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Ethanol and erythrocyte membrane interaction: a hemorheologic perspective

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Abstract. Previous studies have documented structural and functional changes induced by ethanol–erythrocyte membrane interaction. In order to perform an *in vitro* study on the effect of different ethanol concentrations on erythrocyte hemorheologic properties, blood samples were collected from 21 male donors at the Hospital of Santa Maria. Whole blood aliquots were incubated with ethanol solutions of rising concentrations. The following parameters were measured: erythrocyte aggregation, haemoglobin, carboxyhaemoglobin and methaemoglobin concentrations, hematocrit, plasma osmolality and erythrocyte membrane fluidity (fluorescence polarisation probes TMA-DPH and DPH). With ethanol blood concentrations of 45 mM a rise in plasma osmolality (0.352 Osm/kg H_2O vs 0.310 Osm/kg H_2O; $p < 0.001$) was verified. With 67 mM concentration a decrease of erythrocyte aggregation (11.03 vs 12.81; $p < 0.05$) and an increase in plasma osmolality (0.380 Osm/kg H_2O vs 0.310 Osm/kg H_2O; $p < 0.001$) were obtained. In conclusion, ethanol only changes erythrocyte aggregation for a concentration of 67 mM. These data could lead to future changes in therapeutic approaches to situations such as alcoholic coma.

1. Introduction

Interest in alcohol consumption and its role in vascular diseases has grown due to epidemiological and experimental reports that moderate or light alcohol consumption reduces the risk of myocardial infarction, stroke and associated mortality [5,9].

Some authors [13] studied ethanol induced changes on hemorheologic parameters on alcoholic individuals. However, it seems important to study the effect of the ethanol on erythrocyte, excluding any factor associated with alcoholic disease.

In vitro ethanol can change water-channel CHIP 28 permeability [6], Na^+-transporter [2] and Ca^{2+}-ATPase pump [1] of erythrocytes of non-alcoholic individuals.

In previous studies [4], ethanol 10^{-3} M was mentioned to penetrate the lipid bilayer into biological membranes to a significant extent, affecting its physicochemical structural properties. On the other hand, some specific ethanol actions on glutamate receptors on central nervous system biological membranes have been referred [14]. However, at erythrocyte level, these specific actions have not been described.

The aim of this study is to evaluate the effect of different ethanol concentrations – 0 mM, 5 mM (25 mg/dl), 10 mM (50 mg/dl), 20 mM (100 mg/dl), 45 mM (225 mg/dl) and 67 mM (335 mg/dl) – on erythrocyte hemorheologic properties.

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2. Materials and method

2.1. Population

Venous blood from 21 apparently healthy male adult donors (mean age 39 years) was collected at the Hospital de Santa Maria. Blood was preserved with heparin (2 drops of a solution 5000 UI/10 ml).

2.2. Methodology

12 ml of blood were divided by six equal aliquots. Blood was centrifuged at 12000 rpm for 1 minute (5414 Centrifuge Eppendorf Sotel). 10 μl of plasma was replaced by 10 μl of an ethanol solution in order to obtain the final concentrations: 0, 5, 10, 20, 45 and 67 mM.

After incubating for 30 minutes at room temperature with slight agitation and partial mixing, the following parameters were assessed:

1. Erythrocyte aggregation was assessed with the agregometer Myrenne MA2, with 20 μl of blood (the value presented is the mean of three parameters) [11].
2. Haemoglobin, carboxyhaemoglobin and methaemoglobin concentrations were measured with the hemoximeter Osm3 [3].
3. Hematocrit was assessed with the 4223 Centrifuge (hematocrit micromethod) [3].

The blood aliquots were centrifuged again under the same conditions and the plasma osmolality measured (osmometer Osmomat 030) using 75 μl of plasma [3]. Plasma osmolality was also measured before incubation procedure.

Two fluorescent probes were used to determine fluorescence polarization (high levels meaning lower membrane fluidity): TMA-DPH (2,4-trimethylamino-phenyl-1,6-phenylhexa-1,3,5-triene) to study the membrane external layer and DPH (1,6-diphenyl-1,3,5-hexatriene) to study the hydrophobic portion [12].

2.3. Stastitical analysis

Results are expressed in average $\pm$ standard deviation. Manova repeated measures tests and variance analysis with Bonferoni correction were applied to the results. For interpretation of ethanol effect the bilateral t-Student test for paired samples was used. The null hypothesis was rejected for a significance level of $p = 0.05$. The data were analysed with the software "SPSS for Windows".

3. Results

Table 1 shows the mean values and standard deviations of studied parameters. The results for haemoglobin, carboxyhaemoglobin, methaemoglobin concentrations, hematocrit, DPH and TMA-DPH were not found to be statistical significant.

The results obtained for plasma osmolality showed a significant rise in 45 mM (0.352 Osm/kg H_2O) and 67 mM (0.380 Osm/kg H_2O) for a value of $p < 0.001$, when compared to the control (0.310 Osm/kg H_2O) (Fig. 1). However, no changes were verified comparing obtained values before and after the incubation procedure.

As for the erythrocyte aggregation a statistically significant decrease ($p < 0.05$) was observed for the highest ethanol concentration studied (11.03 vs 12.81) (Fig. 2).

