Journal of Thrombosis and Haemostasis, **9** (Suppl. 1): 364–373 DOI: 10.1111/j.1538-7836.2011.04370.x

INVITED REVIEW

Historical perspective and future direction of thrombolysis research: the re-discovery of plasmin

V. J. MARDER
Division of Hematology and Medical Oncology, Department of Medicine, David Geen School of Medicine at UCLA, Los Angeles, CA, USA

To cite this article: Marder VJ. Historical perspective and future direction of thrombolysis research: the re-discovery of plasmin. *J Thromb Haemost* 2011; **9** (Suppl. 1): 364–373.

Summary. Two issues have held the focus of thrombolysis research for over 50 years, namely, choosing between a plasminogen activator (PA) or plasmin as the best therapeutic agent and choosing between systemic or local administration. The original plasmin product of the 1950s was both ineffective and contaminated with PA, and catheter technology was not yet developed for routine clinical use. For decades, clinical practice has focused on PA and systemic administration, but today, PAs are often administered by catheter into thrombosed vessels, notably for peripheral arterial and graft occlusion and deep vein thrombosis, and increasingly for acute ischaemic stroke. Despite using catheter-delivered therapy, bleeding complications still occur, most severely expressed as symptomatic intracranial haemorrhage. New experimental data indicate that we should now reconsider plasmin as a viable, even preferable, thrombolytic agent. Plasmin requires catheter delivery to achieve thrombolysis, but this technical issue has been solved with modern technology and widespread presence of interventional suites. After local administration, plasmin will lyse thrombi; thereafter, any plasmin in the circulation will be rapidly neutralised. Pre-clinical studies confirm that plasmin has marked haemostatic safety advantage over t-PA. After more than 50 years, the field has come full circle, and plasmin as the thrombolytic agent and catheter use for local delivery of agent may represent a step forward in thrombolytic therapy.

Keywords: plasmin, plasminogen activators, thrombolysis, historical.

Introduction

The modern era of fibrinolysis research and clinical development began in 1933 with the serendipitous discovery by Tillett and Garner [1] of a fibrinolytic component contained within a broth culture of haemolytic Streptococci. Tillett moved from Johns Hopkins University to New York University, and in 1949, he and Sol Sherry administered this fibrinolytic component, called streptokinase (SK), to patients – not for vascular thrombosis, but rather into the pleural space for treatment of fibrinous adhesions, often with strikingly beneficial results [2]. A very active interest also developed for the therapeutic potential of plasmin, at the time called 'fibrinolysin', and by 1957, Cliffton's [3] group had administered this preparation to patients with all manner of arterial and venous thrombotic disease. Questions arose regarding the agent of choice, either a plasminogen activator (PA) or the active enzyme plasmin, and there was also considerable interest in the best route of administration for therapy, either by direct infusion into a thrombosed vessel or systemically by the intravenous (IV) route. In 1960, Boyles *et al*. [4] showed that plasmin recanalised the occluded canine coronary artery when administered locally, but not when administered intravenously. In retrospect, this observation explains the variable pre-clinical and clinical results of IV-administered plasmin, but the reasons for these conflicting data were not evident until later.

Correspondence: Victor J. Marder, Division of Hematology and Medical Oncology, Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.
Tel.: +1310 825 4469; fax: +1310 825 0914.
E-mail: vmarder@mednet.ucla.edu

Milestones of fibrinolysis research

It is appropriate to recount the biochemical, pre-clinical and clinical milestones that have brought us to our current understanding of fibrinolysis, but finding consensus for what were the important events, and especially for what the future holds is problematic. As to the published historical narratives (Table 1), those of Koller [5] in 1960 and Macfarlane [6] in 1964 could be called 'dispassionate overviews', while the historical summaries of Cliffton in 1960 [7], Sherry in 1981 [8] and 1989 [9] and Braunwald in 2002 [10] could be titled 'personal narratives'. Other narratives were as much directed to a specific agent, often recombinant t-PA (rt-PA), as to the person's involvement in events [11–15], or on a specific therapeutic target, for example, the coronary artery [16,17], or the perspective of a specific medical specialty, such as one devoted to vascular intervention [18,19].

Perhaps the field of fibrinolysis originated with Morgagni in 1761 [20] noting that blood was uncoagulable after sudden death, with Denis in 1838 [21] observing spontaneous clot

dissolution, and Denys and De Marbaix in 1889 [22] postulating a dormant blood fibrinolytic enzyme, but the phenomenon of post-mortem fibrinolysis was exploited by Skundina *et al.* [23] and by Yudin [24] in the mid-1930s to provide a source of unclotted blood for transfusion. The term 'fibrinolysis' was coined by Dastre in 1893 [25], but the modern era of fibrinolysis began with the observations of Tillett and Garner in 1933 and 1934 of the fibrinolytic potential of 'streptococcal fibrinolysin' [1,26,27]. Many investigators, including Milstone [28], Tagnon [29], Christensen [30,31], Astrup and Permin [32,33], Mullertz [34,35], Williams [36], Sobel *et al.* [37], Lack [38], Ratnoff *et al.* [39], Kline [40], Guest [41] and others elucidated and purified fibrinolytic system components (Table 2A). A balanced scheme of fibrinolytic activation and inhibition evolved, described eloquently by Macfarlane and Biggs in 1948 [42], Macfarlane being the same person who along with Davie and Ratnoff modernised our concept of coagulation as a cascade [43] or waterfall [44], depending on which brand of English one uses.

