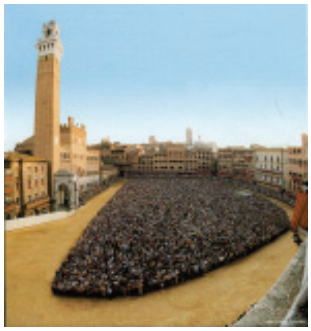
13th Conference of the European Society for Clinical Hemorheology



RELATION BETWEEN THE ERYTHROCYTE NITRIC OXIDE AND HEMORHEOLOGICAL PARAMETERS

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OBJECTIVE

The aim of this work was to study the association / relation between the nitric oxide production, achieved by *in vitro* erythrocyte acetylcholine (ACh) stimulation, and changes on hemorheological parameters, of blood patients with hypercholesterolemia, renal transplantation or hypertension.

EXPERIMENTAL DESIGN

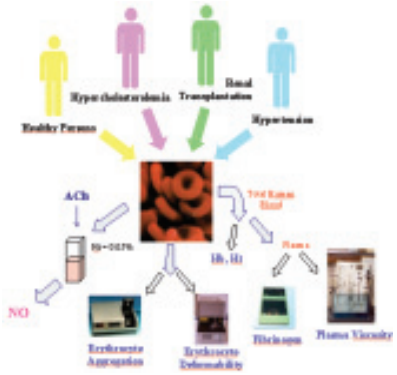

Figure 1: Venous blood samples were collected to tubes with sodium heparin 10 UI/mL, from healthy humans and patients with hypercholesterolemia, renal transplantation or hypertension. We quantified the levels of hemoglobin, hematocrit from the different total blood samples. The blood was centrifuged and the plasma and erythrocytes were separated. We measured the plasma viscosity and the fibrinogen concentration of the plasma samples of each patient or control sample. We also measured the erythrocyte aggregation and deformability on each blood sample. For amperometric NO quantification we used the amiNO-IV sensor (*Innovative Instruments Inc. FL, USA*). The erythrocyte suspensions were obtained adding sodium chloride 0.9% at pH 7.0 to the erythrocytes to reconstitute a hematocrit of 0.05%. The suspensions were incubated for 30 minutes at room temperature and after that, we stimulated the erythrocytes with ACh 10 mM and monitorized the erythrocytic nitric oxide production.

RESULTS

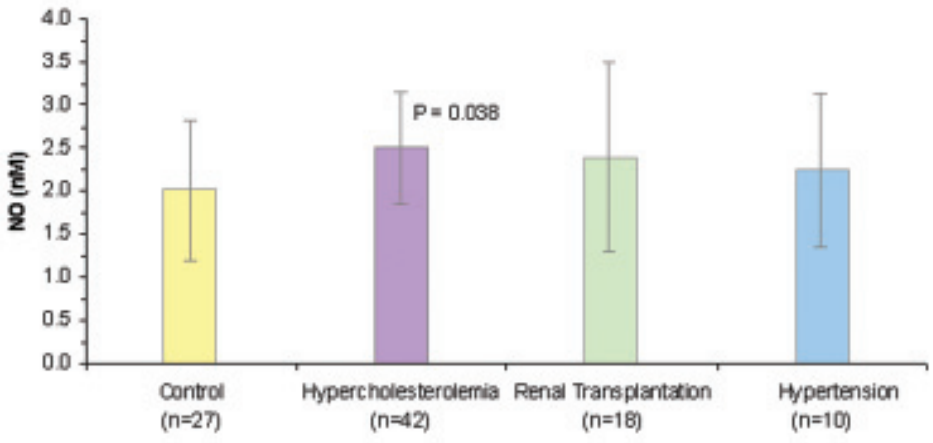

Figure 2: Changes on the NO production of erythrocytes suspensions incubated with ACh 10μM from control (healthy group) and patients with hypercholesterolemia, renal transplantation and hypertension. Significant value between the NO of the control and the others samples was of P = 0.038 for hypercholesterolemia, and for renal transplantation and hypertension the values were not significant. Values are in mean ± SD.

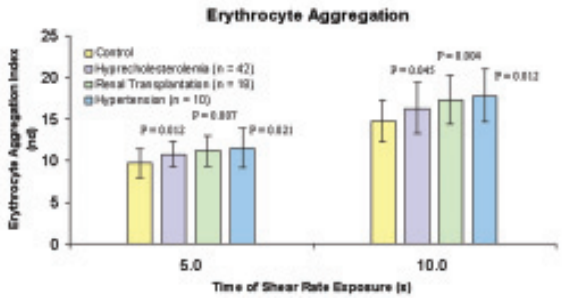

Figure 3: Erythrocyte aggregation of patients with hypercholesterolemia, renal transplantation and hypertension *versus* control values. The erythrocyte aggregation was measured at 5 and 10 seconds of shear rate exposure. Values are in mean ± SD.

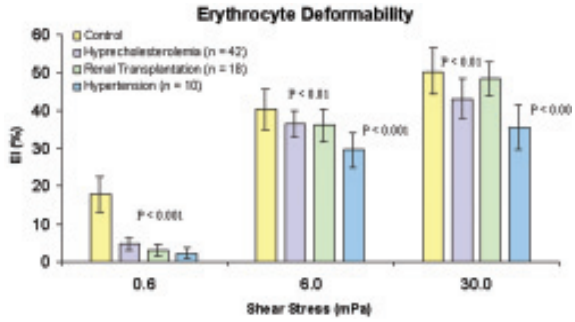

Figure 4: Erythrocyte deformability at shear stress values of 0.6 mPa, 6 mPa and 30 mPa, of patients with hypercholesterolemia, renal transplantation and hypertension *versus* control values. Values are in mean ± SD.

