Anti oxidant activity of [6-chloro-2-(methylsulfanyl) pyrimidine-4-amine]cobalt(II) complex

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ABSTRACT

Sodium nitrite is widely used as color fixative and preservative in meat and fish. Impairment of hepatic function and disturbances in lipid metabolism are well recognized adverse effects of sodium nitrite. The aim of this study is to investigate the role of [6-chloro-2-(methylsulfanyl) pyrimidine-4-amine]cobalt(II) complex, in preventing the oxidation of hemoglobin to methemoglobin in vitro, and preventing the disturbances in lipid metabolism induced by sodium nitrite toxicity in mice. The cobalt(II) complex of pyrimidine derivative causes inhibit sodium nitrite-induced methemoglobin formation. Moreover the complex causes significant increase of total cholesterol, TG and LDL, while HDL significantly reduced.

Key words: Cobalt(II) complex of pyrimidine derivative, sodium nitrite, Cholesterol, Antioxidant

INTRODUCTION

In the early 1900s, irregular curing was common place. This led to further research surrounding the use of sodium nitrite as an additive in food, standardizing the amount present in foods to minimize the amount needed while maximizing its food additive role (1), and in medicine as antidote for cyanide poisoning (2). A principal concern about sodium nitrite is the formation of carcinogenic nitrosamines and nitric oxide via metabolites of sodium nitrite (3). About 1970, it was found that ascorbic acid (vitamin C), an antioxidant, inhibits nitrosamine formation (4). A moderate and significant acceleration of leukemia development was observed in sodium nitrite treated mice (5). Sodium Nitrite is an oxidative agent that causes methemoglobinemia in many species. Ruminants are especially vulnerable because the ruminal flora reduces nitrate to ammonia, while nitrite as an intermediate product is 10 times more toxic than nitrate. Acute intoxication is manifested primarily by methemoglobin formation and resultant anoxia (6). A major concern considering the toxicology of NaNO₂ is the induction of methemoglobinemia, a condition in which there is reduction in oxygen transport ability of hemoglobin (7). Various medicinal plants and vitamins protect liver from damage induce by sodium nitrite administration in mice (8,9). Moreover, some pyrimidine derivatives also possess hepatoprotective effect which could be attributed to their antioxidant activity (10). The present study was designed to investigate the role of cobalt(II) complex of (6-chloro-2-(methylsulfanyl) pyrimidine-4-amine) in preventing the reaction of NaNO₂ with hemoglobin and disturbances in lipid metabolism by sodium nitrite toxicity in mice.
MATERIALS AND METHODS

Nitrite-induce methemoglobinine in hemolysate:
Blood samples were collected from healthy volunteers, and centrifuged (2,500 g x 20 mins) remove plasma and the buffy coat of white cells. The erythrocytes obtained were washed thrice with phosphate- buffered saline. The washed cells were lysed by suspending in 20 vols of 20mM phosphate buffer, pH 7.4. The hemolysate was centrifuged at 25,000xg for 60 min to remove the membrane, and then diluted to give a 150 µM concentration of oxyhemoglobin. The reaction was initiated by the addition of sodium nitrite (final conc. 0.6 mM) to the solution of hemolysate and the formation of methemoglobin was measured by monitoring absorbance at 631 nm using a Shimadzu Graphicord UV 240 Spectrophotometer. Cobalt(II) complex of pyrimidine derivative was synthesized and characterized according to the method described by Wasfi et al (11), cobalt (II) complex dissolved in DSMO and injected in a dose of 0.2 mg/kg body weight corresponding to 1/20 of its LD₅₀(12), and added either before or at various time-interval after the addition of nitrite. The control experiments were conducted without cobalt(II) complex and all experiments were in triplicate and were repeated many times. The data given are for one set.

Antioxidant in vivo
Animal and experimental design:
Mice are divided into 3 groups (5 mice in each group) as following:
1. Control group: mice were injected daily intraperitoneal with 0.2 ml distilled water, for 15 days
2. NaNO₂ group: mice were injected daily intraperitoneal by 0.2ml of 2.1 mg/kg NaNO₂ for 15 days.
3. Group three which treated by NaNO₂, 0.2 ml of 2.1mg/kg, i.p then after one hour the animal injected by cobalt (II) complex of pyrimidine derivative, 0.2 ml of (1/20 LD₅₀) of the complex, daily for 15 days.

At the end of the experiment period (15 days), the mice were sacrificed under light chloroform anesthesia, a ‘Y’ shaped cut in the mice abdomen was done, blood was collected from posterior vena cava as it enters the right ventricle(13), blood samples was transferred into plain tubes and centrifugated at (3000 rpm for 15 minutes) to obtain the serum which stored at -4°C till used for measurement of different parameters.

Biochemical test:
Total cholestrol estimated by the enzymatic method described by Allian et al (14). Triglyceride (TG) was measured according to the method described by Fossati and principle (15). Associated with trainder reaction (16). HDL – cholestrol obtained in supernatant after precipitation of LDL chylomicrons from specimens by phosphotungstic acid (PTA) and mangesium chloride was measured with total cholesterol reagent (17). Serum LDL was calculated according to Friedewald formula (18).

Statistical analysis:
Statistical analysis was performed by a one-way ANOVA (followed by LSD test). Data were expressed as mean ± SDM. Statistical significant was set at p≤0.05.

