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**Theme Issue Article**

# Turbulent axially directed flow of plasma containing rt-PA promotes thrombolysis of non-occlusive whole blood clots *in vitro*

Gregor Tratar[1], Aleš Blinc[1], Mitja Štrukelj[1], Urša Mikac[2], Igor Serša[2]

[1]Department of Vascular Medicine, University of Ljubljana Medical Centre, Ljubljana, Slovenia
[2]Condensed Matter Physics Department, Jožef Stefan Institute, Ljubljana, Slovenia

**Summary**

The rate of thrombolysis markedly decreases after a thrombosed vessel is partly recanalized and the remaining clot poses serious risk for rethrombosis. We studied *in vitro* how thrombolysis depends on penetration of plasma containing thrombolytic agents – 0.2 μg/ml rt-PA or 250 IU/ml streptokinase (SK) - and the magnetic resonance contrast agent Gd-DTPA (at 1 mmol/l) into non-occlusive clots under conditions of fast (turbulent) or slow (laminar) axially directed flow. Cylindrical non-retracted (fresh) or retracted (aged) whole blood clots were pierced lengthways and connected to a perfusion system. Dynamical spin-echo MRI was used for measuring the penetration of labeled plasma into clots and for assessing the remaining clot size. In both types of clots fast flow enhanced the penetration of Gd-DTPA-labeled plasma into clots in comparison to slow flow. In non-retracted clots, lysis with rt-PA and to a lesser extent also lysis with SK followed the path of plasma penetration into clots. After 40 minutes of fast axially directed flow rt-PA resulted in almost complete lysis and SK left only about a third of the clot undissolved, whereas with slow flow lysis was much slower (undissolved clot: 86 ± 5 % with rt-PA and 95 ± 1 % with SK). In retracted clots, substantial lysis was possible only with rt-PA and rapid flow (53 ± 28% of the clot undissolved after 60 min), whereas the use of SK or slow flow precluded meaningful lysis. We conclude that rapid (turbulent) axially directed flow of plasma along non-occlusive blood clots causes forceful exchange of serum inside the clot with outer plasma which enhances both fibrin-specific and non-fibrin-specific lysis of fresh clots. Dissolution of non-occlusive retracted (aged) clots occurs only under fibrin-specific conditions combined with adequate transport of rt-PA into clots.

**Keywords**

Flow, turbulent, thrombolysis, blood clots

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## Introduction

Fibrinolytic therapy is increasingly used in the early treatment of ischemic stroke (1-3) and the management of hemodynamically significant pulmonary embolism (4, 5). It also plays a role in the treatment of acute and subacute arterial thrombosis (6), and remains the mainstay of treatment of myocardial infarction in hospitals that do not provide emergency percutaneous transluminal angioplasty (7).

The success of thrombolyis depends on properties of the thrombolytic agent, structure of the thrombus and characteristics of molecular transport into the thrombus (8). Non-occlusive clots are initially recanalized by slowly penetrating channels caused by permeation of plasma containing thrombolytic agents, but are almost never completely dissolved when blood flow is re-established along the remaining clot (8-10). The residual clot impedes normal blood flow and poses a risk for rethrombosis. The mechanisms of mass transport by diffusion

Correspondence to
Dr. Aleš Blinc
Department of Vascular Medicine
University of Ljubljana Medical Centre
Zaloška 7, 1525 Ljubljana
Slovenia
Tel.: +386 1 522 80 32, Fax: +386 1 522 80 70
E-mail: ales.blinc@trnovo.kclj.si

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and permeation through occlusive clots have been fairly well elucidated (11-17), but relatively little is known about the influence of axially directed flow along blood clots on thrombolyis. Nishino et al. have reported that increasing axially directed flow of plasma containing non-fibrin specific plasminogen activators impedes clot lysis except when it mechanically fragments the clot by high shear forces (18). Komorowicz et al. have reported that increased flow of plasma with plasmin, mini-plasmin or elastase increases fibrin clot dissolution (19). Sakharov and Rijken have used compacted plasma clots where rapid, turbulent flow accelerated fibrin specific-lysis up to 10-fold in comparison to lysis without flow. Paradoxically, it retarded non-fibrin-specific lysis because of flow-enforced "plasminogen steal" (20). By photographing the distribution of FITC-plasminogen in the lysing clot through a fluorescent microscope, they have demonstrated that rapid flow enhances transport of outer plasma into the clot (20).

Our aim was to directly measure *in vitro* the radially directed penetration of flowing, pramagnetically labeled plasma into non-occlusive whole blood clots and the ensuing clot lysis under conditions of fast or slow axially directed flow using magnetic resonance imaging. Streptokinase and recombinant tissue plasminogen activator were used as fibrinolytic agents, and whole blood clots were either non-retracted, simulating fresh thrombi, or retracted, simulating older thrombi.

# Materials and methods

## Blood clots

Blood was collected from the cubital vein of a healthy male volunteer into tubes (Vacutainer, Becton-Dickinson, Germany), containing 1 volume part of 0.129 mol/l Na-citrate and 9 volume parts of blood. Clotting was induced *in vitro* by adding calcium (50 µl $CaCl_2$ at 2 mol/l per ml of blood) and thrombin (Thrombin, Sigma, Germany) in a final concentration of 1 NIH unit/ml of blood.

### Non-retracted clots

In our first series of experiments clot retraction was inhibited by the phosphodiesterase inhibitor UDCG 212 (Boehringer, Germany) in a final concentration of 20 µmol/l (21) that was added to the citrated blood before mixing it with thrombin. Non-retracted clots were formed in cylindrical glass tubes with an inner diameter of 3 mm and length of 3 cm. The inner surface of glass tubes was mechanically etched and covered by a layer or purified fibrin in order to assure good adherence of the clots (22). After at least an hour at room temperature to allow for fibrin formation, the clots were pierced lengthways by a needle with a diameter of 0.7 mm to create a channel for the plasma flow. The tubes were connected to the perfusion system.

