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Differences Between Cation-Osmotic Hemolysis and Filterability in Exaprolol- and Glutaraldehyde-Treated Human Red Blood Cells

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Summary

The changes in human red blood cell microrheology in different glutaraldehyde (3.0 and 5.0 x 10^{-6} mol.l^{-1}) and exaprolol (2.5 and 5.0 x 10^{-4} mol.l^{-1}) concentrations were studied. The method of millipore filtration was compared with the method of cation-osmotic hemolysis. Both drugs prolonged the filtration time. Cation-osmotic hemolysis in glutaraldehyde-treated cells was significantly lower in comparison with the control group. On the other hand, there was a significant increase in cation-osmotic hemolysis in exaprolol-treated cells. Besides cation-osmotic hemolysis and filterability of erythrocytes, we evaluated the medium cell volume (MCV) and the medium cell hemoglobin concentration (MCHC). No changes in MCV and MCHC in glutaraldehyde-treated cells were observed. However, the MCV was significantly lower and the MCHC was significantly higher in exaprolol-treated cells. In conclusion, we suggest that the method of cation-osmotic hemolysis is more sensitive than the filtration method for determination of red blood cell microrheology.

Key words
Exaprolol • Glutaraldehyde • Erythrocytes • Filtration • Cation-osmotic hemolysis

Introduction

Red blood cell (RBC) deformability was defined as the cell ability to undergo deformation during flow in the microcirculation (Mohandas *et al.* 1983). There are three factors influencing erythrocyte deformability: large surface area-to-volume ratio, viscosity of the intracellular hemoglobin solution and viscoelastic properties of the membrane (Mokken *et al.* 1992). It was observed that changes in erythrocyte deformability often resulted from metabolic or other disorders. The hemorheological effect of several drugs and chemical substances has repeatedly been reviewed (Lowe 1984, Rhoads *et al.* 1985, Schneider 1989, Hayakawa 1992, Bilto and Abdala 1998).

Exaprolol, 1-(2-cyclohexyl[2,4-(3) H] phenoxy-3-isopropylamino-2-propanol was found to display a potent β-adrenergic blocker. From the biophysical point of view, the enhanced liposolubility and membrane fluidization are characteristic features. The membrane fluidization effect of exaprolol was described on isolated rat mast cells (Nosál *et al.* 1989). Preclinical observations

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were focused on pharmacokinetics and cardiovascular studies of exaprolol (Trnovec *et al.* 1982, Hughes *et al.* 1984).

On the other hand, glutaraldehyde was described as an artificially hardening factor which reduces RBC deformability (Arevalo *et al.* 1992).

A number of methods were developed to evaluate erythrocyte deformability. The filtration method using positive or negative pressures has become to be the most widely used method (Reid *et al.* 1976).

Few years ago, we developed the method of cation-osmotic hemolysis (Nicák and Mojžiš 1992, Mojžiš and Nicák 1993). Subsequently, we have proved that the membrane deformability and cation-osmotic hemolysis (COH) are closely related (Mirossay *et al.* 1997). On the basis of these results and our previous experiences, we suggest that cation-osmotic hemolysis provides basic information about erythrocyte deformability.

Material and Methods

The blood used for the *in vitro* experiments was obtained in a group of 50 healthy donors aged 20-48 years. Blood was withdrawn in our Transfusion Center according to the rules of The International Hemorheological Committee (ICSH Expert Panel on Blood Rheology 1986). Heparin was used as an anticoagulant.

Fifteen microliters of blood were added into 3 ml of the incubating medium, which contained different concentrations of NaCl and glucose. The concentrations of NaCl (ionic strength) were as follows (in mmol.l^{-1}): 15.4; 30.8; 46.2; 61.6; 77.0; 92.4; 107.8; 123.2; 138.6 and 154.0. On the other hand, the concentrations of glucose were (in mmol.l^{-1}): 258.3; 229.6; 200.7; 172.2; 143.5; 114.8; 86.1; 57.4; 28.7 and 0.0. The osmolality of the solutions ranged from 289.1 to 308.0 mOsm. Hemolysis was induced by 0.15 mmol.l^{-1} $HgCl_2$, present in the incubating media.

Samples of blood were incubated in five different sets. In the first and second set, the concentrations of exaprolol were 2.5 or 5.0 x 10^{-4} mol.l^{-1}, respectively. Glutaraldehyde was present in the third and fourth set, in concentrations 3.0 or 5.0 x 10^{-6} mol.l^{-1}, respectively. In the fifth set, the blood was incubated without glutaraldehyde or exaprolol and served as a control.

