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Fibrinolytic activity of subretinal fluid after cryopexy

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ABSTRACT: Purpose. *To measure the tissue plasminogen activator antigen (t-PA Ag) content and plasminogen activator inhibitor type 1 (PAI-1) activity of SRF and blood.*
Methods. *22 patients aged from 20 to 77 years (median: 57.3 years), were studied, undergoing retinal detachment surgery. Excluded were patients with vein or arterial disease and any other factors that could change the parameters evaluated. Subretinal samples were obtained at the time of routine drainage during retinal detachment surgery, after cryopexy. Venous blood samples were taken from the cubital vein into sodium citrate solution (9: 1) immediately after induction of anesthesia but before surgery. T-PA Ag concentration and PAI-1 activity in subretinal fluid and citrated plasma and their relation to patients' age and sex, the duration and extent of retinal detachment, and degenerative changes of the retina were assessed.*
Results. *The median t-PA Ag concentration in 22 samples of SRF was 6.7 ng/ml (interquartile range 3.6 ng/ml) and PAI-1 activity 14.0 IU/ml (interquartile range 7.5 IU/ml). The median levels of t-PA Ag and PAI in plasma were respectively 10.7 ng/ml (interquartile range 8.6 ng/ml) and 15.7 IU/ml (interquartile range 12.2 IU/ml). There were no differences between the t-PA Ag concentration and PAI-1 activity in SRF and blood. We found no correlation between the levels of t-PA Ag and PAI-1 activity in SRF and age, sex, the degree of myopia, the duration and extent of retinal detachment, or retinal degenerative changes.*
Conclusions. *The parameters of the firbinolytic cascade studied here indicated the presence of high levels of t-PA Ag and PAI-1 activity in SRF. (Eur J Ophthalmol 1999; 9: 291-6)*

KEY WORDS: Subretinal fluid, Fibrinolysis, Tissue plasminogen activator and inhibitor

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INTRODUCTION

Physiological fibrinolysis depends on the activation of plasminogen into plasmin under the influence of plasminogen activator (PA) (1). These activators are rapidly inactivated by PA inhibitors (PAI). Therefore, fibrinolysis depends on the balance between PA and PAI. Furthermore, free PA are also active proteases involved in tissue remodelling and cell migration, and the initiation of angiogenesis in areas of tissue damage or inflammation (2-5). PA activity is associated with a number of ocular structures (6, 7) but there is not much information about fibrinolytic activity in eyes with retinal detachment (8-11).

The purpose of this study was to measure the t-PA Ag content and PAI-1 activity of subretinal fluid (SRF) and blood in patients with rhegmatogenous retinal detachment and to establish whether these components of the fibrinolytic cascade were related to the patients' age and sex, degree of myopia, the duration and extention of the retinal detachment, and degenerative changes of the retina.

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METHODS

SRF was collected from 22 eyes of 22 patients with rhegmatogenous retinal detachment undergoing retinal detachment surgery. There were 9 females and 13 males, aged from 20 to 77 years (median age 57.3 years). The operations included encircling combined with segmental scleral buckling procedure, all under general anesthesia. All patients gave their written informed consent before admission to the study. The study had been approved by the Ethics Committee, of the Faculty of Medicine, University School of Medical Sciences of Bydgoszcz.

Exclusion criteria were other present illness or medical treatment besides retinal detachment, surgical treatment during the past two years, tobacco smoking, pregnancy during the past two years, menopause with the last menstruation not longer than a year ago, diabetes mellitus, previous or present venous thrombosis in the patient or close relatives. None of the patients received any medication that could affect the hemostatic mechanism. No perioperative thromboembolic prophylaxis was used.

Were analyzed the degree of myopia, duration and extent of detachment, changes in the fundus oculi such as degeneration of the retina (cystic, lattice and retinoschisis) and number of tears of the affected eye.

SRF samples were obtained at the time of routine drainage during retinal detachment surgery, after cryopexy had been done and the encircling band and buckle had been placed. The area around the proposed site of scleral puncture was carefully dried and the puncture was made at the point of the greatest projection of the detached retina. The SRF was slowly drawn from the perforation site into a dry syringe, using the plunger, without penetrating the subretinal space. No clinical complications were observed. Within 15 min of sampling the SRF was centrifuged.

Venous blood samples were taken from the cubital vein into sodium citrate solution (9:1) immediately after induction of anesthesia but before surgery. All venipunctures were done with minimal venostasis following a 15-min rest in recumbent position.