### Retracted clots

In our second series of experiments blood clots were formed in tubes with an inner diameter of 1 cm and length of 4 cm, and were detached from the tube wall 15 minutes after the addition of thrombin in order to allow for clot retraction at room temperature during the next 3 hours. The surface of retracted clots was gently wiped by tissue paper and the clots were glued into tubes with an inner diameter of 6 mm and length of 3 cm by a tixhotropic, low reactive type unsaturated polyester (Heliopol, Helios, Slovenia) that was activated by cobalt naphtenate and organic peroxyde catalysts. After hardening of the polyester which occurred overnight (for at least 12 hours), the clot bases were partly exposed and the clots were pierced lengthways by a needle with a diameter of 1.2 mm to create a channel for the plasma flow. A larger needle diameter was needed to reliably provide for uninterrupted flow through the channel after connection to the perfusion system than in non-retracted clots.

### Extracellular space in blood clots

Blood clots were weighed before retraction ($m_1$) and after retraction ($m_2$). The difference between $m_1$ and $m_2$ represented the mass of the extruded serum and blood cells ($m_{ex}$). All masses were converted to volumes assuming a specific density of $1 g/cm^3$. The haematocrit of the donor blood (Ht) – taking into account the dilution by citrate, calcium and thrombin solutions – and the haematocrit of the extruded fluid ($Ht_{ex}$) were measured. The fraction of extracellular volume of the non-retracted clots was 1- Ht, and the fraction of extracellular volume of retracted clots was $V_e/V_2$, where the extracellular volume ($V_e$) was calculated as $V_e = V_2 - V_c$, and the cellular volume ($V_c$) as $V_c = V_1 \cdot Ht - V_{ex} \cdot Ht_{ex}$.

## Perfusion system and the velocity of plasma flow along the clot

Tubes with non-occlusive blood clots were connected via plastic hoses to a reservoir of plasma and an electric rotor pump that drove heparinized plasma with 10 U/ml of unfractionated heparin (Krka, Slovenia) through the perfusion system (Fig. 1).

Rapid flow of plasma was generated by a pump generating a static pressure equivalent to a water column of 1.5 m, i.e., 113 mmHg, whereas slow flow was generated by a weaker pump, able to lift a water column 0.3 m high, i.e., 22.5 mmHg, that was in addition connected to a serial flow resistor, i.e., a needle with an outer diameter of 0.7 mm and length of 45 mm.

Outdated frozen human plasma (ABO and Rh-matched to the blood group of the donor) was provided by the Blood Transfusion Centre of the Republic of Slovenia. Plasminogen was measured in plasma by a chromogenic substrate method (27) before adding the thrombolytic agent after 60 minutes. The values were expressed as a percentage of the normal, pooled plasma value that is about 200 mg/ml. After testing by MRI imaging that blood clots were stable in the running perfusion

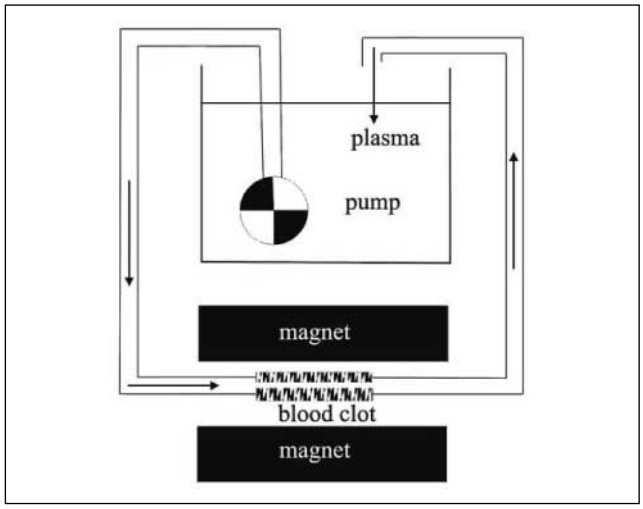



**Figure 1:** Scheme of the perfusion system. The tube containing a non-occlusive blood clot was placed into the MRI magnet and connected via plastic tubing to a reservoir containing plasma and a rotor pump.

system for 30 min, plasma was mixed with 1 mmol/l of the paramagnetic contrast agent Gd-DTPA (Magnevist, Berlex Lab., Germany) and the thrombolytic agent. We used either streptokinase (Streptase, Behring, Germany) in a concentration of 250 IU/ml or recombinant tissue type plasminogen activator – rt-PA, (Actilyse, Boehringer,Germany ) at 2 μg/ml. Control clots were perfused for 60 min without adding the thrombolytic agent.

Volume flow ($\Phi_v$) of plasma was measured through non-occlusive blood clots and clay models that were pierced by a 0.7 mm or 1.2 mm diameter needle, and empty tubes, corresponding to clots after complete dissolution. Knowing the volume flow ($\Phi_v$) and the cross-section of the flow channel (S) we calculated the average flow velocity (v) as: $v = \Phi_v / S$. The shear rate (SR) which would result with laminar flow was calculated as $SR = 4 \cdot v/R$, where R was the radius of the channel.

## Conditions of clot lysis

Each type of clot, the non-retracted and retracted ones, were subjected to 4 regimes of clot lysis: applying rapid or slow tangential flow of plasma, and the fibrin-specific thrombolytic agent rt-PA or the non-fibrin-specific streptokinase. There were 4-7 clots in each group.