Preclinical studies assessed the potential of thrombolytic agents to recanalise occluded vessels in animal models. In 1952, Johnson and Tillett [45] showed that a rabbit ear vein clot could be dissolved by IV-administered SK, and groups led by Cliffton [46,47] and Ambrus [48] in the mid-1950s showed encouraging results with IV 'fibrinolysin'. In an impressive experiment that compared IV vs. intra-arterial (IA) injection of 'fibrinolysin' in canine models of coronary and cerebral artery occlusions, Boyles *et al.* in 1960 [4] showed that only local IA administration was successful. Their conclusion was in conflict with a concurrent study that reported successful lysis of canine coronary artery thrombi after IV administration [48]. These glaringly discrepant results regarding the efficacy of IV-'fibrinolysin' are explained as follows: failure of 'fibrinolysin' would be due to neutralisation by antiplasmin [49], and successes would be due contamination of 'fibrinolysin' with PA (SK) [50].

In a prescient clinical experiment reported in 1960, Boucek and Murphy [51] devised a catheter method of delivering 'fibrinolysin' into the aortic root of eight patients with myocardial infarction (MI), effectively injecting agent into the coronary arteries. They noted that 'segmental arterial infusion appears to offer distinct advantages', but catheter technology in 1960 was not sufficiently developed for routine thrombolytic use, especially as concerned time-sensitive pathologies such as acute MI and acute ischaemic stroke. Studies of the same era tried IV 'fibrinolysin' for various thrombotic disorders [3,52], but in retrospect, it is unlikely that the supposed active agent (plasmin) ever reached or dissolved the target thrombus.

Table 1 Types of published historical accounts of fibrinolysis research

Type of review	Examples
Dispassionate overview	Koller [5]; Macfarlane [6]
Personal narrative	Cliffton [7]; Sherry [8,9]; Braunwald [10]
Specific agent focus (rt-PA)	Collen & Lijnen [11–14]; Verstraete [15]
Therapeutic target (Coronary artery)	Kennedy [16]; van de Werf *et al.* [17]
Medical specialty	Interventional Radiology: Rosch *et al.* [18] Vascular Surgery: Ouriel [19]

Controversies

Two issues have emerged, regarding the choice of agent (plasminogen vs. plasmin) and route of administration (systemic vs. local) (Fig. 1). As of 1960–1970, plasmin (as exemplified by 'fibrinolysin') was discredited, knowing that it was rapidly inhibited upon IV administration [49] and that its beneficial thrombolytic activity was likely due to contaminating PA [50]. Furthermore, a powerful foundation for the superiority of PA over plasmin was formulated by Sherry in 1954 [53] and by Alkjaersig, Fletcher and Sherry in 1959 [54], based upon two principles. First, plasmin is neutralised by antiplasmin after IV administration, a reaction that was later shown to be extremely rapid and irreversible [55]. Second, PA (in sufficient dosage) circulates after IV administration, activating thrombus plasminogen to plasmin, which degrades fibrin in an environment protected from antiplasmin.

This scheme provided a foundation for IV administration of PA, a situation that had been exploited in humans in 1959 for lysis of experimental venous thrombi [56], and which was especially suited for treating acute MI, for which the St. Louis group assessed SK therapy in 1958 [57]. Although better lysis may have been possible with IA administration, there was no feasible catheter technology or support facility available at the time and trials of thrombolytic therapy focused on IV administration of PA.

Meanwhile, vascular surgeons initiated a new era of catheter-directed therapy (CDT), starting with the landmark study by Dotter *et al.* in 1974 [58] that showed successful lysis of a peripheral arterial thrombosis by IA SK. This report noted a somewhat surprising and still largely overlooked occurrence, bleeding complications despite 'local' therapy. We now know that CDT improves thrombolysis, but this delivery route does not restrict PA presence to the site of thrombus. Bleeding at remote sites still occur, just as with systemic therapy.

Major clinical trials (1980–2000)

The era of multicentre study of thrombolytic therapy was initiated by studies of pulmonary embolism coordinated by Sherry and colleagues, reported in the early 1970s. Results showed better recanalisation with UK than with heparin [59] and equal results with UK and SK [60], most evident in patients with symptom onset of < 48 h [61].

From 1980 to the present time, there has been an explosion of work in thrombolytic therapy, and successful demonstrations of relevant clinical success, all with PA, delivered both systemically and CDT.

