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Hemapheresis

Rheopheresis in vascular diseases

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ABSTRACT: Background: Endothelial dysfunction is a common condition in many microvascular diseases, such as Age-related Macular Degeneration (AMD) and Peripheral Arterial Occlusive Disease (PAOD). Rheopheresis therapy improves ematic viscosity, shear stress and endothelial function while decreasing fibrinogen, LDL-cholesterol and alpha-2-macroglobulin levels.
Objective: To evaluate the therapeutic efficacy of rheopheresis in patients with microcirculatory diseases.
Materials and Methods: Eight patients (7 male and 1 female) were treated with rheopheresis: 3 males were affected by AMD, 4 male and 1 female by uremia and PAOD. We used Membrane Differential Filtration (MDF) with an ethinylvinylalcohol copolymer membrane as plasmafiltrator. Patients with AMD were treated once a week for ten weeks. Patients affected with PAOD were treated twice weekly for 3 weeks and then were placed on a once-a-week program.
Results: In all treated patients with AMD, visual acuity improved. In all patients affected with PAOD, we observed a complete resolution of pain; 3 out of 5 had a complete remission of ulcers. There was partial reduction of ulcers in the other patients and no adverse effects were observed.
Conclusion: Rheopheresis is a safe, effective form of hemorheotherapy. (Int J Artif Organs 2007; 30: 923-9)

KEY WORDS: Rheopheresis, Membrane differential filtration, AMD, PAOD

INTRODUCTION

The alteration of microcirculation is a common condition in several diseases, causing impaired organ perfusion with metabolic alterations and tissue ischemia leading to degeneration or necrosis.

Organ perfusion is determined from interrelated blood vessel function and blood rheology. It is well recognized that blood vessel function is influenced by mechanical factors. Indeed, the cardiocirculatory system is constantly exposed to mechanical stresses produced by blood flow that is modulated and checked by the elastic recoil of the heart and vessels. These stresses influence vessel wall function, especially endothelium activity. The endothelium plays a role in the regulation of the cardiovascular system and there are ongoing studies to improve endothelial dysfunction.

Blood rheology is the compound effect of the dynamic interaction of blood proteins, (such as fibrinogen, LDL-cholesterol, α-2-macroglobulin) and cells under a given flow and stress field. Organ perfusion is strongly interrelated with alteration of blood rheology (hemorheology). In particular, previous studies have demonstrated a correlation between the alteration of biochemical molecules with a role in blood viscosity, and vascular diseases (1-5) such as Age-related Macular Degeneration (AMD) and Peripheral Arterial Occlusive Disease (PAOD).

AMD is a leading cause of blindness in people over 60 years old. It affects the macula, responsible for seeing fine details. AMD is characterized by the collections of debris (dry-drusen) beneath the retinal pigment epithelium which disrupt retinal function (6). There are two forms of AMD: dry-drusen, with amorphous debris, and wet-drusen, in which there is choroidal neovascularization. The pathogenesis of AMD is not yet fully understood but new hypotheses put forward suggest that a dysfunction of the microcirculatory blood supply is one major factor contributing to the pathogenesis (7). AMD is the hemody-

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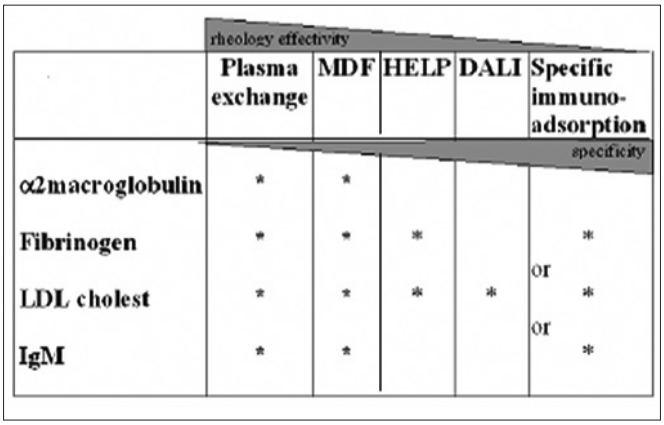

Fig.1 - *Plasma protein elimination profile in different techniques of extracorporeal hemorheopheretic therapy (modified from Klingel et al) (10). * Reduction >60%*

namic consequence of impaired blood flow in the choroid with accumulation of intracellular material and extracellular deposit (drusen). The initial dry AMD, characterized by drusen, may progress to wet, a more advanced form characterized by neovascularization (8).

