Blood rheological changes in rodents treated with metal salts

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Abstract

The rheological properties of blood are an important marker of changes that occur in various pathological conditions. Changes in the rheological properties of blood and the factors that determine them: number and size of blood cells, their membrane and morphological properties, serve as an indicator for early detection of many diseases. The aim of this study is to investigate the influence on the blood rheological properties of some metal compounds: cadmium acetate [Cd(CH\textsubscript{3}COO)\textsubscript{2}x2H\textsubscript{2}O], cobalt chloride (CoCl\textsubscript{2} x 6H\textsubscript{2}O) and sodium nitrite (NaNO\textsubscript{2}), which are known to be present in the environment or daily absorbed by the body from the atmosphere or food. Plasma viscosity of rats after treatment with NaNO\textsubscript{2} is measured too. Disturbances in the rheological properties, blood and plasma viscosity of the treated rodents are observed in comparison with a control group of non-treated rodents, depending on the type, concentration of metal salts, the animal species and the type treatment. The observed blood and plasma viscosity changes have been confirmed by morphological studies of the same blood samples, performed simultaneously.

Keywords: Rodents, blood and plasma viscosity, metal salts

1. Introduction

The results of the study of comparative mammalian haemorheology show remarkable variation in the rheological properties of the blood of the mammalian species [1-3]. Each species has its own rheological fingerprint. The physiological significance of these variations in different mammalian species is not entirely clear at present [3].

It is well known that environmental contamination increase as a result of all human activities – processes of production, transportation, feeding, etc. This fact leads to life condition changes and increase of negative factors for healthy human environment. The emission of metals and metal salts in the atmosphere results in their accumulation in the living organism and this directly affects people’s health. This is the reason for our interest in investigation the changes in rheological properties of blood in rodents as a result of metal salts intake.

Hameed Bataineh et al. [4] demonstrate that the short-term exposure of female rats to industrial metal salts during the early stages of gestation would cause failure of pregnancy and produce fetotoxic or fetal resorptive potentials. In an another paper the same authors assess aggression, sexual behavior and fertility in adult male rats following long-term ingestion of four industrial metals salts - manganese sulfate, aluminum chloride, lead acetate and copper chloride. Their results show that sexual behavior was suppressed, male rat aggression was also abolished. Data suggest that the long-term ingestion of manganese sulfate, aluminum chloride, lead acetate and copper chloride would have adverse effects on sexual behavior, territorial aggression, fertility and the reproductive system of the adult male rats [5].

Cobalt chloride (CoCl\textsubscript{2}) is very toxic to aquatic organisms and may cause long term adverse effects in the environment. Its role has also been elucidated in cardiovascular and excretory systems and angiogenesis [6]. Data show that CoCl\textsubscript{2} is used by athletes for stimulating erythropoietin production and as micro additives in animal
feed [7]. In animals, tumor genesis is detected after inhalation, muscle and/or subcutaneous injections. Treatment of rats and mice with CoCl₂ protects heart and kidneys from ischemic disturbances [8]. Inhalation of cobalt chloride can result in asthma-like breathing problems, persistent breathing problems like pulmonary fibrosis. Frequent contact with this compound may cause skin irritation, rashes, skin sensitivity. Ingestion of the salt can result in nausea, vomiting, cardiomyopathy, thyroid problems, nerve problems, thickening of the blood.

Cadmium (Cd) is a toxic environmental pollutant, ranked the 7th toxicant in the Priority List of Hazardous Substances of the Agency for Toxic Substances and Disease Registry. Food, drinking water and inhalation of smoke from tobacco products are the main sources of daily exposure to Cd thus oral administration seems to be the most appropriate in long-term experiment of cadmium as it enters the animal/human body through food and water. It accumulates in living organisms causing severe DNA damage, renal and hepatic dysfunction, anemia, diabetes, peripheral artery disease, myocardial infarction, lung, prostate, pancreas, breast, kidney cancer, etc [9].