Thrombosis was definitively established as the central pathologic event of acute MI by DeWood *et al.* in 1980 [62]. With this foundation, Boucek and Murphy's [51] concept of 'segmental perfusion of coronary arteries', expounded in 1960, was exploited by intra-coronary infusion of PA by Chazov

Table 2 Milestones of fibrinolysis research

Year	Investigator	Observation
(A) Biochemical observations		
1761	Morgagni [20]	Uncoagulable blood after sudden death
1838	Denis [21]	Spontaneous dissolution of post-mortem clot
1889	Denys & Marbaix [22]	Dormant fibrinolytic enzyme
1893	Dastre [25]	Coined term 'fibrinolysis'
1933–34	Tillett & Garner [1,26,27]	Fibrinolytic principal in haemolytic Streptococcal broth
1935–36	Skundina [23], Yudin [24]	Transfusion of post-mortem liquefied blood
1941–45	Milstone [28], Tagnon [29], Christensen [30,31]	Precursor of plasmin converted by streptococcal agent to active enzyme
1947 on	Astrup & Permin [32,33]	Fibrinolytic activator in animal tissue
1948	Macfarlane & Biggs [42]	Concept of balanced clot formation and dissolution
1948–54	Mullertz [34], Williams [36], Sobel *et al.* [37], Lack [38], Ratnoff *et al.* [39]	Fibrinolytic inhibitors and activators (t-PA, UK, staphylokinase)
1953	Kline [40], Mullertz [35]	Purification of plasminogen
1961	Guest & Celander [41]	Urokinase
1978	Wiman and Collen [55]	$Alpha_2$-antiplasmin
1981	Rijken & Collen [66]	Activator purified from melanoma line
1983	Pennica *et al.* [67]	Cloning and expression of rt-PA
1990s	Multiple investigators	Recombinant mutant derivatives of rt-PA
(B) Notable pre-clinical studies		
1952	Johnson & Tillett [45]	Clot lysis in rabbits by streptokinase
1955–59	Cliffton group [46,47], Ambrus group [48]	Lysis of arterial clots with intravenous fibrinolysin
1960	Boyles *et al.* [4]	Intra-arterial vs. intravenous fibrinolysin for canine coronary artery occlusion
1981	Matsuo *et al.* [68]	t-PA in experimental pulmonary embolism
2001	Marder *et al.* [132]	Plasmin superior to rt-PA in haemostatic safety
(C) Notable clinical studies		
1949	Tillett & Sherry [2]	Streptokinase use in humans (fibrinous pleural adhesions)
1957	Cliffton [52]	Plasmin (fibrinolysin) in human thrombotic disease
1958	Fletcher *et al.* [57]	Streptokinase in patients with acute MI
1959	Johnson & McCarty [56]	Lysis of artificial clots in man by streptokinase
1960	Boucek & Murphy [51]	Intra-aortic vs. intravenous fibrinolysin for MI
1970–74	Sherry *et al.* [59,60]	Streptokinase and urokinase in pulmonary embolism
1974	Dotter *et al.* [58]	Catheter-directed thrombolysis in peripheral arterial occlusion (streptokinase)
1976	Chazov *et al.* [63]	Intra-coronary artery fibrinolysin for MI
1979, 1983	Rentrop *et al.* [64]; Schroder *et al.* [65]	Intracoronary and intravenous streptokinase for acute MI
1981	Weimar *et al.* [69]	t-PA for human thrombosis (deep vein thrombosis)
1984	van de Werf *et al.* [70]	Recombinant t-PA in acute MI
1986–88	GISSI, ISIS-2, ASSET, AIMS [71–74]	Survival benefit with IV streptokinase, rt-PA or anistreplase vs. placebo in acute MI
1990–93	GISSI-2, ISIS-3, GUSTO-1 [75–77]	Head to head comparisons of streptokinase, rt-PA, and anistreplase in acute MI
1994	Ouriel *et al.* [86]	Survival advantage for urokinase (vs. surgery) in peripheral arterial graft occlusion
1995, 2008	NINDS [80], Hacke *et al.* [83]	Recombinant t-PA in ischaemic stroke

<table>
<tr><th></th><th>Plasmin vs
plasminogen activators (PA)</th><th>Local vs systemic
route of administration</th></tr>
<tr><td>1960–1970</td><td>"Fibrinolysin" discredited.</td><td>Primitive catheter technology.</td></tr>
<tr><td colspan="3">Theoretical advantage for PA, especially with systemic use.</td></tr>
<tr><td>1970–1980</td><td>Emphasis on PAs (SK, UK).
Large clinical trial in PE.</td><td>Development of sophisticated catheters.
Local treatment of PAO (SK).</td></tr>
<tr><td colspan="3">No studies of plasmin as a viable thrombolytic agent.</td></tr>
<tr><td>1980–present</td><td>t-PA and recombinant mutants.
Pharmacological and physical adjuncts.
Trials of PA in MI, stroke, PAO, DVT.</td><td>Expansion of interventional technology
for arterial and venous thrombosis.
(endovascular procedures).</td></tr>
<tr><td colspan="3">Persistent problems with plasminogen activators, especially bleeding complications.</td></tr>
</table>

Fig. 1. Status of two major issues regarding thrombolytic therapy (1960 to present).