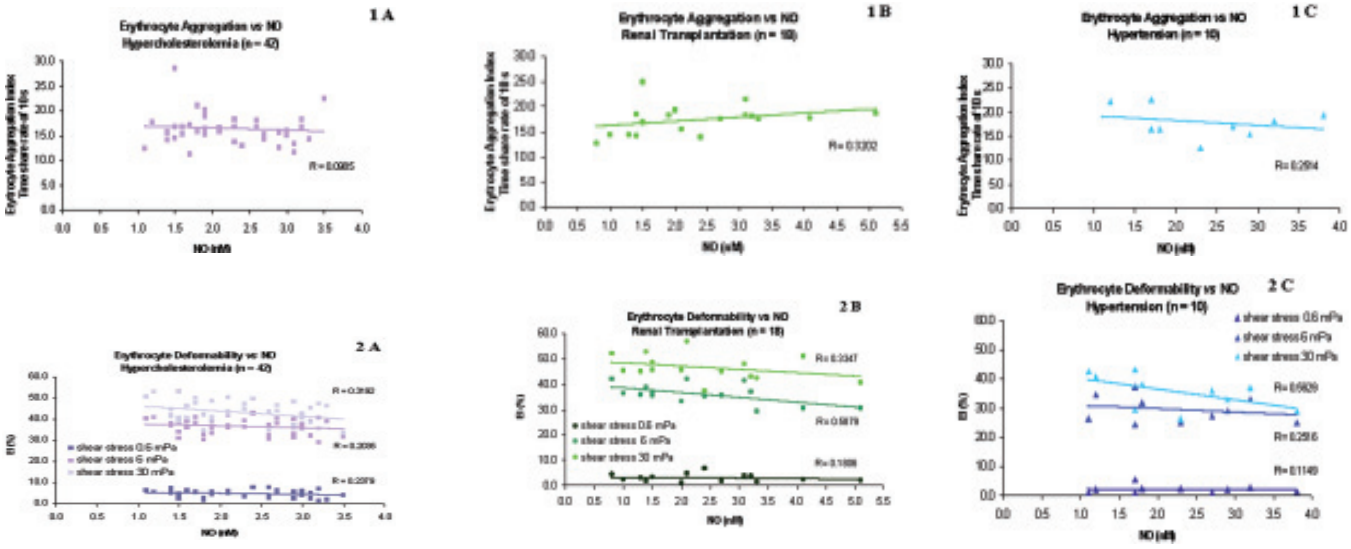

Figure 5: Values of hemorheological parameters *versus* erythrocyte NO concentration. Erythrocyte aggregation levels at 10 seconds of shear rate exposure (1), erythrocyte deformability at shear stress values of 0.6 Pa, 6 Pa and 30 Pa (2) of hypercholesterolemic (A), renal transplantation (B) and hypertensive (C) patients

Table 1: Hemoglobin (g/dL), hematocrit (%), plasma viscosity (mPa.s) and fibrinogen concentration (mg/dL), of hypercholesterolemic, renal transplantation, hypertension and control (healthy persons) blood samples. Values are in mean ± SD.

Groups	Hb (g/dL)	Ht (%)	Plasma Viscosity (mPa.s)	Fibrinogen (mg/dL)
Control (n=27)	13.7 ± 1.2	41.9 ± 3.6	1.24 ± 0.04	272.7 ± 55.4
Hypercholesterolemia (n=42)	13.9 ± 1.2	42.5 ± 3.5	1.26 ± 0.04[1]	310.0 ± 64.7[4]
Renal Transplantation (n=18)	13.2 ± 1.8	41.2 ± 4.9	1.26 ± 0.04[2]	294.0 ± 65.0
Hypertension (n=10)	14.5 ± 1.3	42.9 ± 3.8	1.27 ± 0.03[3]	287.0 ± 65.0

[1] Significant difference relatively to the control samples (p = 0.035); [2] Significant difference relatively to the control samples (p = 0.045); [3] Significant difference relatively to the control samples (p = 0.034); [4] Significant difference relatively to the control samples (p = 0.027);

CONCLUSIONS

- ACh increases NO production on "*in vitro*" in erythrocytes of different diseases.
- Hypercholesterolemia was the disease with the most increased erythrocyte NO production.
- At every shear stress value and on each studied group, there was a decrease in erythrocyte deformability and an increase of NO concentration.
- The erythrocyte aggregation levels significantly increase compared with the control group. Higher tendency of erythrocyte to aggregate, the lower ability to the NO production after ACh stimulation.
- The plasma viscosity significantly in-

creased in all patient groups.

- The fibrinogen concentration increased significantly only in hypercholesterolemic patients.

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- Different diseases have different physiological responses to erythrocyte ACh stimulation that leads to changes on erythrocyte NO production.
- This fact could be related with changes on the erythrocyte membrane that influence directly the erythrocyte aggregation and erythrocyte deformability.
- Erythrocyte with reduced deformability (as seen in diseases groups) were better stimulated by ACh producing higher NO levels than the healthy controls. This may represent a compensation of the impaired hemorheological behaviour by NO production in the presence of ACh.
- We could hypothesize that cholinergic drugs could be used as a co-adjuvant of specific therapeutic compounds in hypercholesterolemia, renal transplantion and hypertension. This fact suggestes a future target for vasodilation therapeutic action on a microcirculatory homeoatatic network, damaged by different types of pathological insults.