## Magnetic resonance imaging and image analysis

Magnetic resonance imaging (MRI) was conducted in a 2.35 T superconducting magnet (Oxford, England) using Bruker's RF and gradient coils and TecMag's NMR spectrometer. We used T1-weighted spin-echo MRI, single scan, repetition rate TR = 400 ms, interecho time TE =12.5 ms, field of view 2 × 2 cm and resolution 256 × 256 pixels, slice thickness 2 mm. Image acquisition time was about 2 min. Dynamical imaging was performed for 40 – 60 minutes. The MR signal intensity was recorded on a grey scale from 0 – 255.

Penetration of Gd-DTPA labeled plasma into the extracellular space of the clots was determined by MRI signal intensity that was higher in the presence of Gd-DTPA than in its absence (9). MR images of intact clots and clots perfused with plasma containing 1 mmol/l Gd-DTPA were recorded (Fig. 2). The Gd-DTPA penetrated parts of the clot were defined as areas of increased MR intensity ($I_{pen}$) that was equal or higher than the arithmetic mean of the signal intensity of the completely perfused clot ($I_{max}$) and the intact clot ($I_{min}$): $I_{pen} > (I_{max} + I_{min}) / 2$ . In each analyzed image we measured $I_{max}$ in the part of the clot adjacent to the flow channel, and $I_{min}$ in the part of the clot that was remote from the flow channel and had the lowest MR

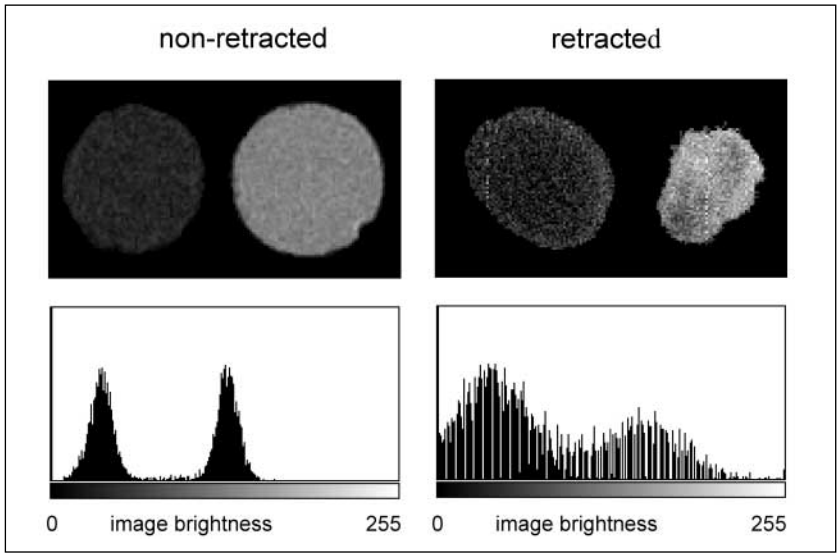



**Figure 2:** Transverse axial MR images of non-retracted and retracted blood clots that were either intact (left cross-section) or completely perfused with plasma containing 1 mmol/l Gd-DTPA (right cross-section). Below, the histograms of pixel intensities on a gray scale from 0 to 255 are shown, indicating that perfusion with Gd-DTPA strongly enhanced image intensity in both non-retracted clots (from 36 ± 7 to 131 ± 8) and in retracted clots (from 60 ± 26 to 146 ± 35 ).

image intensity. With rapid flow of plasma the channel in the clot was completely dark due to the flow-void phenomenon (26), whereas with very slow flow or no flow the Gd-DTPA labeled plasma was exhibiting a bright MR signal intensity.

The transverse sections through the middle of the clot were taken as index slices for measuring penetration of plasma and clot size because they represented the clot most reliably. In pilot experiments we noted that the proximal and middle clot sections behaved similarly, whereas the distal sections displayed more variability, presumably due to "embolised" fragments from the proximal parts of the clot. Longitudinal clot slices were viewed only in pilot experiments because it was difficult to align the slice exactly along the path of the flow channel. Thus, clot size was measured as the remaining part of the initial cross section on transverse sections through the middle of the longitudinal axis of the clot. For image analysis, we used the program ImageJ (National Institute of Health, USA).

### Statistical methods

For statistical analysis, Statistica 4.5 software (StatSoft, Tulsa, USA) was used. Variables were tested for normal distribution by the Kolmogorov-Smifrnoff test and are presented as means and standard deviations. Differences between groups were tested by the Student's t-test, and the p values were corrected for multiple comparisons by Bonferroni's correction.

## Results

### Non-retracted clots

With the stronger pump, the flow through the perfusion system with clots pierced by a 0.7 mm wide needle was initially 1.63 ± 0.05 ml/s which corresponded to an initial velocity of 426 ± 13 cm/s through the 0.7 mm diameter channel (estimated average shear rate of $48.6 \cdot 10^3\ s^{-1}$). The flow increased to 6.07 ± 0.21 ml/s with complete lysis, but the velocity through the widened, 3 mm diameter channel fell to 86 ± 3 cm/s. With the weaker pump and the serial flow resistor the initial flow was 0.12 ± 0.01 ml/s which corresponded to an initial velocity of 31 ± 4 cm/s through the 0.7 mm diameter channel (estimated average shear rate of $3.5 \cdot 10^3\ s^{-1}$). The flow slightly increased to 0.15 ± 0.01 ml/s with an empty tube which corresponded to a velocity of about 2 cm/s through a 3 mm diameter channel.