Table 1
Results of studied parameters as mean ± standard deviation

	Control	5 mM	10 mM	25 mM	45 mM	67 mM
EAI	12.81 ± 4.84	12.53 ± 5.33	12.35 ± 4.65	12.01 ± 4.44	12.19 ± 5.25	11.03 ± 4.03
[Hb] (g/dl)	12.80 ± 1.11	13.00 ± 1.14	12.87 ± 0.88	12.85 ± 1.14	12.83 ± 0.95	13.00 ± 0.81
[MetHb] (g/dl)	0.71 ± 0.45	0.69 ± 0.43	0.71 ± 0.47	0.72 ± 0.47	0.74 ± 0.49	0.69 ± 0.48
[COHb] (g/dl)	2.44 ± 2.39	2.43 ± 2.37	2.40 ± 2.39	2.43 ± 2.46	2.43 ± 2.35	2.45 ± 2.37
Ht (%)	39.57 ± 3.34	40.05 ± 3.14	40.38 ± 2.40	39.90 ± 4.05	40.33 ± 2.80	39.81 ± 3.33
Osm (Osm/kg H_2O)	0.310 ± 0.04	0.300 ± 0.01	0.310 ± 0.03	0.331 ± 0.01	0.352 ± 0.01	0.380 ± 0.01
TMA-DPH	0.322 ± 0.02	–	0.331 ± 0.02	0.330 ± 0.01	0.320 ± 0.01	0.320 ± 0.01
DPH	0.301 ± 0.03	–	0.302 ± 0.02	0.310 ± 0.04	0.290 ± 0.05	0.280 ± 0.03

EAI, erythrocyte aggregation index; [Hb], haemoglobin concentration; [MetHb], methaemoglobin concentration; [COHb], carboxyhaemoglobin concentration; Ht, hematocrit; Osm, plasma osmolality; TMA-DPH, fluorescent polarisation probe (1,4-(trimethylamino)-phenyl-6-phenylhexa-1,3,5-triene); DPH, fluorescent polarising probe (1,6-diphenyl-1,3,5-hexatriene).

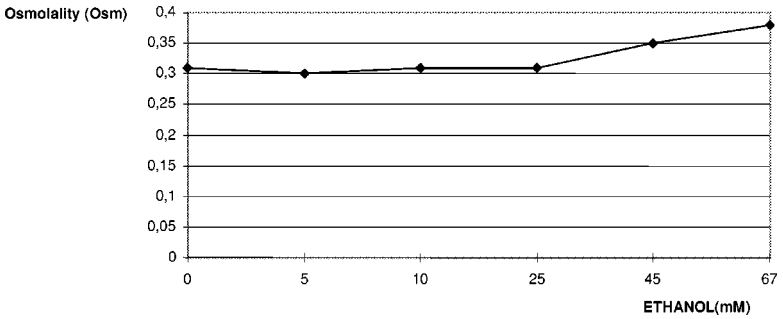

Fig. 1. Plasma osmolality mean values for different ethanol concentrations.

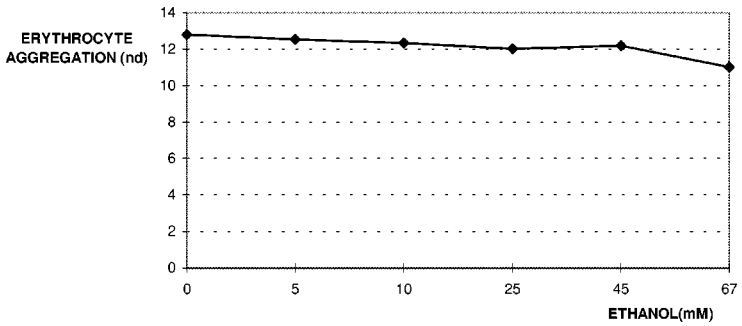

Fig. 2. Erythrocyte aggregation mean values for different ethanol concentrations.

4. Discussion and conclusion

The aim of this work was to study the *in vitro* effect of different ethanol concentrations on hemorheologic parameters.

Ethanol concentrations lower than or equal to 20 mM (100 mg/dl) showed no significant changes in any parameters suggesting that ethanol does not interact with erythrocyte membrane or its presence in plasma is compensated by any unknown mechanism.

Ethanol concentration of 45 mM showed a significant rise in plasma osmolality when compared to the control ($p < 0.001$), even though the erythrocyte aggregation remains unchanged. A previous study [10] documented that erythrocyte aggregation increases when levels of plasma osmolality rise. Therefore, it can be suggested that ethanol inhibits this plasma osmolality effect.

For ethanol concentration of 67 mM there was a decrease in erythrocyte aggregation ($p < 0.05$) and a rise in plasma osmolality ($p < 0.001$). These results suggest that ethanol affects erythrocyte aggregation by a different mechanism of plasma osmolality. The increased values of plasma osmolality were verified just after replacing plasma by ethanol solution and were maintained during incubation time. They suggest that ethanol may have little or no effects in membrane permeability, which means that the dynamic electrolytic equilibrium remains unchanged.

The ethanol induced change in erythrocyte aggregation may be justified by erythrocyte membrane and cytoskeleton changes interfering with unspecific adsorption of macromolecules by membrane cortex, superficial charges, membrane lipid/protein packing and electrolytic pumps abnormalities [7,8].

It seems important to refer that 45 mM and 67 mM ethanol corresponds to alcoholic coma. This study suggests that ethanol have no important hemorheologic effects at blood concentrations corresponding to light to moderate levels of consumption. However its effect on erythrocyte aggregation may be important for therapeutic issues of acute alcoholic intoxication.

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