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In Vitro Effect of Temperature and Cobalt Chloride Treatment on Human Red Blood Cells' Morphology and Indices

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Changes of erythrocyte morphology induced by exposure of blood to cobalt chloride ($CoCl_2$) and/or various degrees of temperature are poorly studied. The aim of the study was to investigate the *in vitro* effect of $CoCl_2$ on erythrocyte morphology and indices of red blood cell suspensions after incubation at different temperatures. Human erythrocyte (RBC) suspensions were treated with 50 μM or 500 μM $CoCl_2$ and incubated for one hour at 4 ºC, room temperature and 40 ºC. After incubation the morphological changes were studied. Morphological studies showed RBC aggregates at 4 ºC while elyptocytes were mainly observed at 40 ºC. Thermal treatment induced anisocytosis leading to increased RDW and decreased MCV. The results indicate that both $CoCl_2$ and temperature affect erythrocyte morphology and indices of RBC suspensions possibly by inducing structural, biomechanical and biochemical changes in the erythrocyte membrane.

Key words: RBC suspensions, *in vitro* treatment, cobalt chloride, erythrocyte morphology, temperature incubation.

Introduction

Cobalt chloride ($CoCl_2$) is a water soluble hypoxia-mimicking agent shown to improve hematological parameters [1]. Recent data show that athletes use the compound to stimulate endogenous erythropoietin production, thus erythropoiesis [4].

Temperature, on the other hand, induces changes in cell membrane composition and permeability. The literature data suggest that it affects primarily the membrane protein component and not the lipids [3]. No significant morphological changes are observed when erythrocytes are exposed *in vitro* to 42 ºC [2].

Changes in the hematological parameters induced by exposure of blood to varying degrees of temperature are poorly studied. Alterations in red blood cell (RBC) properties – morphology and viscoelasticity is one of the basic indicators for human health, disease diagnosis, treatment, etc [6].

The aim of the present study was to investigate the *in vitro* effect of $CoCl_2$ treatment on erythrocyte morphology and some erythrocyte indices of RBC suspensions

after incubation at different temperatures.

Materials and Methods

Human erythrocytes (RBC) from a healthy donor, obtained from the National Center for Hematology and Transfusiology in Sofia, Bulgaria were washed in 0.9% NaCl, hematocrit was adjusted to 40% and treated with 50 μM or 500 μM $CoCl_2 \times 6H_2O$ (Reahim). The RBC suspensions (n = 5) were incubated for one hour at 4 ºC, room temperature and/or 40 ºC. The morphological changes were studied on blood smears stained with May-Grünwald-Giemsa (Merck, Germany) and observed on a light microscope Leica DM 5000B (Leica Microsystems, USA). Erythrocyte indices such as hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red blood cell distribution width (RDW) were obtained using automated hematological analyzer BC-2800Vet (Mindray, China). Changes were compared against control samples of RBC suspensions (n = 5) incubated at the same temperatures.

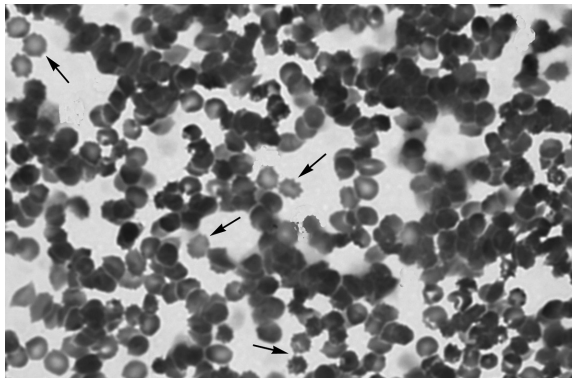
Statistical Analysis

The obtained results are presented as mean value ± SD. Statistical significance between the experimental groups was determined using Student's *t*-test. Difference was considered significant at $p < 0.05$.