Problem	Resolution
Limited efficacy	Catheter-delivered therapy
Bleeding complications	Plasmin as thrombolytic (delivered by catheter)

Fig. 2. Problems with plasminogen activators and solution approaches.

et al. in 1976 [63] and by Rentrop *et al*. in 1979 [64]. Lysis of coronary artery thrombi was documented in real time, and the potential of IV administration to decrease delay from event to treatment was shown by Schroder *et al*. in 1983 [65]. The development of tissue-type PA (t-PA) coincided with these remarkable observations, with ultimate purification and synthesis in recombinant systems by the efforts of Rijken and Collen [66] and Pennica *et al*. [67], demonstration of efficacy in an animal model of pulmonary embolism by Matsuo *et al*. [68] (Table 2B), first use of t-PA in humans [69], and IV-administered rt-PA in patients with acute MI [70]. An enormous number of patients with acute MI ($>$ 100 000) were subjected to clinical trial of IV PA vs. placebo from 1986 to 1988, showing survival advantage for SK [71,72], rt-PA [73] and anistreplase [74]. Head-to-head comparative trials showed equivalent mortality for SK and rt-PA in two studies (GISSI-2 AND ISIS-3) [75,76] and an advantage favouring rt-PA over SK (6.3% vs. 7.2%) in a third trial (GUSTO) [77]. Although there was controversy regarding interpretation of these comparative trials [78], the studies clearly document the strong survival benefit of thrombolytic reperfusion. Mortality benefit is greatest if initiated in the first 2 h (40% reduction), less so at 6 h (25%), but still with measurable at 12 h [79]. A similar effort to establish clinical benefit of PAs in acute ischaemic stroke was expended in the 1990s, with significant functional improvement established for rt-PA when administered IV in the 0–3 h time window [80]. There was a suggestion of similar effect with SK by subgroup analysis of patients treated in the 0–3 h period [81], but study results did not meet statistical significance [82]. Subsequent study of rt-PA in acute stroke has extended the time window to 4.5 h [83]. In the early 1980s, CDT with SK or UK was used increasingly [84,85] for acute peripheral arterial (graft) occlusion. In 1994, Ouriel *et al*. [86] showed that IA CDT with UK (vs. early surgical repair) for occluded peripheral arterial graft occlusion showed a dramatic decrease in the need for invasive surgery and more importantly, a decreased 1-year mortality (16% vs. 42%). Subsequent comparison of UK with t-PA showed no difference in clinical outcome [87].

Persistent problems: limits of efficacy and bleeding complications

Significant problems with PA therapy persisted, specifically, practical limits of efficacy and bleeding complications, especially intracranial haemorrhage (ICH) (Fig. 2). Efforts to improve recanalisation and clinical results include CDT with or without other recanalisation techniques, ultrasound [88] and micro-vesicle tools, adjunctive pharmacologic agents (anticoagulant and anti-platelet) and novel PA such as recombinant derivatives of rt-PA [15], staphylokinase [89], and recombinant bat salivary derivative desmoteplase [90] (Table 3). An immense literature attests to these efforts, and a review of anticoagulants, anti-platelet agents and novel PA, whether recombinant varieties of t-PA or of bacterial or haematophagous origin are not the subject of this review. Rather, this narrative will focus on the confluence of two developments, namely, the progress made using catheter technology and a renewed consideration of plasmin as a potentially safer thrombolytic agent than PA.

Improved recanalisation with CDT and endovascular procedures

In the decades since the first documented advantages of local delivery of agent in 1960 [4], CDT has become the preferred approach for the management of arterial and venous thrombosis, with referral to the Interventional Radiology suite now routine for agent delivery and for combined mechanical recanalisation approaches.

Thrombolytic therapy for acute ischaemic stroke is currently limited to a small portion of patients [91,92] during a window of opportunity of up to 4.5 h [80,83]. To maximise cerebral artery recanalisation, a 'multimodal reperfusion therapy' approach may be applied [93] wherein all potential advantages provided by catheters can be brought to bear, including IA administration, thrombus disruption and extraction, angioplasty and stent deployment. To this end, IA administration of PA induces significant vascular recanalisation [94], clot retrieval devices have been deployed at up to 8 h after symptom onset [95], including one that also serves as an expandable stent [96], and micro-bubble infusion has been used as adjunct to IA-rt-PA as rescue for occluded cerebral arteries [97]. Recanalisation is also accelerated by transcranial ultrasonography administered during systemic rt-PA therapy [98].

Controlled trials in patients with acute MI show that early coronary artery angioplasty provides superior clinical benefit than is achieved by IV rt-PA, provided that patient transfer to a specialised facility can be accomplished efficiently ($<$ 2 h) [99,100]. Of special value is the decreased risk of symptomatic ICH (sICH), the report by Grines *et al*. [100] showing a 2% incidence in 200 t-PA-treated patients vs. 0% in 195 patients who underwent angioplasty ($P = 0.05$).

Significant ($>$ 90%) thrombolysis can be achieved in about 50% of patients with deep vein thrombosis (DVT) treated with only IV PA [101,102]. However, current practice often combines CDT with 'minimally-invasive endovascular strategies' [103], and observational studies using CDT show high patency rates of up to 70% [104,105], with 75% of occluded vessels also subjected to angioplasty, stent placement, mechanical thrombectomy and/or surgery [105]. Although CDT has not been compared with IV PA alone, CDT has been compared with heparin alone for ileofemoral DVT, reporting a superior 6-month patency rate of 64% (vs.

36% for heparin) [106]. The endovascular therapies provided in addition to PA likely explain the very high patency rates with CDT [105,107]. More thrombolysis likely translates to fewer cases of post-thrombotic syndrome [108], and the additional endovascular strategies may be especially useful for older occlusions that are not amenable to thrombolysis, and for conditions with intractable extravascular compression, such as the May-Thurner syndrome [109].