The main use of sodium nitrite (NaNO₂) is for the industrial production of organonitrogen compounds. It converts amines into diazo compounds, which are key precursors to many dyes, such as diazo dyes. NaNO₂ can be used as part of an intravenous mixture to treat cyanide poisoning. However it has now been made obsolete by hydroxocobalamin if this newer medicine is available. There is also research investigating its applicability towards treatment of heart attacks, brain aneurysms, pulmonary hypertension in infants, and Pseudomonas aeruginosa infections. Sodium nitrite is known to inhibit the growth of disease causing microorganisms, to give taste and color to meat and to inhibit lipid oxidation that leads to rancidity [10]. The ability of NaNO₂ to address the above mentioned issues has led to the production of meat with improved food safety, extended life storage and desirable color and taste [10]. In the European Union it may be used only as a mixture with salt containing at most 0.6% sodium nitrite. It has the E number of E250.

In our previous studies hematological changes in cases of chronic cadmium intoxication and monensin detoxication and their relationship with rheological variables were investigated in [9]. Influence of temperature incubation and CoCl₂ concentration on blood hemorheological properties and erythrocyte morphological parameters was shown in [6]. Cobalt(II)-induced changes in hemoglobin content and iron concentration in mice from different age groups are reported in [11]. In spite of the above mentioned results the literature data are insufficient about the influence of metal salts on the blood rheological properties. Our recent investigations and the aim of this work is to provide a more comprehensive study on the influence of metal salts CoCl₂, Cadmium acetate and NaNO₂ on hemorheological properties of rodents.

2. Materials and methods

Our experimental model includes rodents (rats and mice) treated in vivo with metal salts of Co, Cd and Na. Blood viscosity changes of the samples from the treated and control animals have been evaluated. Different experimental models of rodents’ treatment with metals were applied:

- Cobalt chloride (CoCl₂x6H₂O) influence was estimated on two 60 days old mice subjected to daily dose of 125 mg/kg b.w. until day 60 of the newborn pups. Cobalt (II) was obtained from drinking tap water. Animals were fed a standard diet and had access to food ad libitum. The experimental animals were weighed weekly and the experimental cobalt concentration was adjusted accordingly. Mice drinking distilled water served as a control group (n=8, Hct=44.2±2.6%).

- Cadmium acetate [Cd(CH₃COO)₂x2H₂O] influence was estimated on six adult male mice subjected to subacute daily treatment with 20 mg/kg b.w. for 14 days. Mice drinking distilled water served as a control group (n=8).

- Adult male Wistar rats (n=33) were given single intraperitoneal NaNO₂ injection of 50 mg/kg body weight and after this blood samples were prepared. At different time periods (1h, 5h, 24h, 48h, days 5, 10 and 20) the examined animals were sacrificed and blood and plasma samples were prepared in relation with examination time period. Each experimental group included 5 animals (group 1h, n=5, Hct=24.3±3.81%; group 5h, n=5, Hct=31.5±1.82%; group 24h, n=4, Hct=28.75±4.19%; group 48h, n=5, Hct=31.8±2.59%; group 5d, n=5, Hct=32.6±2.96%; group 10d, n=4, Hct=35.25±4.11%; group 20d, n=5, Hct=32.8±1.48%). The control group included five animals as well with Hct=34.4±2.6%.

Whole blood viscosity (WBV) and plasma viscosity of the experimental rodents was measured with the
rotational viscometer LS30 Contraves at eleven different shear rates ranging from 0.0596 s\(^{-1}\) to 94.5 s\(^{-1}\) at 37 °C.

For plasma viscosity measurements blood is collected in heparinized tubes and centrifuged for 20 min at 2500 rpm in Hereus centrifuge. All measured samples are with native hematocrit and this parameter was determined by microhematocrit centrifuge with microhematocrit tubes for 5 min at 12 000 rpm.

Hematological parameters were measured on automated hematological analyzer BC-2800Vet (Mindray, China).

For real analysis of WBV changes for rats group, treated with NaNO\(_3\) we standardized the native Hct values to reference hematocrit of 40%. This calculation was performed according to Weaver equation [12]:

\[
\log_{10} WBV = \log_{10} WBV_0 + kHct(\%),
\]

where \(\log_{10} WBV_0\) is the intercept when \(Hct \to 0\) and \(k = 0.03 - 0.076 \log_{10} \), i.e. \(k\) introduces the shear rate \(\gamma\) dependence.