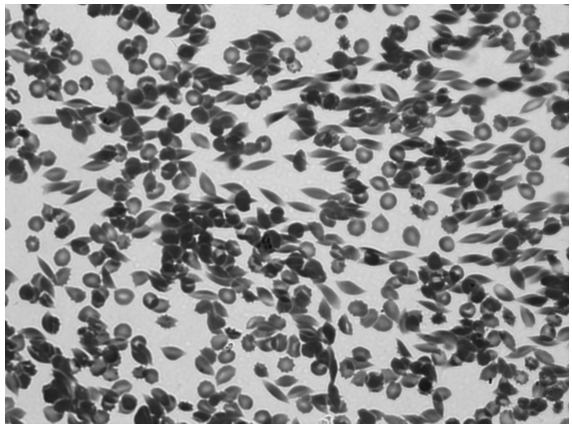
Results and Discussion

Preliminary results showed that RBC morphological alterations were detected when different $CoCl_2$ concentrations were added. Thermal treatment induced anisocytosis leading to increased RDW and decreased MCV. The highest RDW values were measured at 40 ºC. Incubation at room temperature or 40 ºC decreased MCH and MCHC ($p < 0.03$). Addition of $CoCl_2$ to the RBC suspensions increased insignificantly both parameters when the samples were incubated at 4 ºC. On the other hand, at room temperature and at 40 ºC MCH and MCHC decreased significantly in the presence of the cobalt compound compared to the samples incubated at 4 ºC with 50 and/or 500 μM $CoCl_2$. The results suggest a relationship between cobalt and hemoglobin content which is temperature dependent. Changes in MCH and MCHC could be explained by the results of Simonsen et al. [5] demonstrating that ^{57}Co co-migrates with hemoglobin when added *in vitro* to a lysate of unlabeled cells or to a solution of purified hemoglobin. Addition of $CoCl_2$ increased MCV in samples incubated at 4 ºC or 40 ºC compared to controls. The parameter though was significantly decreased when samples treated with the compound but incubated at different temperatures were compared. Morphological studies showed RBC aggregates (**Fig. 1a**) and echinocytes at 4 ºC while elyptocytes were mainly observed at 40 ºC (**Fig. 1b, c**). Incubation at different temperatures and treatment with $CoCl_2$ did not affect Hct values of RBC suspensions compared to the control samples. Dose-dependent effect was not observed.

According to the literature data changes in hematological parameters and erythrocyte morphology are found when blood is exposed to 50 ºC [2]. These results indicate significant stability of the RBC membrane at high temperature. Incubation of RBC with $CoCl_2$ leads to accumulation of cobalt in the cells in a time-dependent manner [5]. The



(a)



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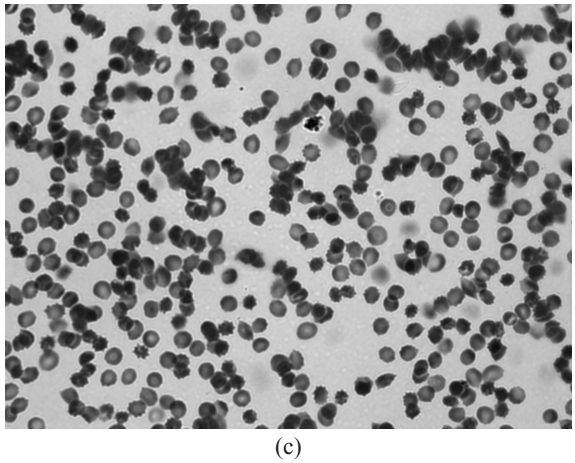

(c)

Fig. 1. RBC smears stained with May-Grünwald-Giemsa, × 200. RBC aggregates and echinocytes (arrow) are observed after incubation at 4 °C (a); Eliptocytes are found mainly in control smears incubated at 40 °C (b); Individual echinocytes are observed in smears treated with $CoCl_2$ (c)

temperature treatment possibly affects cobalt ion influx in the cells. Cobalt ion concentration up to 0.1 mM is regarded as non-cytotoxic. Incubation of RBC suspensions with 500 μM $CoCl_2$ for one hour in our experiment possibly is not long enough to induce apoptotic changes. Therefore, significant changes in erythrocyte morphology are not expected. The effect in this case would be mainly due to heat treatment. The experimental data suggest that the temperature of the solution is an important factor and should be considered when applying $CoCl_2$ treatment.

Conclusions

The results indicate that both $CoCl_2$ and temperature affect erythrocyte morphology and indices of RBC suspensions possibly by inducing structural, biomechanical and biochemical changes in the erythrocyte membrane.

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