PAOD is an emerging disease basically due to the increase in the aging population as well as the increase of atherosclerosis and diabetes. Fibrinogen and lipoproteins are considered to be the most crucial pacemakers in the pathogenesis of atherosclerosis (9). The pathogenetic role of lipids in atherosclerotic plaque is reported extensively in the literature. Nevertheless, there is strong evidence that fibrinogen may hold a key position in the development of obstructive vascular diseases. In clinical studies, patients with PAOD have elevated plasma viscosity and concentration of fibrinogen as compared with control groups. In patients with critical limb ischemia, plasma viscosity, fibrinogen, cholesterol, and α-2-macroglobulin were significantly elevated (10). Rheopheresis decreases fibrinogen, LDL-cholesterol, α-2-macroglobulin levels and plasma viscosity. The biochemical changes in these diseases could provide clinical effects already reported in literature.

In order to remove a defined spectrum of high molecular weight proteins with a significant impact on blood rheology, there are various hemorheotherapy techniques to consider: Membrane Differential Filtration (MDF), selective system of fibrinogen adsorption (RheoSorb), Heparin-induced Extracorporeal LDL Precipitation (HELP), dextran sulphate adsorption, Direct Adsorption of Lipoproteins (DALI) hemoperfusion system. None of these techniques has been demonstrated to have a clear-cut advantage over the others. However, selective techniques do offer the advantage of removing specific high molecular weight proteins (e.g. LDL cholesterol, fibrinogen). Nevertheless, these apheresis techniques are considered to be more specific and less rheologically effective (11) (Fig. 1). Moreover, selective rheopheresis is a costlier procedure. For this reason, we chose MDF over the others. MDF is a semiselective technique able to select plasma proteins based on molecular weight. The plasma is pumped into a hollow fiber filter where high molecular weight proteins and lipoproteins are captured, while returning to the patient low molecular weight proteins ("Cascade Technique"). The cut-off of the molecular weights is determined by the pore size of the fiber.

The aim of this study was to assess the efficacy of removal of several high molecular weight proteins using the Membrane Differential Filtration (MDF) technique, and to evaluate its effectiveness in patients with AMD and PAOD.

MATERIALS AND METHODS

We performed rheopheresis in 8 patients: three males affected with dry AMD, and five uremic patients with PAOD (4 males and 1 female). Patient characteristics at admission are summarized in Tables I and II.

AMD patients were evaluated for visual function before and after the treatment protocol. Visual Acuity (VA) was tested by using LogMAR scale (Logarithm of the Minimum Angle of Resolution), that allows simple conversion of reading acuity between different viewing distances. It measures visual acuity loss, converting the geometric sequence of a traditional chart to a linear scale (1.7; -0.6): positive values indicate vision loss, while negative values denote normal or better visual acuity.

In uremic patients with PAOD, the Fontaine classification (Tab. III) was used for the clinical stage. All of them were characterized by critical limb ischemia with pain at rest and/or tissue lost (Fontaine stage IV).

We used an MDF technique ("Cascade technique") with a polyethylene-ethinylvinylalcohol hollow fiber filter (PLASMAFLO OP 05 W(L) ASAHI®, 0.5 m^2 surface) as plasmaseparetor, and an ethinylvinylalcohol copolymer hollow fibres filter (Rheofilter ER 4000 ASAHI® 2 m^2 surface) as plasmafiltrator, with a molecular weight cut-off of 600 kD. We treated 1.5 plasma volumes per session. The description

TABLE I - BASELINE CHARACTERISTICS OF AMD PATIENTS

Pts*	Age	Gender	Visual acuity§ (AMD lesion)		Fibrinogen	Tot Cholesterol	LDL Cholesterol	Albumin
	(years)		RE+	LE+	(mg/dL)	(mg/dL)	(mg/dL)	(gr/dL)
1	66	male	0.9 (dry)	0.9 (wet)	231.4	174	103	4.4
2	60	male	0.8 (wet)	0.9 (dry)	339.7	109	68	4
3	79	male	0.9 (wet)	0.9 (dry)	262	170	104	4.5

* Pts = patients; + RE = right eye; LE = left eye; § with LogMAR scale (range -0.6; 1.7).

TABLE II - BASELINE CHARACTERISTICS OF UREMIC PATIENTS WITH PAOD

Pts*	Age	Gender	Leriche-Fontaine stage		Fibrinogen	Tot Cholesterol	LDL Cholesterol	Albumin
	(years)		Right foot	Left foot	(mg/dL)	(mg/dL)	(mg/dL)	(gr/dL)
1	71	male	IV	IV	461.8	108	42	4.1
2	80	male	IV	I	390	149	108	3.9
3	57	male	IV	I	502	186	112	4.2
4	62	male	IV	I	339	109	68	4
5	65	female	IV	IV	375	170	98	4

* Pts = patients.

of the MDF treatment is summarized in Table IV.

