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# HEMORHEOLOGICAL DISTURBANCES ARE PREDICTORS OF CARDIOVASCULAR EVENTS IN TRANSMURAL ACUTE MYOCARDIAL INFARCTION. A 60 MONTHS PROSPECTIVE STUDY*

*C Saldanha, L Sargento, HS Rosário, J Monteiro ,C Perdigão, J Martins e Silva*

Inst. Bioquímica, Fac. Med. Lisboa. UTIC-AC. Portugal

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The aim of this work was to evaluate the relationship, in transmural acute myocardial infarction (AM1) survivors, between hemorheological disturbances and the cardiovascular events (CVE) curve during 60 months of prospective follow-up. Sixty-four patients (59 men), 58.0 ± 12.0 years old transmural AMI survivors, were prospectively followed during 60 months. The following CVE were collected: death, AMI, unstable angina, stroke. Twenty nine patients had a CVE (9 died). Hemorheological parameters measured were plasma viscosity, fibrinogen, C protein (Prot. C), PAI-l inhibitor and III antithrombin, leukocyte count, elastase, erythrocyte aggregation and the erythrocyte membrane fluidity. Kaplan-Meier survival curve (with Log Rank test), Chi square, and relative risk determination and the Cox regression were used. The leukocyte count ($p$<0.01), C protein ($p$<0.05) and the erythrocyte membrane fluidity ($p$<0.05) were predictors of CVE during the follow-up. The higher the leukocyte count, and the lower the value of the C protein, the higher the risk for CVE patients. The higher the value of the erythrocyte membrane fluidity, the higher the risk for CVE. By multivariate analysis (Cox regression) the leukocyte count ($p$<0.01), C protein ($p$<0.01) and the outer erythrocyte membrane fluidity ($p$<0.01) were identified as independent predictors of CVE. In this group of transmural AMI survivors a relationship between hemorheological factors and the patient's 60 months event free survival curve was established.

# ACETYLCHOLINESTERASE ENZYMATIC INHIBITION BY VELNACRINE MALEATE AND ITS EFFECT ON HEMORHEOLOGICAL PROPERTIES IN DIFFERENT PATHOLOGIES*

*S Hilário, P Pessegueiro, C Saldanha, J Martins e Silva*

Institute of Biochemistry, Faculty of Medicine,
University of Lisbon, Lisbon. Portugal

* Apresentação no 11th International Congress of Biorheology and 4th Conference on Clinical Hemorheology

The aim of this work was to study the repercussion of acetylcholinesterase enzyme activity inhibition by velnacrine maleate (VM) on erythrocyte membrane fluidity and other hemorheological properties.

30 adult donors were divided in 3 groups of 10 subjects each, according to their pathology: control group, Insulin Dependent Diabetes Mellitus (IDDM) group and Chronic Renal Failure (CRF) group.

Afterwards, blood was drawn and the aliquots obtained were incubated for 15 minutes, with and without VM $10^{-5}$ M. The following hemorheological parameters were determined: erythrocyte AChE enzymatic activity, hydrophobic erythrocyte membrane fluidity, erythrocyte aggregation, plasma osmolality and pH. Data analysis was performed using the student *t*-test ($p$=0.05). The VM inhibitory action was confirmed in this study ($p$<0.0001), being 95% in the control group and 93% in both IDDM and CRF groups. A significant decrease in erythrocyte aggregation values ($p$<0.05) was observed in all the groups studied under the influence of VM. Yet, it was shown that the use of this inhibitor was associated with an increase in erythrocyte fluidity but only in control donors because in the other groups, VM had achieved the opposite effect. In this i*n vitro* study we observed a high percentage of AChE inhibition by VM. This suggests that an enzyme-inhibitor complex was formed and that this complex is at the root of the hemorheological variations found and on membrane fluidity, not only in healthy donors as well as in people with IDDM and CRF.

## INTRODUCTION

Velnacrine Maleate (MV) inhibits the acetylcholinesterase (AChE) enzymatic activity in a reversible way. Being this enzyme in contact with the remaining erythrocyte membrane elements, it would be possible, modula-ting its activity, to modify the fluidity and consequently to influence the functional level of another membrane proteins.