3. Results

The results show that the apparent blood viscosity of two 60 days old mice (\(n=2\)) following CoCl\(_2\) administration is lower in comparison with the control group (mean Hct=44.16±2.56%) (Fig. 1) at shear rates – 0.1102 s\(^{-1}\) n 0.0596 s\(^{-1}\) (with statistical significance \(p=0.0302\), \(p=0.001\) respectively). Statistical significant differences obtained by unpaired Student’s t test are shown in Table 1.

![Graph](image)

Fig.1. Whole blood apparent viscosity-shear rate dependence after CoCl\(_2\) treatment of two 60 days old mice, controls (mice drinking tap water) - Hct=44.2±2.6%, \((n=8)\).

In our previous work [6] we have found that \textit{in vitro} incubation with different doses of CoCl\(_2\) and at various temperatures affect hemorheological properties and erythrocyte morphology of human RBC suspensions in phosphate buffered saline (PBS). The results with normal human RBC suspensions in PBS shown increased apparent viscosity [6] which is dependent on the concentration and temperature of incubation in CoCl\(_2\). Biochemical analysis of mouse blood samples show that hemoglobin content was increased in a time-dependent manner in mature mice (day 45 to day 90), while it was reduced in immature mice (day 18 to day 30) [11]. We also found increased Co (II) concentration in plasma and changes of plasma Fe concentration. Results indicate that the solubility of the compounds is an important factor for bioaccumulation in blood plasma and in the organs respectively [11].

WBV of six adult male mice subjected to subacute daily treatment with 20 mg/kg b.w. for 14 days was significantly elevated (Fig. 2) in the treated with cadmium acetate (Cd(CH\(_2\)COO)\(_2\)\(x\)H\(_2\)O) animal group at shear rates lower than 2.37 s\(^{-1}\) [9] (Table 1). The increased WBV is found in parallel with increased hematocrit Hct and changes in the morphological properties of blood cells as increased RDW (red blood cell distribution width, which is a measure of the variation of red blood cell width that is reported as part of a standard complete blood count), RBCs (red blood cell count), reduced MCV (mean cell volume is a measure of the average red blood cell
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size that is reported as part of a standard complete blood count) and reduced hemoglobin Hb [11].

Table 1
Probability density function (p) obtained with Student’s t test for whole blood (WBV) and plasma viscosity between controls and groups after CoCl₂, Cadmium acetate and NaNO₂ treatment

<table>
<thead>
<tr>
<th>Shear rates, s⁻¹</th>
<th>p, CoCl₂ (WBV)</th>
<th>p, Cadmium acetate (WBV)</th>
<th>p, NaNO₂ (WBV)</th>
<th>p, plasma viscosity, NaNO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>94.5</td>
<td>0.158</td>
<td>0.054</td>
<td>---</td>
<td>1d: p=0.013; 5d:p=0.013, 10d:p=0.004, 20d:p=0.017 *</td>
</tr>
<tr>
<td>51.2</td>
<td>0.155</td>
<td>0.076</td>
<td>---</td>
<td>5d: p=0.02; 10d:p=0.026 *</td>
</tr>
<tr>
<td>20.4</td>
<td>0.117</td>
<td>0.074</td>
<td>---</td>
<td>10d:p=0.039 *</td>
</tr>
<tr>
<td>11.02</td>
<td>0.123</td>
<td>0.093</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>5.96</td>
<td>0.154</td>
<td>0.068</td>
<td>---</td>
<td>5d:p=0.033 *</td>
</tr>
<tr>
<td>2.37</td>
<td>0.101</td>
<td>0.051</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1.285</td>
<td>0.349</td>
<td>0.029 *</td>
<td>---</td>
<td>2d: p=0.052; 5d:p=0.02 *</td>
</tr>
<tr>
<td>0.512</td>
<td>0.324</td>
<td>0.013 *</td>
<td>---</td>
<td>2d: p=0.004 **</td>
</tr>
<tr>
<td>0.277</td>
<td>0.064</td>
<td>0.022 *</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>0.1102</td>
<td>0.03 *</td>
<td>0.014 *</td>
<td>5d, 0.018 *</td>
<td>---</td>
</tr>
<tr>
<td>0.0596</td>
<td>0.001 **</td>
<td>0.013 *</td>
<td>5d, 0.045 *</td>
<td>---</td>
</tr>
</tbody>
</table>

*p≤0.05, **p≤0.005

Significant decreased hemoglobin content Hb (p<0.001) following Cd administration was found in [11].