The samples were incubated at 37 °C for 60 min. After incubation, the samples were centrifuged for 5 min at 700 x *g*. Hemolysis was established using the spectrophotometrical methods at 540 nm and expressed as the hemolytic ratio in relation to the hemolysis in distilled water which was arbitrary set as 100 %.

The filtration time (FT) was measured according to the method of Reid *et al.* (1976). Under standard conditions blood was passed through the membrane filter (Sartorius, 5.0 μm pore size) using a negative pressure of 20 cm of water. The blood in the volume of 1 ml was mixed with 1 ml of an isotonic saline solution. One control sample, exaprolol and glutaraldehyde-treated samples were used in the same conditions as in the cation-osmotic method.

The mean cell volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC) was measured automatically using Müller-SYSMEX K 80 equipment in concentrations as described above.

Statistical analyses were performed by using unpaired Student's t-test. The $p<0.01$ value was selected as the point of the minimal statistical significance.

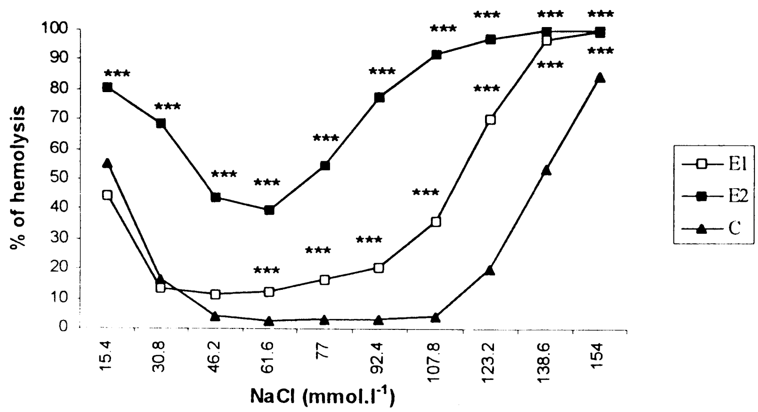


Fig. 1. *Comparison of cation-osmotic hemolysis in exaprolol-treated erythrocytes (2.5 x 10^{-4} mol.l^{-1} - E1, 5.0 x 10^{-4} mol.l^{-1} - E2) and non-treated erythrocytes (C). Ordinate: % of hemolysis. Abscissa: concentration of NaCl in mmol.l^{-1}. Statistically significant differences for ***$p<0.001$.*

Results

The results of COH in exaprolol-treated cells are shown in Figure 1. A lower concentration of exaprolol caused a significant increase of COH in the region from 61.6 to 154.0 mmol.l^{-1} NaCl in relation to the control ($p<0.001$). A higher concentration of exaprolol caused a significant increase of COH during the whole course of hemolysis ($p<0.001$).

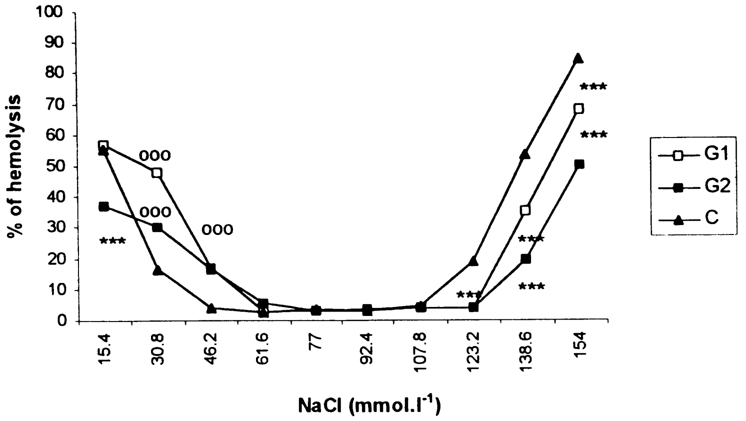
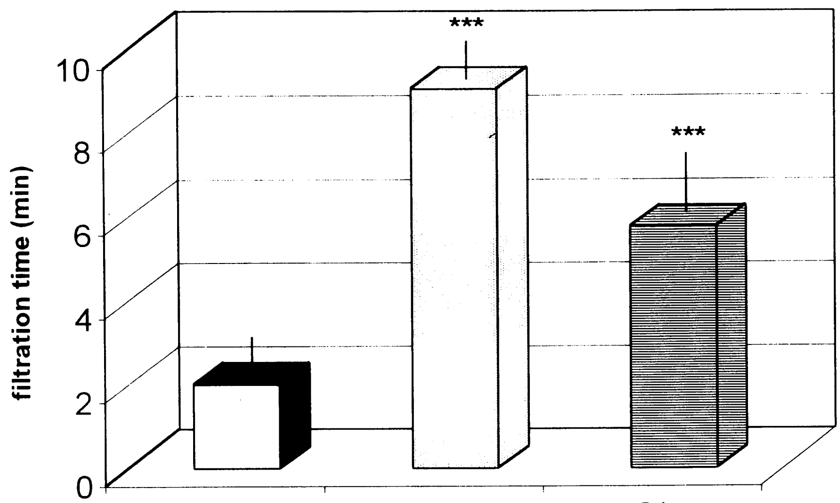