Bleeding complications with PA therapy

Every thrombolytic agent currently approved by federal regulatory agencies for treatment of pathologic thrombi is a PA [110], and haemorrhagic complications have been and continue to be the most dangerous adverse events associated with PA use [111]. CDT has improved the vascular recanalisation results achieved with PA but the agent is not restricted to the thrombus locale, especially as vessel recanalisation occurs. Thus, bleeding complications are anticipated, based upon lysis of susceptible haemostatic plugs at sites of vascular injury [110–113]. Despite the varied molecular structure and source of PA, they all have in common the properties of (i) capacity to circulate to sites of thrombosis or haemostatic plug presence despite plasma inhibitors, and (ii) conversion of plasminogen to plasmin to lyse a thrombus or haemostatic plug [113]. Not surprisingly, adjunct anticoagulant and anti-platelet therapy exaggerate the bleeding risk [110]. Among the PA, more potent agents such as t-PA and its recombinant variants carry a greater risk of inducing ICH after IV administration than does SK [111]. This effect is clearly documented in comparative trials of IV PA in patients with acute MI, with rates of 0.7% for t-PA vs. 0.35% for SK in GISSI-2 and ISIS-3 [75,76].

The risk of inducing sICH by PA use is greatest in the treatment of ischaemic stroke, exemplified in the pivotal NINDS study that showed a 10-fold greater rate for rt-PA-treated patients (6.5% vs. 0.6% for placebo) [80]. The pathologic causation of sICH is multifactorial, involving ischaemic vasculopathy and loss of microvascular integrity, leading to extravasation of blood and parenchymal injury [114], to a great degree initiated directly by toxic effects of t-PA on the blood-brain barrier and neural cells [115]. The data suggest that sICH occurs at the same rate at 0–3 and 3–4.5 h [116,117], albeit still 10-fold more often than with placebo [83]. The incidence of 'any' ICH is also higher for t-PA than for placebo (27% vs. 17.6%, $P = 0.001$) [83], a worrisome increase, as asymptomatic ICH (haemorrhagic transformation rather than parenchymal haemorrhage) may be a harbinger of poor clinical outcome [118]. Advanced age (> 80 years) does not necessarily connote a higher risk of sICH [119]. However, IV-rt-PA is associated with a lower rate of sICH (5.2%) than IA-rt-PA (12.5%) or combined IV + IA rt-PA (20%) [120]. Recent reports show sICH for tenecteplase (0.4 mg kg^{-1}) at 15.8% (vs. 3.2% for rt-PA) [121] and of desmoteplase (90 or 125 μg kg^{-1}) at 3.5% and 4.5% (vs. 0% for placebo) [122], reinforcing the association of sICH with any PA used for stroke treatment.

Although CDT using PA is an effective approach for acute peripheral arterial and graft occlusion, serious haemorrhagic complications occur not-infrequently. In the TOPAS report, major haemorrhage was noted in 12.5% of 256 patients who received recombinant UK, including four episodes of ICH (1.6%) [123] and similar rates of major haemorrhage and ICH were reported for the STILE [87] and 'Rochester' [86] reports. A retrospective compilation of experience in 125 cases of peripheral vascular disease (90% of which were arterial or graft occlusions) from 2005 to 2008 showed haemorrhagic complications in 22.4%, a 5.6% 'stroke rate', and mortality because of haemorrhagic complications in 4 (3%) of patients [124].

Therapeutic success with CDT for DVT without a high rate of major haemorrhage has been reported as only two of 53 (3.8%) [105], three of 50 (6%) [106], and seven of 178 (3.9%) [125]. However, serious bleeding is still a significant limitation to PA use, as shown by six of 30 (20%) patients with major haemorrhage or large haematomas requiring cessation of treatment [126], significant haematoma formation in up to 11% of published studies [127], and a persistent occurrence of fatal ICH in one of 68 (1.5%) [128] and one of 103 (1%) patients [129]. About 10% of patients with pulmonary embolism who received PA therapy had in-hospital bleeding complications [130] and 21.4% of such patients receiving CDT had 'treatment-related haemorrhagic complication' [131]. The overall risk of serious bleeding is less in patients with DVT than in patients with peripheral arterial thrombotic disease, but bleeding nevertheless represents a clinical complication of significant degree that warrants search for an improved (safer) thrombolytic agent.

The re-discovery of plasmin and its proposal for use as a safer thrombolytic

A proposal made in 2001 by Marder *et al.* [132] on the basis of pre-clinical data, supported by editorial commentary [133], stated that plasmin administered by catheter could have the desired pharmacologic characteristics of both thrombolytic efficacy and haemostatic safety (Fig. 3).