Penetration of contrast agent-containing plasma depended on the velocity of plasma flow along the clot. After 6 minutes, Gd-DTPA -permeated areas were found 2.0 ± 0.4 mm from the border of the flow channel with rapid flow, but only 0.4 ± 0.13 mm with slow flow ($p < 0.001$). Typical images of Gd-DTPA-labeled plasma penetration into non-retracted clots are shown in Figure 3 and the corresponding graph is shown in Figure 4a. There were no differences in the initial rate of Gd-DTPA penetration between clots lysed with rt-PA and those lysed with SK. After 6 min clot lysis with rt-PA became pronounced, thus the diameter of the flow channel increased and the distance from the channel border was no longer a valid measure for Gd-DTPA penetration.

Clot lysis was much faster with rapid flow than with slow flow (Fig. 5a). With rt-PA the differences in the remaining cross-sections became significant at 8 min: 62 ± 23 % with rapid flow and 98 ± 0.1 % with slow flow ($p = 0.02$). After 40 min with rapid flow and rt-PA, lysis was nearly complete with only 5 ± 4 % of the clot cross- section remaining, whereas in the slow flow regime there was still 86 ± 5 % of the clot cross-section remaining. With SK lysis was less efficient, but still much faster when flow was rapid than when it was slow. After 40 minutes of exposure to rapid flow there was 34 ± 18 % of the clot left, whereas with slow flow lysis was negligible with 95 ± 1 % of the clot still in place ($p = 0.02$). Typical examples of clot lysis with turbulent and laminar flow, in both cases with either rt-PA or streptokinase, are shown in Figure 6.

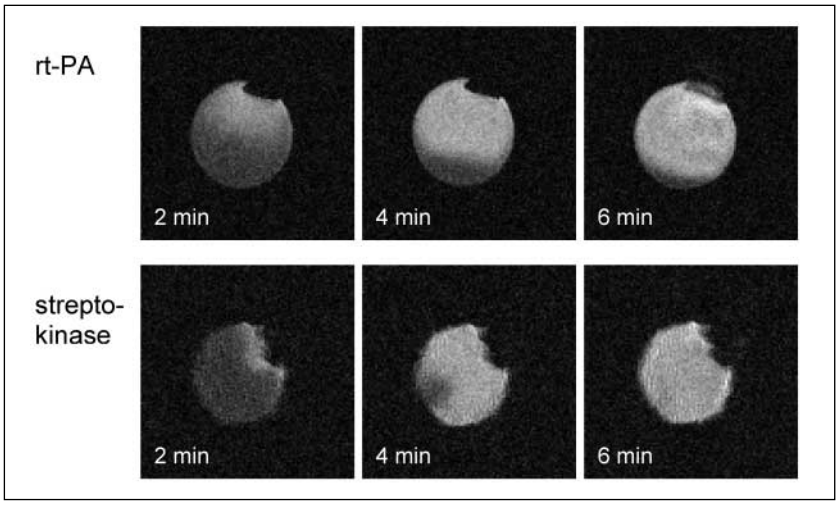



**Figure 3:** MR images of plasma penetration into non-retracted blood clots under conditions of fast (turbulent) flow. The images were recorded after 2, 4, and 6 minutes of fast tangential flow of plasma mixed with thrombolytic agent (rt-PA or streptokinase) and the MRI contrast agent Gd-DTPA at 1 mmol/l. Bright areas correspond to regions permeated by plasma containing MRI contrast agent. No difference was observed between penetration of Gd-DTPA-labeled plasma containing rt-PA or streptokinase.