**The aim of this work** was to study the repercussion of membrane protein activity inhibition on erythrocyte membrane fluidity and other

hemorheological properties.

## POPULATION AND METHODS

The studied **population** consisted of 30 men donors, divided in 3 groups of 10 elements each, according to their pathology: Control (C) group, Insulin Dependent Diabetes Mellitus (IDDM) group and Chronic Renal Failure (CRF) group. (Table I)

**The adopted method is presented in the next organisational Tables**

**Determination of:**

- **AChE enzymatic activity** *(Ellman' spectrophotometric method modified by Kaplan'*);
- **Erythrocyte aggregation** *(Myrenne agregometer***)**;
- **Plasma osmolality** *(Osmomat 030 osmometer);*
- **pH** *(Copenhagen ABL$^{TM}$ 500 Radiometer);*
- **Hydrophobic erythrocyte membrane fluidity** *(fluorescence polarization DPH).*

**Data analysis** was performed using Student t-test ($\alpha$=0.05).

## RESULTS

Initial AChE enzymatic activity values (279.2 IU/min/mgHb) are higher in IDDM group than the remaining groups (Fig. 1).

The VM inhibitory action was confirmed on this study ($p \leq 0.0001$), being of 95% in control group and 93% in both IDDM and CRF groups, (Fig. 2).

It was attended a significant decrease ($p \leq 0.05$) on erythrocyte aggregation values in all the studied groups under influence of VM, (Fig. 3).

Initial osmolality values are different when compared among groups. Both chronic insufficient renal ($p \leq 0.0001$) and diabetic type I ($p \leq 0.01$) groups had higher osmolality initial values than control group, (Fig. 4).

No statistical variations were observed in pH values.

DPH initial values of CRF group are lower than control group ones ($p \leq 0.01$), (Fig. 5).

## CONCLUSIONS

In this "*in vitro*" study we verified a high percentage of AChE inhibition by VM which meaning an enzyme-inhibitor (EI) complex formation suggesting the hypothesis that this EI complex should be in the origin of the hemorheological variations verified (even in membrane fluidity), not only in healthy donors as well as in IDDM and CRF people.

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**TABLE 1**

Characterization of the studied population C group: Healthy men; IDDM group: 130 ± 10 mg/dL of blood glucose values (fasting); CRF: Blood samples were collected before the regular intermittent haemodialysis

| Population | N | Age (*Years old*) | Hemoglobin (*g/L*) | Hematocrit (*%*) |
|---|---|---|---|---|
| Control (C) Group | 10 | 43 ± 2 | 14.1 ± 1.2 | 41.3 ± 3.2 |
| Insulin Dependent Diabetes Melitus (IDDM) Group | 10 | 53.2 ± 12 | 13.6 ± 1.4 | 40.8 ± 3.8 |
| Chronic Renal Failure (CRF) Group | 10 | 60.9 ± 5.1 | 11 ± 1.7 | 33.6 ± 5.02 |

**Fig. 1 –** Velnacrine Maleate effect on AChE enzymatic activity (C: Control group; IDDM: Insulin Dependent Diabetes Mellitus; CRF: chronic renal failure); ($p \leq 0.0001$)

**Fig. 2 –** Erythrocyte AChE enzymatic inhibition by Velnacrina Maleato. (C: Control group; IDDM: Insulin Dependent Diabetes Mellitus ; CRF: chronic renal failure); ($p \leq 0.0001$)

**Fig. 3 –** Velnacrine Maleate effect on erythrocyte aggregation (C: Control group; IDDM: Insulin Dependent Diabetes Mellitus; CRF: chronic renal failure); ($p \leq 0.05$)

**Fig. 4 –** Velnacrine Maleate effect on osmolality (C: Control group; IDDM: Insulin Dependent Diabetes Mellitus ; CRF: chronic renal failure)

**Fig. 5 –** Velnacrine Maleate effect on erythrocyte membrane fluidity (C: Control group; IDDM: Insulin Dependent Diabetes Mellitus; CRF: chronic renal failure)

**AChE**
**IU/min/mgHb**

**All tubes were incubated 30 minutes**