Table 3 Approaches to improve efficacy of plasminogen activator therapy

Catheter-directed thrombolysis (CDT)
PA infusion alone
PA infusion plus other recanalisation approaches (thrombectomy, angioplasty, stenting)
Adjunctive support
Pharmacologic
Anticoagulants (glucose-aminoglycans, direct anti-thrombins)
Anti-platelet agents (aspirin, ADP and IIb/IIIa inhibitors)
Physical modalities (echogenic vesicles, ultrasound)
Novel plasminogen activators
Recombinant rt-PA mutants (reteplase, tenecteplase)
Bacterial origin (Staphylokinase)
Haematophagous animal salivary gland (desmoteplase)

1 For efficacy: Plasmin delivered by catheter binds to fibrin, and protected from inhibition by antiplasmins, induces thrombolysis.
   (i) As catheters are now almost ubiquitous for thrombolytic administration, plasmin can be utilised routinely.
2 For safety: Plasmin that enters the circulation is neutralised by antiplasmin, so bleeding is prevented.
   (i) Bleeding complications would still occur with rt-PA, even when administered by catheter.

Thus, the biochemical property that nullifies the thrombolytic effect of IV-administered plasmin [49,53] would be turned to advantage for haemostatic safety of catheter-administered plasmin [132]. Furthermore, the wide use of CDT by the 1980s eliminated the technical limits to local plasmin use that existed in the 1950s and 1960s. As to the production flaw of 'fibrinolysin' that allowed contamination with PA [50], the plasmin used in current study is free of PA [132,134].

Pre-clinical studies that compared plasmin with t-PA have supported these hypothetical advantages for plasmin. Whereas rt-PA in thrombolytic dosages caused bleeding in an animal model of fibrinolytic haemorrhage [132], and in a dose-related effect, even in sub-therapeutic dosages [135], plasmin was free of haemorrhagic complications even at dosages up to 4-fold needed for vascular recanalisation. Purposely-toxic dosages of plasmin, which totally depleted plasma fibrinogen, induced prolongation of primary bleeding after vascular trauma, but so