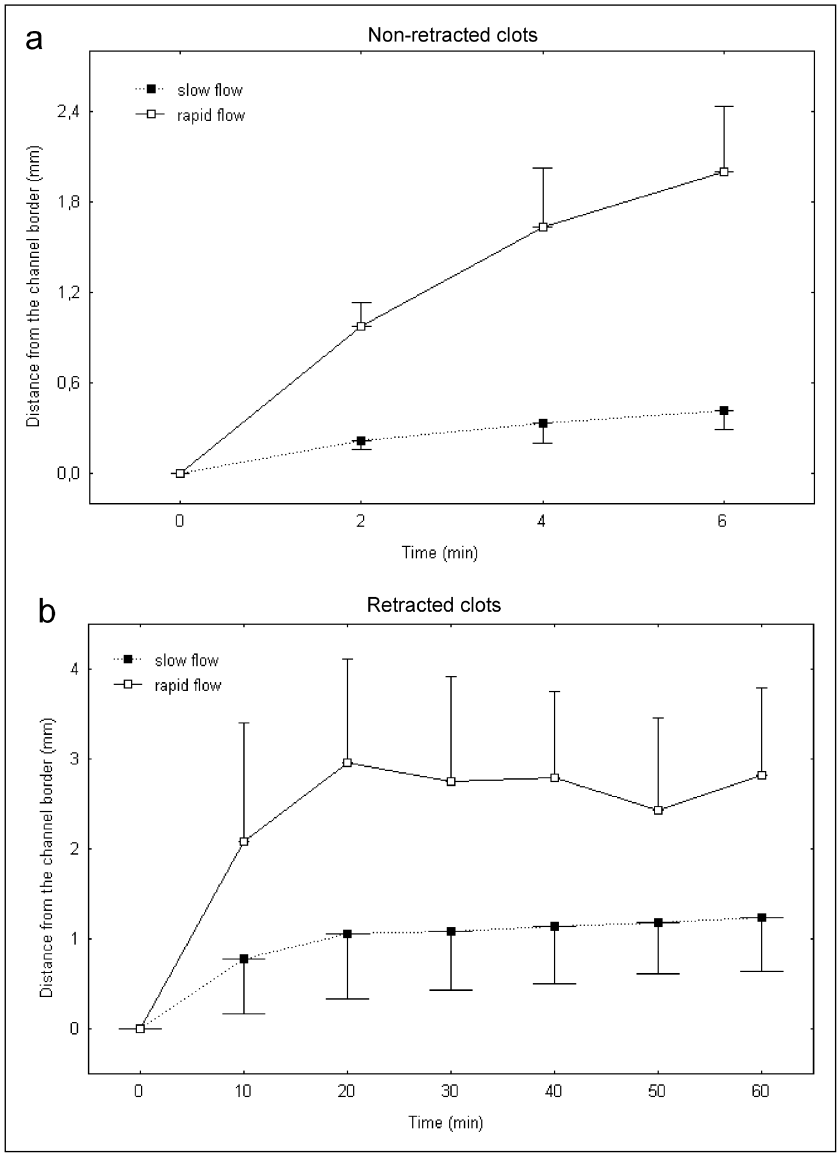



**Figure 4:** Graphs of Gd-DTPA-labeled plasma penetration into (a) non-retracted and (b) retracted clots. The maximum distance from the perfusion channel to area with contrast-enhanced MR image intensity ($I_{pen}$) was plotted against time. Means and standard deviations are shown. In non-retracted clots only the first 6 minutes of perfusion are shown because by that time rapid flow of Gd-DTPA labeled plasma resulted in almost complete permeation of clots. In retracted clots the penetration of plasma with rapid flow was also much faster than with slow flow and reached a plateau, corresponding to the outer clot border, after about 20 min. With slow flow, the penetration was slow and consistent with the laws of diffusion. The observed penetration distances corresponded reasonably well to the expected transport by diffusion, calculated as $\sqrt{2 \cdot D \cdot t}$, where D is the diffusion constant of Gd-DTPA in water, i.e., $3.3 \cdot 10^{-10}$ $m^2 s^{-1}$, and t is the time from the beginning of the diffusion process.

## Retracted clots

Retraction reduced the mass of blood clots from $m_1$ = 3.4 ± 0.2 g to $m_2$ = 1.2 ± 0.1 g, which represented 34 ± 2 % of the initial mass. The haematocrit of the donor blood, diluted by the volume of the citrate, calcium and thrombin solutions (Ht) was 37 %, whereas the haematocrit of the extruded fluid ($Ht_{ex}$) was 14 ± 1 %. The extracelluar compartment of the clot was thus reduced from more than 60 % before retraction to 18 ± 4 % after retraction.

With the stronger pump, the flow through the perfusion system with clots pierced by a 1.2 mm wide needle was initially 1.55 ± 0.99 ml/s which corresponded to an initial velocity of 137 ± 88 cm/s through the 1.2 mm diameter channel (estimated average shear rate of $9.1 \cdot 10^3$ $s^{-1}$). With the weaker pump and the serial flow resistor the initial flow was 0.10 ± 0.02 ml/s which corresponded to an initial velocity of 8 ± 2 cm/s (estimated average shear rate of $0.5 \cdot 10^3$ $s^{-1}$). Using pierced clay models mimicking completely lysed retracted clots, the end-stage flow averaged 4.2 ml/s with the stronger pump which corresponded to a velocity of 59 cm/s through a 3 mm diameter channel, whereas with the weaker pump and serial resistor the end-stage flow averaged 0.16 ml/s which corresponded to a velocity of about 2 cm/s through a 3 mm wide channel.

Similar to non-retracted clots, permeation of Gd-DTPA into retracted clots was faster with rapid than with slow flow (Fig. 4b). After 10 minutes Gd-DTPA -permeated areas were found 2.1 ± 1.3 mm from the border of the flow channel when exposed to rapid flow, but only 0.8 ± 0.6 mm when exposed to slow flow ($p = 0.049$). With rapid flow the permeation reached a plateau after approximately 20 minutes, whereas with slow flow there was slow, but steady progression of permeation of Gd-DTPA into clots. The differences were statistically significant throughout the observation period.