Fibrinolytic studies

Immunosorbent assays (ELISA) were used for the following determinations:

1) The concentration of tissue plasminogen activator antigen (t-PA Ag) (Imulyse, Biopool);

2) The activity of plasminogen activator inhibitor type 1 (PAI-1 activity), with an amidolytic method (Spectrolyse, Biopool).

Statistical analysis

Parameters (non-Gaussian distribution) are presented as median, range and interquartile range. The Mann-Whitney U test was used for independent and ordinal data and the Spearman rank correlation test for ordinal variables. A p-value below 0.05 was considered statistically significant.

RESULTS

The main ocular details of the group, such as refractive error, extent and duration of detachment, number of tears, are presented in Table I. Nine eyes were myopic, with refraction over -3.0 D. Degenerative change in the retina were noticed in 11 eyes.

The median concentration of t-PA Ag in 22 samples of SRF was 6.7 ng/ml (interquartile range 3.6 ng/ml) and in blood plasma 10.7 ng/ml (interquartile range 8.6 ng/ml) (Figs. 1, 2). The median value for PAI-1 activity in 22 samples of SRF was 14.0 IU/ml (interquartile range 7.5 IU/ml) and in blood plasma 15.7 IU/ml (interquartile range: 12.2 IU/ml) (Figs. 3, 4). There were no significant differences between the t-PA Ag concentrations and PAI-1 activity values in SRF and blood (Mann-Whitney U test). We found no correlation between the levels of t-PA Ag and PAI-1 activity in SRF and age, sex, degree of myopia, the duration and extent of retinal detachment, retinal degenerative changes and number of tears which, however, was also correlated with the extension of cryopexy applied (Spearman rank correlation).

DISCUSSION

The pathological changes following retinal detachment include accumulation of macrophages and other cell types, such as retinal pigment epithelial cells in the SRF, leakage from ocular vessels and the degeneration of light receptor cells (10, 12-15). SRF con-

tains components characteristic of both the vitreous body and the blood (10, 12, 14-19). Most biochemical research has focussed on the protein composition of the SRF (16-19). Electrophoretic and immunochemical findings are contradictory, some reports stating that SRF contains components of blood serum protein (11-13). However, one of the fractions always present in normal serum (IgM) was not found in any cases studied, indicating that the permeability of the capillary endothelium and Bruch's membrane is limited (11). Some authors found that normal human vitreous body contains a large amount of serum protein, so the presence of such protein in the SRF does not indicate that it comes from blood (13). Also, the discovery of specific vitreous body protein in the SRF shows that the vitreous body has something to do with the formation of the fluid.

The total protein concentration of the SRF tends to rise with the duration of the detachment (10, 15), possibly indicating increasing permeability of the choroidal capillaries. This suggests the formation of SRF is a dynamic process. Some authors, however, found no correlation between the level of protein in the SRF and the duration of the detachment, and the protein content was high even in very recent detachments (17).

There are few studies of other components in the SRF (9, 10, 14, 18, 20, 21). Siren et al showed that the SRF cell cultures had more proteolytic activity and secreted more urokinase-type plasminogen activator than retinal pigment epithelial cells of donor origin (10). Tripathi et al found the extracellular release of tissue plasminogen activator (t-PA) increased with the phagocytic activity of the retinal epithelium (11).

T-PA has been found in cells as well as in the extracellular matrix (ECM) of many of vascularized tissues of the eye, such as the iris, ciliary body, episclera, choroid, and retina (4). There are two possible sources of t-PA in ECM. The first source are the cells which are the normal components of these tissues, and the second may be the vascular endothelial cells, which produce t-PA. T-PA is released into the circulation from vascular endothelial cells after various stimuli: exercise, venous stasis, trauma (22).

The presence of t-PA in the avascular tissues of the eye, especially the corneal endothelium, epithelium and stroma, lens, vitreous, which are normally free from vascular elements, implies its release either by diffusion (passive or facilitated) or by active transport and highlights its role in processes other than fibrinolysis (3-5, 23, 24). T-PA may catalyze localized pericellular proteolysis in a very wide spectrum of physiological and pathological situations. Increased fibrinolysis may lead to damage of extracellular matrix components, while a defective fibrinolytic system in SRF can favour an adhering clot (3, 5, 22).