Fig. 2. *Comparison of cation-osmotic hemolysis in glutaraldehyde-treated erythrocytes (3.0 x 10^{-6} mol.l^{-1} - G1, 5.0 x 10^{-6} mol.l^{-1} - G2) and non-treated erythrocytes (C). Ordinate: % of hemolysis. Abscissa: concentration of NaCl in mmol.l^{-1}. Statistically significant differences for ***p<0.001 and ^{ooo}p<0.001.*

Glutaraldehyde (Fig. 2) in both concentrations used enhanced hemolysis only in the region with low ionic strength (30.8-46.2 mmol.l^{-1} NaCl) ($p<0.001$). However, a significant decrease of COH occurred in the range of high ionic strength (123.2-154.0 mmol.l^{-1} NaCl) ($p<0.001$). The filterability of control samples, exaprolol and glutaraldehyde-treated cells are shown in Figure 3. Both concentrations of exaprolol and glutaraldehyde significantly increased the transit time in comparison with the control group ($p<0.001$). Because the filtration times in higher concentrations of both agents were more than 25 min, filtration times in the lower concentrations of exaprolol (2.5 x 10^{-4} mol.l^{-1}) and glutaraldehyde (3.0 x 10^{-6} mol.l^{-1}) are shown only.

Fig. 3. *Filtration time of exaprolol-treated erythrocytes (2.5 x 10^{-4} mol.l^{-1}- E1) and glutaraldehyde-treated erythrocytes (3.0 x 10^{-6} mol.l^{-1} - G1). Non-treated erythrocytes (C) served as controls. Statistically significant differences for ***p<0.001.*



The MCV in both concentrations of exaprolol and glutaraldehyde can be seen in Figure 4. A significant decrease of MCV was found in both concentrations of exaprolol ($p<0.001$). However, glutaraldehyde, used in either higher or lower concentrations, did not influence MCV.

Figure 5 shows the effect of exaprolol and glutaraldehyde on MCHC. We observed a significant increase of hemoglobin concentration in exaprolol-treated cells ($p<0.001$). On the other hand, glutaraldehyde, similarly to MCV, had no effect on MCHC.

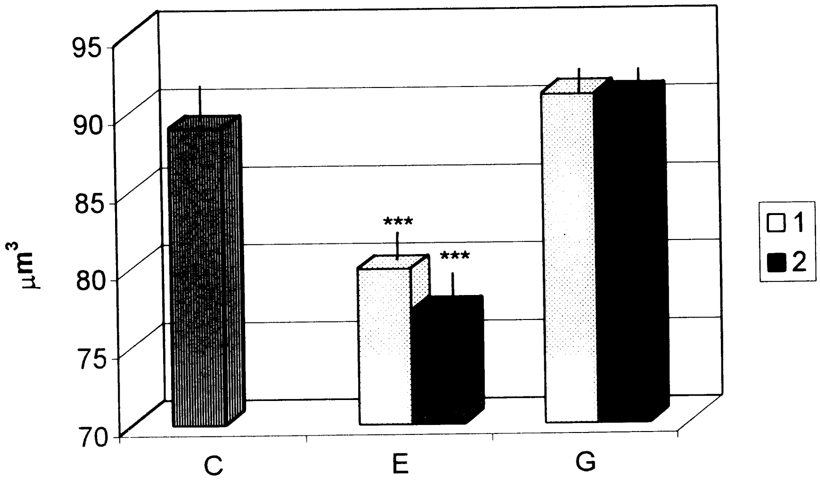


Fig. 4. *Mean cell volume (µm³) in exaprolol-treated erythrocytes (2.5 x 10⁻⁴ mol.l⁻¹- E1, 5.0 x 10⁻⁴ mol.l⁻¹ - E2) and glutaraldehyde-treated erythrocytes (3.0 x 10⁻⁶ mol.l⁻¹ - G1, 5.0 x 10⁻⁶ mol.l⁻¹ - G2) in comparison with non-treated erythrocytes (C). Statistically significant differences for ***p<0.001.*