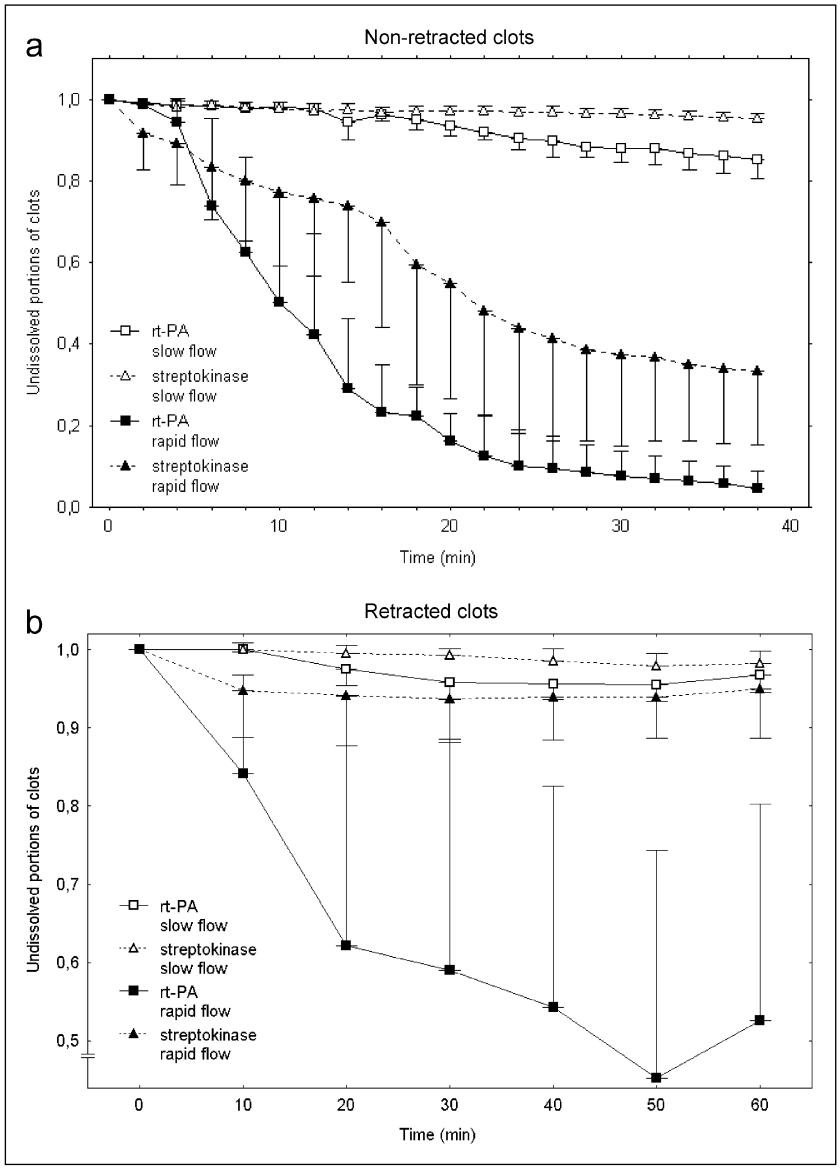



**Figure 5:** Time dependence of (a) non-retracted and (b) retracted clot dissolution with rt-PA and SK, both with rapid or slow tangential flow. In non-retraced clots maximal lysis was achieved with rt-PA and rapid tangential flow, but SK and rapid flow also resulted in appreciable lysis. Slow tangential flow resulted in only very modest lysis with rt-PA and negligible lysis with SK. In retracted clots, substantial lysis, albeit only about 50% in $^3/_4$ of an hour, was achieved only by combining rt-PA and rapid tangential flow, whereas the use of SK or slow flow precluded meaningful lysis of retracted clots.

Substantial lysis of retracted clots was achieved only when rt-PA was used under conditions of rapid tangential flow (Fig. 5b). After 30 minutes of exposure to rapid flow the remaining cross-section was 59 ± 29 % with rt-PA and 94 ± 6 % with SK ($p = 0.03$). At the end of the observation period at 60 min the remaining cross-section was reduced to 53 ± 28 % with rt-PA, whereas SK had virtually no effect with 93 ± 6 % of the clot still intact. With slow flow, neither rt-PA nor SK had any effect on the retracted clots, with 97 ± 2 and 98 ± 2 % of the clot still intact after 60 min. Typical MR images of retracted clots under the four different experimental conditions are shown in Figure 7.

### Plasminogen

The concentration of plasminogen in plasma was not reduced significantly when rt-PA was used: 1.01 ± 0.57 of the normal pooled plasma value before adding 2 μg/ml rt-PA and 0.72 ± 0.04 after 60 minutes. As expected, the concentration of plasminogen was reduced with SK: from 1.35 ± 0.21 of the normal pooled plasma value before lysis to 0.38 ± 0.15 after 60 minutes ($p = 0.003$).

## Discussion

In our study we employed dynamic MRI to simultaneously measure the transport of tangentially flowing Gd-DTPA-labeled plasma into non-occlusive whole blood clots *in vitro* and to follow the ensuing thrombolysis by streptokinase or rt-PA. Clots were either non-retracted or fully retracted and were exposed to axially directed flow of plasma that was either slow and laminar or fast and turbulent. The study was designed as an extension of the work of Sakharov and Rijken who studied effects of tangential flow on fibrin specific and non-fibrin-specific throm-