Patients affected with AMD were given treatment once a week for 10 weeks, while patients with PAOD underwent to treatments twice weekly for 3 weeks and then were placed on a once-a-week program that continued for 6 to14 weeks. The measurement of biochemical parameters was carried out before and after each treatment. To prevent an unintentional hemodiluition related to hypothetical effects of the treatment, hematocrit percentage was evaluated before each session. We were not able apply any statistical evaluation because of the small population of the study. Following explanation of the protocol, informed written consent was obtained from each patient.

TABLE III - FONTAINE CLASSIFICATION OF PAOD

	Fontaine Classification
Stage 1	Asymptomatic
Stage 2	*Claudicatio intermittens*
Stage 3	Rest pain
Stage 4	Ulcers and ischemic lesions

RESULTS

No adverse event occurred during the rheopheresis sessions; arterial blood pressure and heart rate did not shown significant differences from baseline and during the sessions. Table V reports mean values, standard deviations and percentage diminutions for different molecules before and after each rheopheresis session. The hematocrit percentage values were not modified by apheretic therapy. The clinical evaluation revealed that all patients showed an improvement: all results are summarized in Tables VI and VII.

At onset evaluation, the first AMD patient had a 0.9 LogMAR bilateral VA due to an irreversible hemorrhagic lesion of the left eye and dry drusen in the right. The second had 0.9 and 0.8 LogMAR VA in the left and right eyes, respectively, with dry drusen in the left and wet drusen in the right. The third patient presented a bilateral VA of 0.9 LogMAR, with dry drusen on the left eye and wet drusen on the right.

At the end of rheopheresis treatments, we observed an improvement of visual acuity in all the eyes with dry drusen. The first patient improved right eye VA from 0.9 to 0.2 LogMAR; the second and third improved their LogMAR left eye VA from 0.9 to 0.0. In all the patients, the improvement of VA persisted up to the followup at 3 months after the last rheopheretic treatment.

PAOD patients were at Fontaine stage IV at the onset. The first patient was a 71-year-old male with no clinical indication for surgical revascularization due to multiple distal stenosis. At onset, he presented with ulcers on the feet; he was affected with severe pain requiring chronic therapy with tramandole. The second patient (male, 80 years old) had right foot pain and ischemic lesions without tissue loss. He was revascularization candidate, waiting for admission. The third was a 57-year-old male patient, affected with ulcer and pain in the big right toe. The fourth patient was 62 years old, male, with ulcers on the right foot; he had previously undergone percutaneous

TABLE IV - CHARACTERISTICS OF MDF THERAPY

Pts	Disease	Number of treatments	Number of weeks	Vascular access	Plasma treated volume/mL	Anticoagulation Na-heparin bolus/continuous	Blood flow (ml/min)	Plasma flow (ml/min)
1	AMD	10	10	pheripheral veins	1.5/3300	500 U.I./5 UI/kg/h	80	18
2	AMD	10	10	pheripheral veins	1.5/3300	500 U.I./5 UI/kg/h	80	18
3	AMD	10	10	CVC*	1.5/3800	500 U.I./5 UI/kg/h	80	18
4	PAOD	15	12	AVF+	1.5/4500	500 U.I./5 UI/kg/h	200	26
5	PAOD	13	10	AVF+	1.5/4400	500 U.I./5 UI/kg/h	200	26
6	PAOD	20	17	AVF+	1.5/4300	500 U.I./5 UI/kg/h	200	26
7	PAOD	12	9	AVF+	1.5/4100	500 U.I./5 UI/kg/h	200	26
8	PAOD	15	12	AVF+	1.5/4700	500 U.I./5 UI/kg/h	200	26

* Central Venous Catheter; + Arthero-Venuous Fistula.