To characterize the fibrinolytic potential of SRF we searched for some components of the fibrinolytic cascade. We found t-PA Ag and PAI-1 in every sample. Previous investigators have detected t-PA activity in SRF but only in small number of fluid samples. Immonen et al found t-PA activator only in 5 out of 12 eyes and concluded that the plasmin system was activated in SRF in some eyes with retinal detachment,

TABLE I - REFRACTIVE ERROR, EXTENT AND DURATION OF DETACHMENT, NUMBER OF TEARS IN 22 PATIENTS WITH RHEGMATOGENOUS RETINAL DETACHMENT

Refractive error		Extent of detachment		Duration of detachment		No. of tears	
D	No. of eyes	Quadrants	No. of eyes	Days	No. of eyes	No. of tears	No. of eyes
+6.0 ± 3.0	3	1-1.5	4	<20	10	1	12
+2.9 – -2.9	10	>1.5-2	10	21-40	8	2-3	6
-3.0 – -4.0	4						
4.1 – -7.0	3	>2	8	>40	4	>4	4
over –7.1	2						

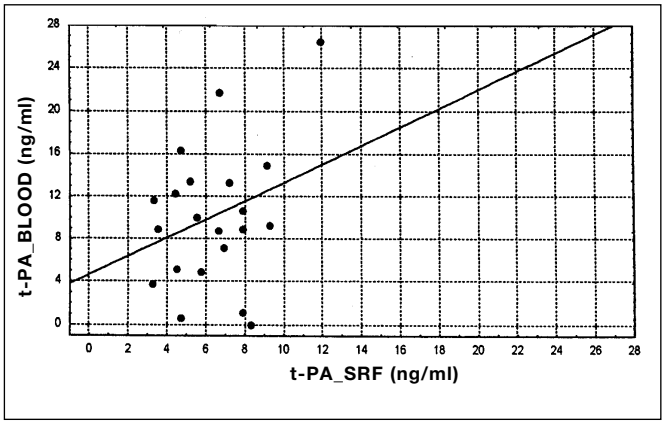

Fig. 1 - *Levels of tissue type plasminogen activator in subretinal fluid (t-PA SRF) and in blood plasma (t-PA blood) in patients with rhegmatogenous retinal detachment.*

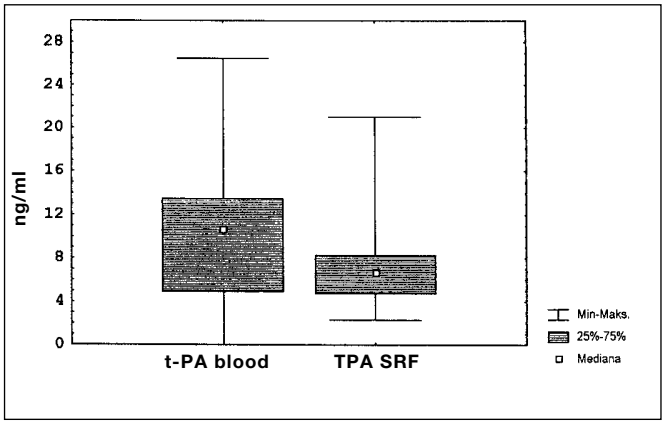

Fig. 2 - *Tissue type plasminogen activator antigen concentration in subretinal fluid (t-PA SRF) and in blood plasma (t-PA blood) in patients with rhegmatogenous retinal detachment (median, range).*

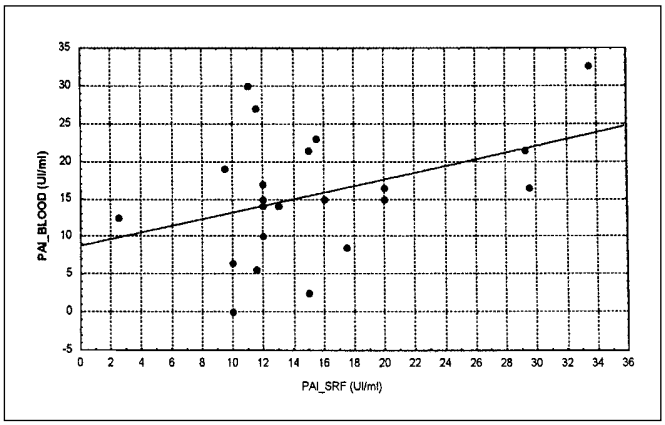

Fig. 3 - *Levels of plasminogen activator inhibitor type 1 activity in subretinal fluid (PAI-1 SRF) and in blood plasma (PAI-1 blood) in patients with rhegmatogenous retinal detachment.*