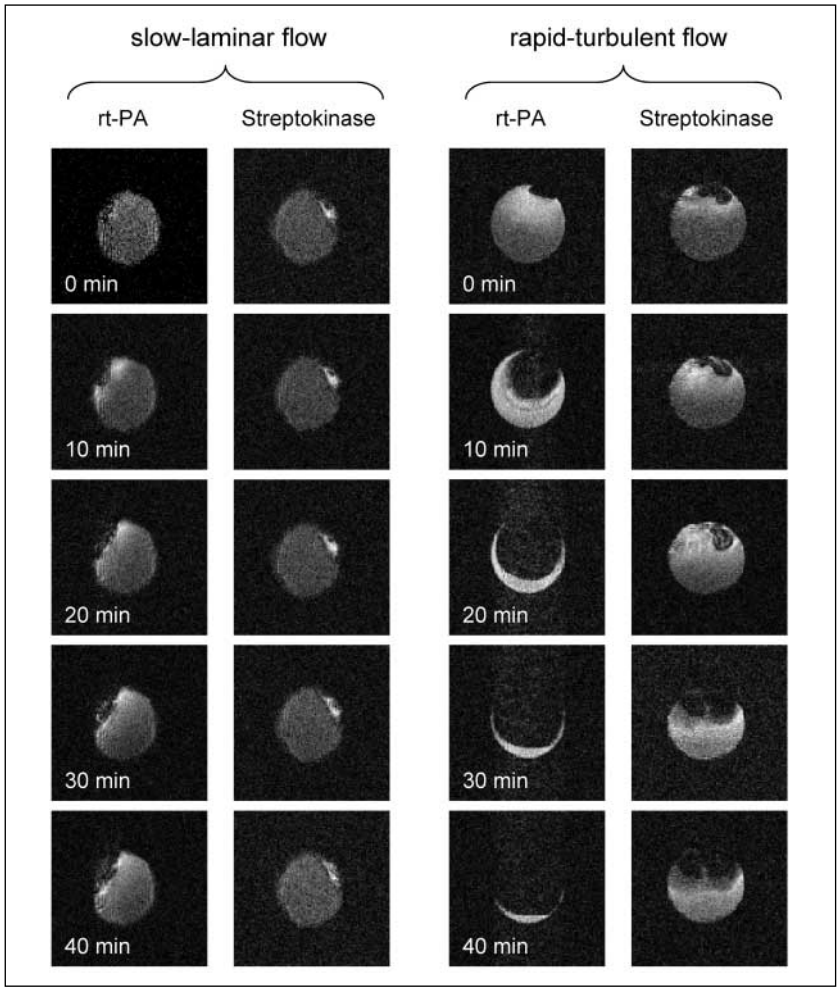



**Figure 6:** Typical MR images of non-retracted clots undergoing lysis by slow – laminar – or rapid – turbulent – tangential flow, in both cases either with rt-PA or with SK. Bright areas around the flow channel indicate the extent of Gd-DTPA labeled plasma penetration into the clot, which was significantly faster with turbulent flow than with laminar flow. With rt-PA the extent of lysis closely followed the path of plasma penetration, whereas with SK lysis did not strictly follow plasma penetration, probably because of plasminogen depletion.

bolyis of thin, perpendicularly shaped non-compacted plasma clots by serial photographing of the clot size and the distribution of Blue dextran or FITC-plasminogen in the clots (20). In our study the clots were cylindrical, consisted not only of the fibrin network and entrapped plasma proteins, but also contained blood cells. Since whole blood clots are not translucent, observation from the outside was no longer possible. MRI has already proven its capability of following the transport of Gd-DTPA-labeled plasma into non-retracted whole blood clots and measuring the ensuing clot lysis by rt-PA (12), therefore it was our method of choice in the experiments that were designed to shed additional light on the mechanisms of clot dissolution after flow through a partly dissolved clot has been reestablished. The measurements were done on transverse sections through the middle of the clot that were found to be the most representative in pilot experiments.

Our first finding is that rapid flow above the threshold of turbulence induces much more rapid exchange of outer plasma with the serum inside the clot than does slow laminar flow. This confirms the results of Sakharov and Rijken who documented accelerated transport of Blue-Dextran containing plasma and FITC-plasminogen into plasma clots exposed to turbulent flow (20). We found that outer plasma penetration into clots did not depend on the choice of thrombolytic agent, but we noted profound differences in the rate of clot lysis between fibrin-specific rt-PA and non-fibrin specific streptokinase. The two thrombolytic agents were used in concentrations that can be expected after intravenous administration into an average-sized person for treatment of myocardial infarction (25), whereas concentrations of locally applied thrombolytic agents during intrarateral lysis are much more variable due to different dosing schemes and variable haemodynamic conditions. Non-retracted clots, mimicking fresh thrombi, were almost completely lysed after 40 min of exposure to turbulent flow of plasma containing 2 μg/ml of rt-PA, whereas with 250 IU/ml of streptokinase about $^{1}/_{3}$ of the clot remained non-dissolved although it had been thoroughly permeated by outer plasma. This is also in accordance with the results of Sakharov and Rijken who described incomplete lysis of non-compacted plasma clots exposed to rapid flow and non-fibrin specific conditions due to plasminogen depletion inside the clot (20). When axially directed flow of plasma was slow, only about 14% of the

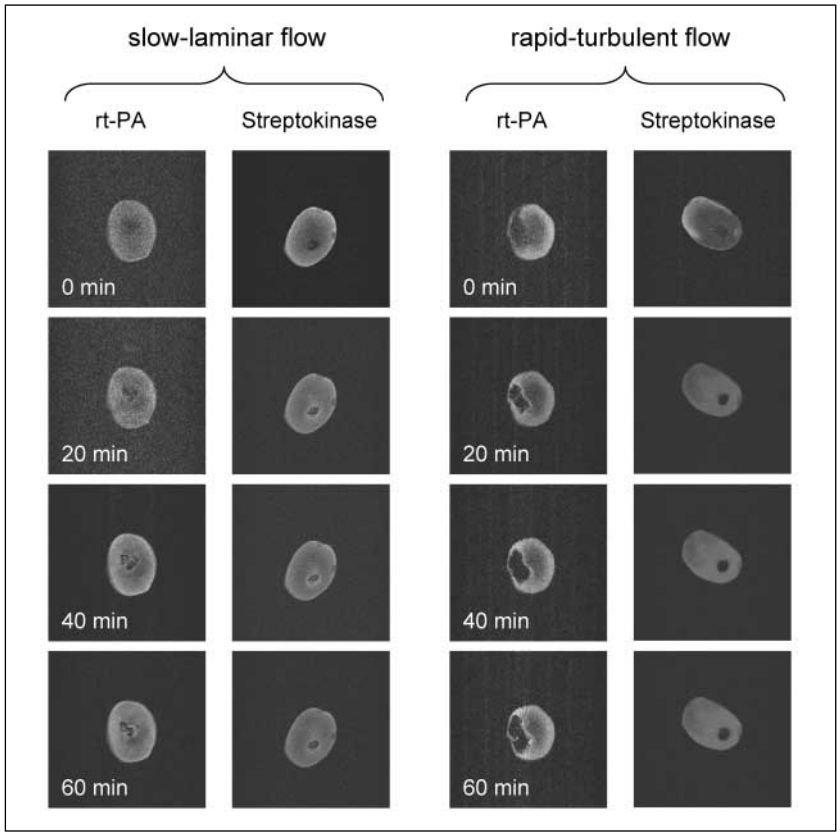



**Figure 7:** Typical MR images of retracted clots undergoing lysis by slow – laminar – or rapid – turbulent – tangential flow, both either with rt-PA or with SK. As with non-retracted clots, the rate of Gd-DTPA-labeled plasma penetration into the clots, indicated by the progression of bright areas around the flow channel, was faster with rapid than with slow flow. With rt-PA clot lysis followed the path of plasma penetration, but with SK there was virtually no lysis irrespective of plasma penetration into the clot. The bright rim at the border of the clot, that was nearly always present from the beginning of the observation and did not change much with time, probably resulted from the interaction of the clot with the polyester glue that was activated by cobalt naphtenate and organic peroxyde catalysts.

