et al.\textsuperscript{17} reported that patients with GSH-Px deficiency, an autosomally inherited disorder, showed drug-induced hemolysis and neonatal jaundice, indicating that the GSH-Px portion of hexose monophosphate shunt represents the major defence of erythrocytes against oxidative damage.

Glutathione appears to play a role in maintaining the activity of sulfhydryl enzymes within the red cells, and in detoxifying small quantities of hydrogen peroxide\textsuperscript{18}. GSH is oxidized glutathione (GSSG) in GSH-Px reactions and GSSG can be reduced to GSH through mediation of the enzyme glutathione reductase with NADPH as the substrate.

Sasame and Boyd\textsuperscript{19} demonstrated that cobalt chloride caused a striking dose-dependent elevation of tissue levels of GSH associated with decreases in hepatic, pulmonary and renal cytochrome p-450.

It is generally believed that GSH plays a significant role in maintaining the structural integrity of the erythrocyte membrane. Prins et al.\textsuperscript{20} reported that glutathione deficiency is associated with a mild non-spherocytic hemolytic anemia.

GSH-Px has been suggested to act in the formation of hydroxy-fatty acids from peroxides accompanied by the reduction in the formation of MDA. However there have been no experiments to measure the formation of hydroxy-fatty acids. McCay et al.\textsuperscript{21} tested this and found that GSH-Px did not prevent MDA formation by converting peroxide to hydroxy-fatty acids, but rather may have acted to prevent the formation of peroxide from fatty acids.

Our results lead us to suggest that cobalt chloride accelerates lipid peroxide formation in erythrocytes and in plasma, but a clarification of the role that GSH-Px and GSH play remains unclear.
SUMMARY

The effects of cobalt chloride administration on the formation of lipid peroxide in the plasma and red blood cells of rabbits, and the alteration of the activity of glutathione peroxidase as well as glutathione content in the red blood cells were studied.

Cobalt chloride raised the concentration of the product of lipid peroxidation, malondialdehyde, not only in the red blood cell but also in plasma and caused a slight tendency towards hemolysis. High correlations were observed between the concentration of malondialdehyde and triglyceride and cholesterol in plasma. Neither the activity of glutathione peroxidase nor glutathione content in red cells was affected by cobalt chloride treatment.

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REFERENCES


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塩化コバルトによる家兎の血液過酸化脂質の形成,
血球グルタチオン・パーオキシダーゼの
活性と還元型グルタチオンに及ぼす影響について

塩化コバルトを家兎に3日間注射すると、赤血球と血漿の脂質酸化物マロンジアルデヒド（MDA）が増加し、
溶血の傾向がみられた。コバルト注射によって上昇した血漿MDAと血漿トリグリセライド、血漿コレステロール
の関には高い相関があり、血漿過酸化脂質は他の脂質と同様に肝臓由来することを示唆している。赤血球の
グルタチオンパーオキシダーゼの活性も赤血球の還元型グルタチオンの濃度もコバルト注射によって有意な変動
を示さなかった。

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