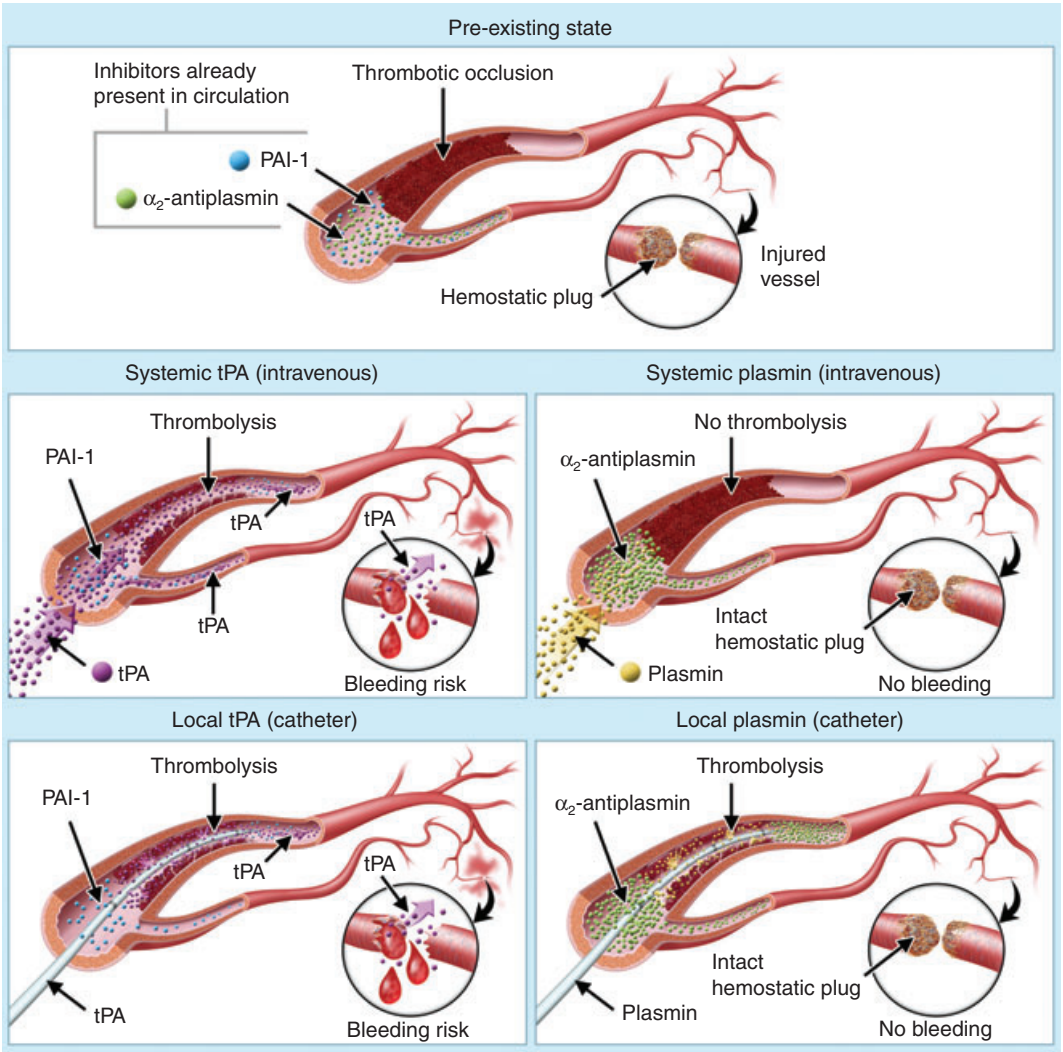

Fig. 3. Schematic representation of plasmin and t-PA modes of action on vascular thrombi and haemostatic plugs at vascular injury sites, after systemic (intravenous) or local (catheter) administration. Modified from references [132] and [138]. Top panel: The pre-existing state shows a thrombus occluding a major vessel (artery or vein) and a haemostatic plug at a site of vascular injury, remote from the thrombosed vessel. Inhibitors of plasmin (α_2-antiplasmin) and of t-PA (PAI-1) are present in the circulation (shown as green and blue spheres, respectively). Middle panels: Systemic delivery of therapeutic amounts of t-PA exceed the inhibitory capacity of PAI-1, allowing it to reach and dissolve thrombus; however, t-PA also reaches and dissolves haemostatic plugs anywhere in the circulation, which can result in a haemorrhagic complication. Plasmin delivered systemically by the intravenous route is safe but also ineffective, as it is efficiently neutralised by α_2-antiplasmin. Bottom panels: Local delivery of t-PA by catheter achieves a high local concentration that is effective for dissolving thrombi, but t-PA enters the circulation despite its 'local' administration and can cause haemorrhage at remote vascular injury sites. Catheter delivery of plasmin allows binding and thrombolysis to occur. Unlike the situation with t-PA, plasmin that enters the circulation after catheter delivery is rapidly neutralised by α_2-antiplasmin, thus preventing lysis of haemostatic plugs and avoiding haemorrhage.

long as fibrinogen levels were not totally depleted, reasonable haemostasis was maintained [135]. As for venous or arterial thrombolysis, pre-clinical models showed plasmin to be as effective as t-PA, and even with possible superiority under conditions of limited plasminogen presence [132,134]. Plasmin has also shown *ex vivo* thrombolysis equivalent to that for rt-PA for thrombi retrieved from the middle cerebral artery of patients with acute ischaemic stroke [136]. This combined result of at-least equal efficacy to go with greater haemostatic safety reflects a more favourable benefit-to-risk ratio for plasmin over rt-PA [137].

Plasmin is the prototypic example of a group of fibrinolytic agents, classified as direct-acting, all of which exert their effect independent of plasminogen as substrate, but with differing properties for fibrin-binding and inhibitor neutralisation. These agents are arbitrarily divided into plasmin and related species and fibrino(geno)lytic molecules derived from haematophagous animals, details of biochemical, preclinical and clinical testing summarised in a recent review [138]. Several agents have been or are under current clinical study, including microplasmin, composed of the serine protease domain alone [139], alfimeprase, a recombinant derivative of the Copperhead snake venom fibrolase [140], and full-length plasmin, prepared in a low pH formulation that activates upon exposure to neutral pH in plasma (Talecris Biotherapeutics, Inc, Research Triangle Park, NC, USA). Plasmin is currently in Phase 2 trial for peripheral arterial and graft occlusion [NCT NCT 00418483) and in a Phase 1 safety study for acute ischaemic stroke [NCT 01014975]. A novel recombinant plasmin molecule has been synthesised, Δ (K2-K5) plasmin, consisting of the first kringle of plasmin adjoined to the serine protease domain, with virtually the same biochemical [141] and haemostatic safety characteristics [142] as full-length, plasma-derived plasmin.

Summary

Plasmin (as an impure preparation called 'fibrinolysin') was first studied more than 50 years ago and found to be either ineffective or inferior to plasminogen activators. With the current widespread use of catheters to deliver thrombolytic therapy, plasmin has been re-discovered as a potentially valuable agent, with a firm pharmacologic foundation for effective and haemostatically safe application in thrombotic disease.

Disclosure of Conflict of Interests

V.J. Marder has received research grants and is a paid consultant for Talecris Biotherapeutics, Inc, Research Triangle Park, NC, USA.