TABLE V - MEAN VALUES, STANDARD DEVIATION (SD) AND PERCENTAGE DIMINUTION (Δ%) BEFORE AND AFTER TREATMENTS OF PLASMATIC AND SERUM PROTEINS

	Normal value	PRE (mean±SD)	POST (mean±SD)	Δ%
IgG (mg/dL)	700 – 1600	648.2 ± 116.1	460.5 ± 116.6	28.9
IgA (mg/dL)	70 – 400	215.1 ± 64.4	124.7 ± 36.6	42.03
IgM (mg/dL)	40 – 230	34.9 ± 17.05	16.4 ± 8.2	53.1
Total cholesterol (mg/dL)	110 – 200	140.1 ± 35.2	67.7 ± 19.7	51.7
HDL (mg/dL)	35 – 60	37.9 ± 11.4	23.5 ± 8.03	38.03
Triglycerides (mg/dL)	40 – 160	144.7 ± 54.1	92.2 ± 55.4	36.3
LDL(mg/dL)	< 160	84.4 ± 34.9	35.08 ± 16.5	58.5
Lp(a)(g/L)	0.01 – 0.74	0.1 ± 0.2	0.07 ± 0.1	46.5
Albumin (g/dL)	3.4 – 4.8	3.9 ± 0.1	3.4 ± 0.2	14.8
Total proteins (g/dL)	6.6 – 8.7	6.1 ± 0.3	4.9 ± 0.5	18.4
Fibrinogen (mg/dL)	200 – 400	329.2 ± 57.2	138.9 ± 22.2	57.8
RCP (mg/L)	0.00 – 5.00	9.04 ± 7.9	5.2 ± 4.6	42.0
α–2Macroglobulin (g/L)	1.30 – 3.00	1.0 ± 0.4	0.5 ± 0.2	49.5

TABLE VI - VISUAL ACUITY AT ONSET (PRE) AND AFTER (POST) THE TENTH TREATMENT SESSION

	Visual acuity* (AMD lesion)			
Pts§	RE+		LE+	
	pre	post	pre	post
1	0.9 (dry)	0.2 (dry)	0.9 (wet)	0.9 (wet)
2	0.8 (wet)	0.8 (wet)	0.9 (dry)	0.0 (dry)
3	0.9 (wet)	0.9 (wet)	0.9 (dry)	0.0 (dry)

* with LogMAR scale; + RE = right eye; LE = left eye; § Pts = patients.

TABLE VII - COMPARISON OF LERICHE-FONTAINE STAGE AT ONSET (PRE) AND AFTER (POST) HEMOPHERESIS TREATMENTS

Pts*	Leriche-Fontaine stage			
	Right foot		Left foot	
	pre	post	pre	post
1	IV	II	IV	II
2	IV	II	I	I
3	IV	IV	I	I
4	IV	II	I	I
5	IV	II	IV	II

*Pts= patients.