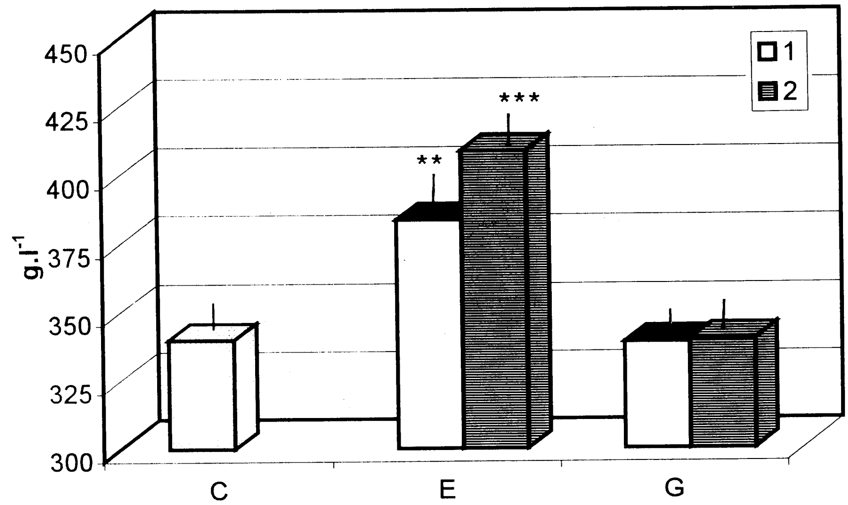


Fig. 5. *Mean corpuscular hemoglobin concentration (g.l⁻¹) in exaprolol-treated erythrocytes (2.5 x 10⁻⁴ mol.l⁻¹- E1 and 5.0 x 10⁻⁴ mol.l⁻¹ - E2) and glutaraldehyde-treated erythrocytes (3.0 x 10⁻⁶ mol.l⁻¹ - G1 and 5.0 x 10⁻⁶ mol.l⁻¹ - G2) in comparison with non-treated erythrocytes (C). Statistically significant differences for ***p<0.001.*

Discussion

On the basis of COH, two different biophysical processes may occur. Exaprolol is a highly lipophilic compound. If its concentration is higher than the beta-blocking doses, when in contact with the lipid bilayer, intercalation was observed (Hughes *et al.* 1984, Ondriáš *et al.* 1987, Pečivová *et al.* 1991). It resulted in a significant increase of hemolysis, predominantly in solutions with high ionic strength. We suggest that it is due to the ability of exaprolol to perturb the membrane. Nosál and co-workers (1989) observed similar results on isolated rat mast cells. On the other hand, glutaraldehyde caused membrane protein cross-linking resulting in hardening of the membrane (Burt *et al.* 1990, Arevalo *et al.* 1992, Mirossay *et al.* 1997). In our experiments, the decrease of COH was observed in glutaraldehyde-treated cells as a result of decreased membrane deformability.

Despite different biophysical processes acting on two different membrane constituents, erythrocyte filterability increased with the significant prolongation of filtration time. These differences between COH and filterability are probably due to two different processes. COH is a biophysical process, reflecting the membrane properties. Moreover, changes of ionic strength make it possible to distinguish the spectrine membrane skeleton properties of the lipid membrane bilayer state (Nicák and Mojžiš 1993). However, the measurement of whole cell deformability by filtration reflects a combination of various determinants, such as the geometric relationship between cell volume and surface area or internal viscosity (Reinhart and Chien 1985).

Surprising results were obtained by using the filtration technique, which revealed that both drugs prolonged the filtration time, despite increased membrane fluidity by exaprolol (Nosál *et al.* 1989). It is possible that exaprolol decreased MCV and increased MCHC. The decreased deformability is believed to be closely related to the surface area. Reduced cell volume, followed by an increase in MCHC and internal viscosity, results in a loss of erythrocyte deformability (Bessis *et al.* 1980, Hardeman *et al.* 1988). In the case of glutaraldehyde-treated cells, of course, these results could be expected because of protein cross-linking caused by glutaraldehyde diminished erythrocyte deformability, so that erythrocytes were less deformable and the transit time was prolonged.

In conclusion, our study indicated that the cation-osmotic hemolysis technique, in comparison with the filtration technique, provides more information about the effects of exaprolol and glutaraldehyde on the cell membrane bilayer in relation to the deformability of the spectrin membrane skeleton.

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Reprint requests

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