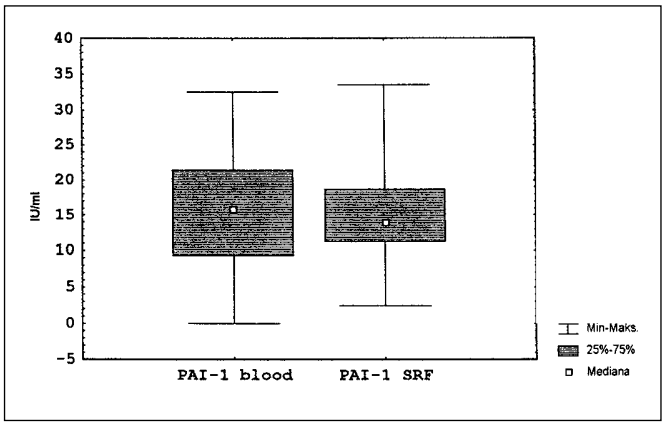

Fig. 4 - *Plasminogen activator inhibitor type 1 activity in subretinal fluid (PAI-1 SRF) and in blood plasma (PAI-1 blood) in patients with rhegmatogenous retinal detachment (median, range).*

t-PA being the main activator (8). Siren et al found active urokinase-type PA production in 11 cell cultures from subretinal fluid (10).

It would be difficult if not impossible to distinguish whether t-PA Ag and PAI-1 are synthesized and secreted by ocular tissues or simply originate from plasma or from, for example, the vitreous humor, anterior chamber fluid or SRF cells (e.g. macrophages or retinal epithelium cells). T-PA Ag and PAI-1 levels in SRF showed they are not significantly different from blood plasma levels. This may indicate that they are exuded from the vascular bed. The cryopexy applied during the surgical procedure may possibly break down the blood-endothelial barrier. The plasma transudate is attributed to a permeability disturbance in the choriocapillaris although leaking vessels of the retina could provide another route (14). However, considering the presence of t-PA in vitreous humor, other possible sources of t-PA Ag and PAI-1 in SRF should not be excluded (3, 4). The retinal pigment epithelium cells or other cells, such as pigmented macrophages, in the SRF may also be a source (10). The release of t-PA associated with phagocytic activity of the retinal pigment epithelium may be involved in the degeneration of photoreceptors, which could also be a source (11).

We found that t-PA Ag and PAI-1 activity levels did not correlate with the duration and extension of the

detachment, nor the extension of cryopexy applied and did not reflect the degree of myopia or degenerative processes in the retina. Thus, t-PA Ag and PAI-1 activity levels in SRF are not indicators of the duration of the disease or the condition of the ocular tissues.

The physiological significance of PA Ag and PAI-1 presence in SRF is unclear. T-PA and PAI are carefully coordinated, providing for prompt control of bleeding with eventual resolution and healing. They certainly play an important role after hemorrhage and clot formation. Tissue degeneration and regeneration are accompanied by fibrin formation and t-PA and PAI regulate the generation of active protease which is plasmin, involved in destructive processes or tissue remodeling (1, 2, 5, 6, 14). Moreover, t-PA plays a role in diseases with an inflammatory component (5, 6, 14).

T-PA binds directly to deposited fibrin and exerts its homeostatic clot-dissolving function by giving rise to clot-localized plasmin. Local secretion of t-PA from endothelial cells in the vicinity of the thrombus may be stimulated by fibrin, by thrombin bound to the thrombus, or by the effects of vessel occlusion. The magnitude and duration of PA activity is a factor that determines whether adhesions are formed and this may play a role in retinal reattachment (1, 2, 5, 6, 14). Plasmin in SRF may also enhance dispersion of pigment ephitelial cells into the subretinal space and the vitreous, a phenomenon seen frequently in eyes with retinal detachment (8, 9). Immonen et al detected plasmin in SRF more often in large detachments, but there was no clear correlation with the duration of the detachment or the characteristics of the retinal holes. They concluded that plasmin in SRF may inhance release of cells from the pigment epithelium by degrading the extracellular matrix and contribute to the development of proliferative vitreoretinopathy (9). That is why the presence of t-PA and PAI-1 in SRF lead us to believe they play a role in the pathophysiology of retinal detachment and reattachment.

From our preliminary observations of the fibrinolytic activity of SRF, it is impossible to determine the extent to which the presence of t-PA and PAI-1 in SRF in the patients studied is caused by breakdown of the blood-retinal barier secondary to cryoapplication. It remains to be verified whether cryopexy does indeed influence the levels of t-PA and PAI-1 in SRF. Although the clinical relevance of our findings is not yet clear we believe they may help to elucidate the role of the local fibrinolytic system in retinal detachment pathophysiology.

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