RESULTS

In vitro study: Nitrite causes a rapid oxidation of hemoglobin to metheoglobin. In the presence of cobalt(II) complex of pyrimidine derivative, the oxidation process was delayed in a dose dependent manner, Fig. 1. The time required to convert 50% of the available hemoglobin to methemoglobin was 6.7 min. in the absence of the complex, whereas with 20 µM cobalt (II) complex present the time was increased to 18.2 min. (Fig. 1).

Fig.1. describe the effect of cobalt (II) complex on the time-course of nitrite oxidation of hemoglobin. Without complex, the time-course of oxidation show a characteristic pattern of slow initial transformation followed by a rapid autocatalytic process. When the complex was added along with nitrite at 0 min, the formation of methemoglobin was inhibited to great extent. However, when the cobalt (II) complex was added at the end of the autocatalytic stage, i.e at 10 mins, no protection was observed. The best result in our study at concentration 2.5 µM concentration of cobalt (II) complex which protect hemoglobin from oxidation by sodium nitrite, Fig. 1.
In vivo study:
The result in Table (1) revealed that HDL-cholesterol was significantly decreased while Total cholesterol, Triglyceride, LDL, significantly increased in NaNO₂ group compared with control group. These changes in lipid profile are reversed and their values become close to values in control group by injection of cobalt (II) complex.

Table (1): Effect of Cobalt (II) complex of pyrimidine derivative and NaNO₂ on lipid profile different letter denote the significant differences between groups (P ≤ 0.05)

<table>
<thead>
<tr>
<th>Group</th>
<th>TG(mg/dl)</th>
<th>HDL(mg/dl)</th>
<th>LDL(mg/dl)</th>
<th>T.Cholesterole(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>212.60 ± 22.3b</td>
<td>53.60 ± 5.1a</td>
<td>53.88 ± 5.10b</td>
<td>150.00 ± 3.80b</td>
</tr>
<tr>
<td>NaNO₂</td>
<td>305.00 ± 25.00a</td>
<td>41.20 ± 1.30b</td>
<td>69.60 ± 7.47a</td>
<td>171.80 ± 11.12 a</td>
</tr>
<tr>
<td>Cobalt (II) complex with NaNO₂</td>
<td>203.08 ± 18.50b</td>
<td>56.80 ± 5.10a</td>
<td>56.84 ± 5.60b</td>
<td>154.40 ± 4.80b</td>
</tr>
<tr>
<td>LSD</td>
<td>92.40</td>
<td>17.40</td>
<td>12.40</td>
<td>12.76</td>
</tr>
</tbody>
</table>

DISCUSSION

Since the cobalt (II) complex of pyrimidine derivative is newly prepared by Wasfi  et al (11), with LD₅₀ (192.8 mg/kg), and significantly anti bacterial activity therefore the experiments focused to determine its antioxidant activity. The present study has shown that cobalt(II) complex pyrimidine derivative can protect hemoglobin from oxidation by sodium nitrite both in hemolysate and in intact erythrocytes. However, it did not to reverse the effect of sodium nitrite if added at a later stage. It is well established that oxidation of hemoglobin takes place in two stages. There is slow initial stage followed by a rapid autocatalytic stage, which carries the reaction to completion (19). Cobalt (II) complex is able to prevent the onset of autocatalytic stage. Since superoxide is implicated in autocatalytic stage (19) and the fact that cobalt (II) complex is a potent scavenger of superoxide (20).

Direct interaction between sodium nitrite and cobalt(II) complex pyrimidine derivative as a reason for protection is ruled out because the concentration of cobalt complex causing protection is very low (< 10µM) compared to the nitrite concentration (0.6mM). This result was disagreement with that Doyle et al (21) who used curcumin as antioxidant and recorded a 20µM. Although cobalt complex pyrimidine derivative can reduce ferric ions Fe³⁺ to the ferrous state Fe²⁺, it fails to reverse the oxidation of hemoglobin, suggesting that protection is not due to reduction of methemoglobin to hemoglobin. Many antioxidants like ascorbic acid, uric acid, 3-ribozyl uric acid, and glutathione protect hemoglobin from oxidation by nitrite (22). These antioxidants also inhibit the onset of the autocatalytic stage of nitrite if added at a later stage (22). Thus, the effect of cobalt(II) complex pyrimidine derivative may be similar to these antioxidants in protecting hemoglobin from nitrite ions.

The best result in our study at (2.5 µM) concentration which protect hemoglobin from oxidation by sodium nitrite (Fig.1 ).
Antioxidant *in vivo.*

Table 1 demonstrated the effect of cobalt (II) complex of pyrimidine derivative and NaNO₂ on lipid profile of mice after two weeks of treatment. The results demonstrated that NaNO₂ group showed significant increase \((P \leq 0.05)\) in total cholesterol, triglyceride and LDL levels while HDL show significant decrease \((P \leq 0.05)\) compared with control group. The results of treated groups shows significant decrease \((P \leq 0.05)\) in total cholesterol, triglyceride, LDL and HDL levels compared with NaNO₂ groups. The results of this study are agreement with the results of Sidoriak and Volgin (8). The changes in lipid profile in this study may be attributed to peroxidation of cell membrane lipids and mobilization of free fatty acids from the adipose tissue to the blood stream resulted from nitrite induced oxidative stress and free radical generation. This hypolipidemic effect of cobalt (II) complex of pyrimidine derivative could be related to its antioxidant and free radical scavenging activity.

REFERENCES