Fig.2. Whole blood apparent viscosity-shear rate dependence in six adult male mice treated with cadmium acetate [Cd(CH₃COO)₂·2H₂O], controls - Hct=44.2±2.6% (n=8), for blood samples treated with cadmium acetate Hct is not measured.

Blood and plasma viscosity data of adult male Wistar rats (n=33), treated intraperitoneally by NaNO₂ injection of 50 mg/kg body weight, are shown on Fig. 3a and Fig.3b. The statistical analysis by Student’s t test shows that significant statistical differences (p≤0.05) exist between plasma viscosity of controls and treated with NaNO₂ group data (Table 1). The statistical differences for plasma viscosity were found in all treated groups (1,5,10 and 20 days after treatment with NaNO₂), but not for all shear rates (Table 1).

Simultaneously with our rheological test we made morphological analysis of blood samples and obtained decrease of the parameters RDW and MCV, increase of MCHC (mean corpuscular hemoglobin concentration, a measure of the concentration of hemoglobin in a given volume of packed red blood cells) as published in
This observation shows that NaNO₂ affects erythrocyte morphology inducing alterations in the parameters related to the hemoglobin content. This could be attributed to the fact that NaNO₂ reacts with hemoglobin to form methemoglobin. Methemoglobin is formed when the heme iron of unoxygtenated hemoglobin is oxidized to the ferric (Fe³⁺) state. In the erythrocyte, equilibrium normally exists between hemoglobin and methemoglobin in relation to the capture and release of oxygen. Methemoglobinemia occurs when the rate of formation of methemoglobin exceeds the rate of reduction as a result of exposure to the nitrite, and seems a relevant reason for the decreased blood viscosity. Moreover, sodium is known to play role in the maintenance of blood viscosity: the interaction between Na atoms and globulin proteins present in the blood leads to the formation of water soluble salts. Salt formation prevents these globulins to increase blood viscosity. In accordance with these considerations, plasma viscosity was also reduced in NaNO₂-treated animals (Fig. 3 b). Further studies are required to elucidate the possible changes in plasma protein content responsible for the reduced plasma viscosity.

![Fig.3a. Whole blood apparent viscosity-shear rate dependence in adult rats after intraperitoneal NaNO₂ injection, Control group included five rats, Hct=34.4±2.6%, T=37ºC.](image)

![Fig.3b. Plasma viscosity-shear rate dependence in adult rats after intraperitoneal NaNO₂ injection, Control group included three rats, T=37ºC.](image)

Data for the hematocrit changes measured on days 1, 2, 5, 10 and 20 following administration of NaNO₂ injection are shown on Fig. 4. The statistical significant difference between hematocrit changes of controls and treated groups (p≤0.05) was found only on the first day after injection. With time hematocrit recovers to the control value (in samples from day 10 following the compound’s administration we measured hematocrit almost equal to the controls). We can conclude that the biggest influence of NaNO₂ is within the first 24 hours following the administration.
Fig. 4. Time variation of hematocrit in adult rats after intraperitoneal NaNO₂ injection and in control group, (n=5).

Fig. 5. Time variation of the mean apparent blood viscosity, adjusted to reference value Hct=40%, by the formula of Weaver et al. (1), at 94.5 s⁻¹ in adult rats after intraperitoneal NaNO₂ injection and in controls, T=37°C.

Fig. 6. Time variation of the mean plasma viscosity at 0.1102 s⁻¹ in adult rats after NaNO₂ injection and in controls, T=37°C.