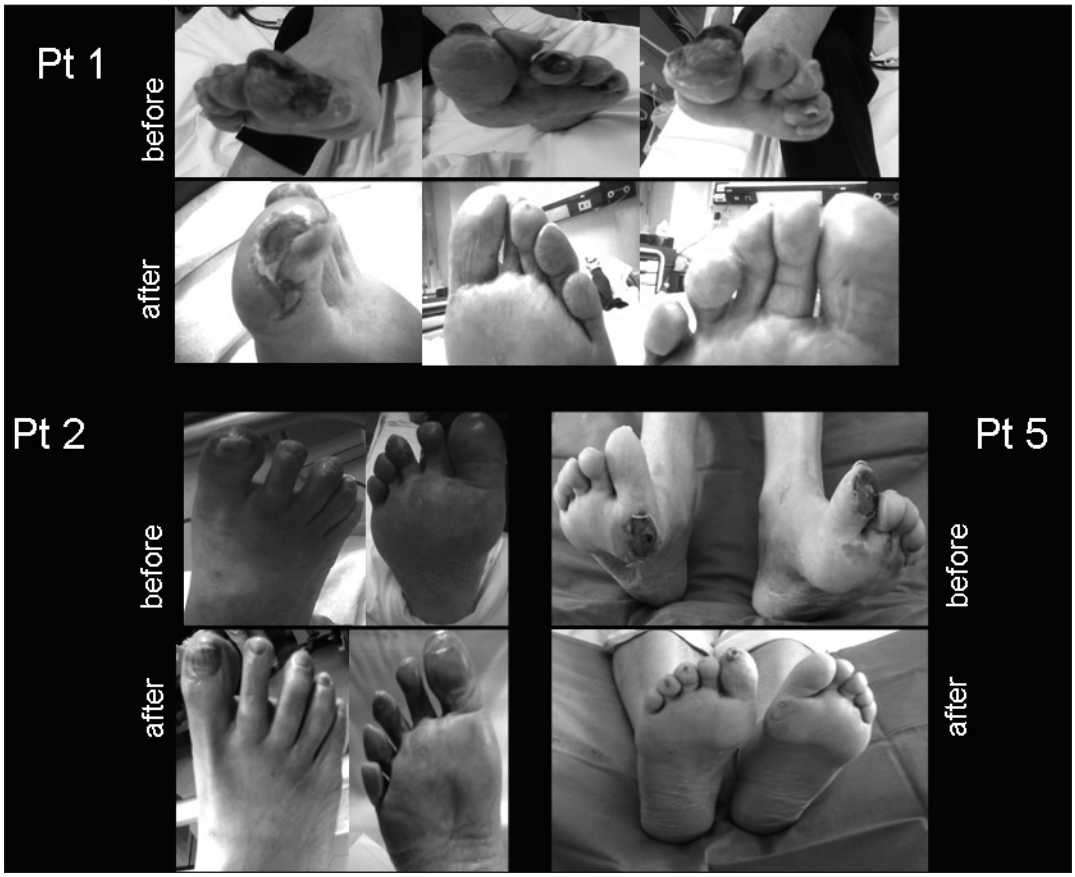

Fig. 2 - *Improvement in ulcers after rheopheretic treatments in patients 1, 2 and 5. Pt = patient.*

transluminal angioplasty of the popliteal artery, however the pain persisted. The fifth was a 65-year-old female patient affected with bilateral ulcers. Since the lesions were distal, there was no indication for revascularization.

In all these patients we observed the complete resolution of pain after three weeks of rheopheresis, with suspension of analgesic therapy and improvement of quality of life. Progressive improvements were observed in four patients, with *restitutio ad integrum* (Fig. 2).

There were no appreciable side effects. However after four treatments, in one AMD case, we removed and repositioned a femoral CVC due to initial deep venous thrombosis, which completely regressed after treatment.

DISCUSSION

The impairment of rheology has been demonstrated to play an important role in the mechanisms of vascular damage. Its clinical role has been evaluated since the 1980s, showing that some high-weight molecules negatively affect tissue oxygenation (12, 13).

Fibrinogen in particular has been demonstrated to represent an independent risk factor for cardiovascular diseases and increases the risk of coronary artery disease, cerebrovascular disease and PAOD (14). Moreover, there is evidence that fibrinogen may hold a key position in the development of obstructive vascular diseases. These observations are confirmed by the Framingham Study (15).

Rheopheresis decreases an exactly defined spectrum of high molecular weight proteins such as fibrinogen, LDL-cholesterol and α-2-macroglobulin. The serial pulses of plasma protein elimination, associated with a possible reduction of plasma viscosity (16-18), can result in an improvement in microcirculation. Randomized, controlled trials on the therapeutic role of rheopheresis in AMD are reported in the literature, confirming its efficacy; however, for PAOD and other cardiovascular diseases, the verdict is still out.

As Klingel has proposed, the goal of rheopheresis is to restore and activate or stabilize the functional reserve,

both in AMD and in PAOD. This is a hypothesis based on preliminary available clinical data but it needs to be confirmed by other clinical studies (19).

Our study confirmed the therapeutic role of rheopheresis in AMD with dry-drusen, with an improvement of visual acuity in all patients.

As far as PAOD is concerned, we observed a complete regression of ischemic pain in all patients after just three weeks of therapy on a twice-weekly program, and a *restitutio ad integrum* of ulcers in 4 out of 5 patients.

The twice-weekly prescription may have the potential to decrease high molecular weight proteins levels and it could be the reason for the quick improvement in ischemic pain. Nevertheless, this program can not be sustained for a long period because of protein loss, such as immunoglobulin and HDL cholesterol. The switch from a twice-weekly to a weekly program prevents the severe loss of these proteins.

In spite of the positive results, we had a great deal of difficulty enrolling more patients. The first reason was vascular access: patients are usually elderly and vasculopathic, so their veins are unable to guarantee a minimum blood flow of 70 to 80 ml/min and femoral vein catheterization is not readily accepted because it exposes the patient to a high risk of deep venous thrombosis and infection. Also, it was difficult to explain rheopheresis therapy to patients without comparing this technique to hemodialytic therapy, with obvious consequent refusal. For these reasons, eleven potential patients refused the informed consent for treatment of their AMD.

CONCLUSIONS

Our experience, however limited, confirms that rheopheresis is a valid and safe therapy for microvascular diseases. In our opinion, rheopheresis will have an important therapeutic role in the future because of the large incidence and prevalence of these kinds of diseases and, in some cases, the lack of other effective therapies.

To date, rheopheresis has shown to have a solid theoretical base, strengthened by controlled randomized clinical trials for dry AMD. Our results justify the attempt to extend the indications of this therapy to other diseases, such as PAOD, and to employ all the resources necessary to conduct larger controlled randomized studies.

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