clot was lysed with rt-PA in 40 min, whereas streptokinase had virtually no lytic effect. This result shows the enormous importance of transport processes for successful thrombolysis. With laminar axially directed flow the components of the fibrinolytic system can enter the clot only by thermally activated diffusion, whereas with turbulent flow in the presence of local geometric non-uniformities local vortices are created that seem to forcefully propel outer plasma into clots in exchange for the serum originally entrapped inside of them. We could observe only the influx of labeled plasma but not the efflux, because the small quantities of out-flowing serum were immediately mixed with the much larger pool of circulating labeled plasma. Pressure driven permeation along the pierced clot did not play an important role in transporting labeled plasma and its fibrinolytic components into the clot although there was still a substantial pressure drop along the clot when the stronger pump was used. Transport of labeled plasma proceeded radially from the flow channel and not along additional channels through the clot. Also, in pilot experiments we did not observe a consistent time lag in radial progression of labeled plasma between the proximal and mid-sections of clots. In pilot experiments with longitudinal slices we saw that labeled plasma entered the clots from the flow channel in wave-like fronts when flow was rapid and turbulent, and in practically linear, slowly progressive fronts when flow was slow and laminar.

Retracted clots, that are much more resistant to thrombolysis than non-retracted ones (24), were used to mimic thrombolyis of older thrombi. Retraction decreased the proportion of extracelluar space in the clots from about 60 % to about 18 %, but this was still sufficient for effective paramagnetic labeling of plasma transport into clots and for measuring clot lysis. Again, penetration of Gd-DTPA labeled plasma was much faster with rapid than with slow axially directed flow, which can be ascribed to the effects of turbulence. The pattern of penetration of outer plasma into retracted clots was less regular than in non-retracted clots, probably because clot retraction is not a uniform process. Successful thrombolysis that decreased the clot cross-section by about $^1/_2$ in 60 min was achieved only by combining rapid, i.e., turbulent, flow with fibrin specific rt-PA. Under these conditions, parts of retracted clots were often detached and either extruded from the field of observation or lodged into the perfusion channel as “emboli”, whereas virtually no lysis was achieved with streptokinase even in the presence of rapid axially directed flow. This proves that clot dissolution was not an artifact of rapid flow itself. Sabovic and coworkers have described that retracted clots can only be dissolved by fibrin specific thrombolytic agents unless they are enriched by additional plasminogen (24). When tangential flow was slow and laminar, neither rt-PA nor streptokinase resulted in any meaningful lysis in 60 min, which can be explained by inadequate

molecular transport into the clot in addition to the lack of non-fibrin-bound plasminogen in the presence of streptokinase.

Our study had several limitations. We did not actually label plasminogen, streptokinase or rt-PA, but rather the water molecules of plasma through their dynamic interaction by Gd-DTPA. The medium-sized, paramagnetic Gd-DTPA remains confined to the extracellular space because it is hydrophilic and does not bind to proteins (26). Theoretically, transport of rt-PA and plasminogen into clots may be hindered by binding to fibrin (14, 15). However, the pores between fibrin strands and entrapped blood cells in whole blood clots are of micrometer size (27) which is huge on the molecular scale and reduces the quantitative importance of binding when plasma is pushed through the pores by bulk flow. However, binding may still be important when molecular transport is limited to diffusion.

Axially directed plasma flow was generated by rotor pumps maintaining a constant pressure gradient. Therefore, flow was not physiologically pulsatile, but rather continuous. However, the average velocities of rapid flow mimicked the maximum velocities of blood at the site of a significant arterial stenosis, and slow flow mimicked the average velocity in a medium-sized non-obstructed artery (28). Rapid flow through non-retracted clots at an initial velocity over 400 cm/s was clearly turbulent with the Reynolds number exceeding 2300 (29). The velocities of rapid flow through retracted clots were lower than in non-retracted clots due to the larger channel diameter required to obtain consistent flow, and were also more variable than in non-retracted clots. Both is related to the greater inhomogeneity of retracted clots in comparison with non-retracted ones. Although flow through a long, straight hose at 130 cm/s would remain laminar, it is likely that the sudden narrowing of the path of plasma flow at the site of the pierced clot and the irregularities of the clot surface contributed to local turbulence (30, 31). It is also known from Doppler ultrasound studies that turbulence occurs in blood vessels at velocities exceeding 120 cm/s (32). The observation time was limited to 40 -60 min because it sufficed for detecting significant differences between the tested regimes and because thrombolytic agents must act relatively rapidly in order to provide clinical benefit.

In conclusion, enhanced transport of components of the fibrinolytic system into clots by turbulent tangential flow promotes thrombolysis of whole-blood clots. This is of course more effective when plasma contains enough plasminogen in addition to the thrombolytic agent. Our *in vitro* results shed additional light on the interpretation of clinical studies of thrombolysis that have proven rt-PA to be more effective than SK, especially when clots are older than several hours (33, 34). In addition, our results suggest that turbulent flow along an arterial thrombus does not only have detrimental effects due to activating platelets; it may in fact promote thrombolysis. Of course, when the clot is dissolved the residual stenosis should be cleared by angioplasty and antiplatelet or anticoagulant medication should be administered in order to preserve the vessel patency.

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