The comparison between WBV of control groups with adjusted hematocrit by the formula of Weaver (Hct=40%) and similar control groups in [3] are shown in Table 2 and Fig. 5. The obtained statistical difference of p≤0.05 (Student’s t-test) between adjusted WBV of Controls and treated animals was significant at the highest shear rate 94.5 s⁻¹ (Fig. 5). Student’s t-test shows no statistical difference for plasma viscosity even though the maximal change of this viscosity was obtained 24 hours following NaNO₂ administration (Fig. 6).
Table 2
Whole blood viscosity (standardized blood samples to Hct=40%) in Wistar rats after NaNO2 injection and in controls, reference literature data [3], T=37°C, Contraves LS30.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Age</th>
<th>WBV(0.512 s⁻¹) Mean ± s.d.</th>
<th>WBV (2.37 s⁻¹) Mean ± s.d.</th>
<th>WBV (94.5 s⁻¹) Mean ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats (n=3) Controls</td>
<td>4 months</td>
<td>17.60±1.91</td>
<td>12.91±0.97</td>
<td>4.62±0.08</td>
</tr>
<tr>
<td>1⁰ day after injection</td>
<td>4 months</td>
<td>15.40±1.90</td>
<td>13.47±0.00</td>
<td><strong>4.35±0.08</strong>*</td>
</tr>
<tr>
<td>2⁰ day after injection</td>
<td>4 months</td>
<td><strong>13.20±0.00</strong>*</td>
<td>13.47±0.00</td>
<td>4.46±0.14</td>
</tr>
<tr>
<td>5⁰ day after injection</td>
<td>4 months</td>
<td>15.68±1.38</td>
<td>13.19±0.69</td>
<td><strong>4.33±0.15</strong>*</td>
</tr>
<tr>
<td>10⁰ day after injection</td>
<td>4 months</td>
<td>18.29±2.16</td>
<td>14.14±1.51</td>
<td><strong>4.21±0.14</strong>*</td>
</tr>
<tr>
<td>20⁰ day after injection</td>
<td>4 months</td>
<td>18.15±1.91</td>
<td>13.47±0.00</td>
<td><strong>4.43±0.07</strong>*</td>
</tr>
<tr>
<td>Rats (n=40) according [3], 9 male, 31 female; Mean (25%/75%)</td>
<td>4 – 6 months</td>
<td>0.7 s⁻¹</td>
<td>2.4 s⁻¹</td>
<td>94.5 s⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35.400(26.33/40.9)</td>
<td>19.748(16.04/22.)</td>
<td>6.288(5.883/6.92)</td>
</tr>
<tr>
<td>Mice (n=40) according [3], BALB/c, 7 male, 33 female; Mean (25%/75%)</td>
<td>4 – 8 months</td>
<td>13.376(10.69/16.5)</td>
<td>10.563(8.929/12.)</td>
<td>4.879(4.51/5.34)</td>
</tr>
</tbody>
</table>

* p≤0.05

The comparison of the mean controls’ whole blood viscosity data of standardized blood samples to Hct=40% by the formula (1) of Weaver et al. [12] in Wistar rats (n=3) with the reference literature data [3] at 37°C, measured by Contraves LS30 are shown in Table 2. The differences observed in our data with the reference literature data from [3] can be explained by the differences in the groups number of animals, sex, anticoagulant, as well as method of sample preparation and adjusting their hematocrit to 40% by the formula or by centrifugation. The control group, used in the study of Windberger et al. [3] included 40 Wistar rats (9 male, 31 female; age, 4–6 months) anaesthetized with 100 mg kg⁻¹ ketamine and 1 mg kg⁻¹ xylazine intraperitoneally before blood was taken by puncture of the right ventricle and collected in K-EDTA-containing tube.

4. Conclusion

Our preliminary results show that blood or plasma viscosity alterations were observed in all measured blood samples of rodents treated with used metal salts (Fig. 1 – Fig. 3): cobalt chloride decreases WBV and increases Hb content. The use of cobalt (II) has a significant impact on hemoglobin biosynthesis possibly due to its effect on iron metabolism [11]. Chronic exposure to Cd induces changes in hematological parameters and leads to elevated WBV. Cadmium treatment reduced RBC parameters and increased RDW. There were no significant differences found between whole blood viscosity of controls and treated with NaNO2 rats, but significant difference (p<0.05) were found between plasma viscosity data of controls and treated with NaNO2 rats. In conclusion we can summarize that the metal salt treatments influence the blood or plasma viscosity of treated animal’s blood samples.

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