Influence of Flow-induced Mechanical Forces on Thrombolysis Studied by MR and Optical Microscopy

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Introduction: Thromobolysis remains the mainstay in treatment of ischaemic cerebrovascular stroke, haemodynamically significant pulmonary embolism, and retains a role in the treatment of myocardial infarction in hospitals that do not provide emergency percutaneous transluminal angioplasty. Biochemo-mechanical mechanisms of thrombolysis are not yet entirely understood. In the literature, it is believed that thrombolysis is predominantly a biochemical process in which thrombolytic agent (rt-PA) selectively activates plasminogen bound to fibrin into its active form (plasmin) that cleaves the fibrin meshwork and dissolves the clot [1]. However, the thrombolytic process cannot progress without an adequate transport of the thrombolytic agent into the clot. This is a diffusion process in the absence of blood flow (occlusive clots) and reverts into a perfusion process when the recanalization channel is formed through the clot (non-occlusive clots). The second, perhaps even more important effect of flow are mechanical forces of the flowing blood to the surface of the clots. Both flow induced mechanisms, i.e., the faster thrombolytic agent delivery to the clot and mechanical clot degradation, act in parallel and significantly promote thrombolysis [2]. In this study, MR microscopy was used to study the delivery of thrombolytic agent into the clot and also its dissolution followed after. The MR microscopy study was complemented by optical microscopy of the same process.

Materials and Methods: Non-retracted clots from venous blood of healthy male volunteer were formed in cylindrical glass tubes. The clots were pierced lengthways on the outside at the wall surface by a needle to create a flow channel along the clot. The glass tube with clot was connected by a flexible hose to a pump that generated a constant pressure of either 15 000 Pa or 3000 Pa, which represent the mean arterial and venous pressures in man. The clots were then inserted in the circulation system connected to the RF coil in the center of the magnet. After adding the thrombolytic agent rt-PA and MRI contrast agent Gd-DTPA to the plasma the circulation system was started simultaneously with dynamic MR imaging. Clots were imaged dynamically in 3D by the 3D-RARE method at isotropic resolution of 200 μm, matrix 32 by 32 by 128 points, RARE factor of 32 and inter-echo-time/TR = 3.3/3000 ms. In 40 minutes 20 images of the clot were acquired. In optical microscopy experiments, non-retracted clots were prepared in a special chamber that was connected to a peristaltic pump with a reservoir of thermoregulated human plasma to which rt-PA was added in a therapeutic concentration. The pump generated plasma flow velocities in the chamber of either 30 cm/s or 3 cm/s that were comparable to those in MR microscopy experiments. Microscopy images were captured dynamical with a frame rate of 0.06 fps and exposure time of 20 ms using a CCD camera connected to an optical microscope with a 10x objective.

Results and Discussion: MR microscopy results (Fig. 1a) showed that thrombolysis is strongly flow dependant process in which the recanalization channel progressively widens. The process is faster at the entrance, where the flow is not fully developed, than further downstream. Comparison between the results of thrombolysis in fast and slow flow indicates that the dissolution rate increase is too big that it can be attributed only to biochemical clot degradation. Therefore our assumption is that thrombolysis progresses by dislodgment of cell agglomerates of which size are flow dependant. The assumption was confirmed by optical microscopy results (Fig. 1b; cell agglomerate is encircled) which showed that the cell agglomerate sizes increase with flow velocity.

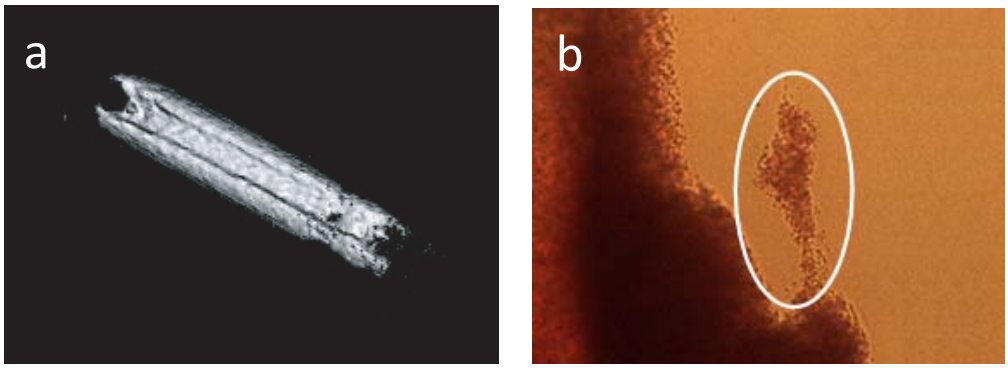

Figure 1: 3D MR microscopy (a) and optical microscopy (b) of thrombolysis.

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2. Sersa, I., et al., *Modelling the effect of laminar axially directed blood flow on the dissolution of non-occlusive blood clots.* Phys Med Biol, 2007. **52**(11): p. 2969-85.