References

1 Tillett WS, Garner RL. The fibrinolytic activity of hemolytic streptococci. *J Exp Med* 1933; **58**: 485–502.
2 Tillett WS, Sherry S. The effect in patients of streptococcal fibrinolysin (streptokinase) and streptococcal deoxyribonuclease on fibrinous, purulent and sanguineous pleural exudations. *J Clin Invest* 1949; **28**: 173–90.
3 Cliffton EE. The use of plasmin in humans. *Ann N Y Acad Sci* 1957; **68**: 209–29.
4 Boyles PW, Meyer WH, Graff J, Ashley CC, Ripic RG. Comparative effectiveness of intravenous and intra-arterial fibrinolysin therapy. *Am J Cardiol* 1960; **6**: 439–40.
5 Koller F. The development of our knowledge of fibrinolysis. *Am J Cardiol* 1960; **6**: 367–70.
6 MacFarlane RG. The development of ideas on fibrinolysis. *Br Med Bull* 1964; **20**: 173–8.
7 Cliffton EE. Review of clinical experience with clot-lysing agents. *Am J Cardiol* 1960; **6**: 476–86.
8 Sherry S. Personal reflections on the development of thrombolytic therapy and its application to acute coronary thrombosis. *Am Heart J* 1981; **102**: 1134–8.
9 Sherry S. The origin of thrombolytic therapy. *J Am Coll Cardiol* 1989; **14**: 1085–92.
10 Braunwald E. Personal reflections on efforts to reduce ischemic myocardial damage. *Cardiovasc Res* 2002; **56**: 332–8.
11 Collen D. Human tissue-type plasminogen activator: from the laboratory to the bedside. *Circulation* 1985; **72**: 18–20.
12 Collen D, Lijnen HR. Tissue-type plasminogen activator: a historical perspective and personal account. *J Thromb Haemost* 2004; **2**: 541–6.
13 Collen D, Lijnen HR. Thrombolytic agents. *Thromb Haemost* 2005; **93**: 627–30.
14 Collen D, Lijnen HR. The tissue-type plasminogen activator story. *Arterioscler Thromb Vasc Biol* 2009; **29**: 1151–5.
15 Verstraete M. The fibrinolytic system: from Petri dishes to genetic engineering. *Thromb Haemost* 1995; **74**: 25–35.
16 Kennedy JW. 50th anniversary historical article. Thrombolytic therapy in acute myocardial infarction. *J Am Coll Cardiol* 1999; **33**: 1829–32.
17 van de Werf FJ, Topol EJ, Sobel BE. The impact of fibrinolytic therapy for ST-segment-elevation acute myocardial infarction. *J Thromb Haemost* 2009; **7**: 14–20.
18 Rosch J, Keller FS, Kaufman JA. The birth, early years, and future of interventional radiology. *J Vasc Interv Radiol* 2003; **14**: 841–53.
19 Ouriel K. A history of thrombolytic therapy. *J Endovasc Ther* 2004; **11**(Suppl II): II128–33.
20 Morgagni JB. *De sedibus et causis morborum per anatomen indagatis*. Lovanii: Apud M.C. Compere, 1761.
21 Denis PS. *Essai sur l'*application de la chimie a l'etude physiologique du sang de l'homme. Paris: JB Ballièere, 1838.
22 Denys J, De Marbaix H. Les peptonisations provoquees par le chloroforme. *Cellule* 1889; **5**: 197–251.
23 Skundina M, Rusakow A, Ginsburg R, Bocarow A. Die biochemischen Veranderungen im Leichenblut. *Sovet Chir* 1935; **6**: 78.
24 Yudin SS. Transfusion of cadaver blood. *JAMA* 1936; **106**: 997–9.
25 Dastre A. Fibrinolyse dans le sang. *Arch Norm Pathol* 1893; **5**: 661–3.
26 Garner RL, Tillett WS. Biochemical studies on the fibrinolytic activity of hemolytic streptococci: I. Isolation and characterization of fibrinolysin. *J Exp Med* 1934; **60**: 239–54.
27 Garner RL, Tillett WS. Biochemical studies on the fibrinolytic activity of hemolytic streptococci: II. Nature of the reaction. *J Exp Med* 1934; **60**: 255–67.
28 Milstone H. A factor in normal human blood which participates in streptococcal fibrinolysis. *J Immunol* 1941; **42**: 109–16.
29 Tagnon JG, Davidson CS, Taylor FHL. The coagulation defect in hemophilia: a comparison of the proteolytic activity of chloroform preparations of hemophilic and normal plasma. *J Clin Invest* 1943; **22**: 127–34.
30 Christensen LR, MacLeod CM. A proteolytic enzyme of serum: characterization, activation, and reaction with inhibitors. *J Gen Physiol* 1945; **28**: 559–83.

31 Christensen LR. Streptococcal fibrinolysis: a proteolytic reaction due to a serum enzyme activated by streptococcal fibrinolysis. *J Gen Physiol* 1945; **28**: 363–83.
32 Astrup T, Permin PM. Fibrinolysis in the animal organism. *Nature* 1947; **159**: 681–2.
33 Astrup T. Activation of a proteolytic enzyme in blood by animal tissue. *Biochem J* 1951; **50**: 5–11.
34 Mullertz S. A plasminogen activator in spontaneously active human blood. *Proc Soc Exp Biol Med* 1953; **82**: 291–5.
35 Mullertz S, Lassen M. An activator system in blood indispensable for formation of plasmin by streptokinase. *Proc Soc Exp Biol Med* 1953; **82**: 264–8.
36 Williams JR. The fibrinolytic activity of urine. *Br J Exp Pathol* 1951; **32**: 530–7.
37 Sobel GW, Mohler SR, Jones NW, Dowdy ABC, Guest MM. Urokinase: an activator of plasma profibrinolysin extracted from urine. *Am J Physiol* 1952; **171**: 768–9.
38 Lack CH. Staphylokinase: an activator of plasma protease. *Nature* 1948; **161**: 559.
39 Ratnoff OD, Lepow IH, Pillemer L. The multiplicity of plasmin inhibitors in human serum, demonstrated by the effect of primary amino compounds. *Bull Johns Hopkins Hosp* 1954; **94**: 169–79.
40 Kline DL. Purification and crystallization of plasminogen (profibrinoloysin). *J Biol Chem* 1953; **204**: 949–55.
41 Guest MM, Celander DR. Urokinase: physiologic activator of profibrinolysis. *Tex Rep Biol Med* 1961; **19**: 89–105.
42 MacFarlane RG, Biggs R. Fibrinolysis: its mechanism and significance. *Blood* 1948; **3**: 1167–87.
43 Macfarlane RG. An enzyme cascade in the blood clotting mechanism, and its function as a biochemical amplifier. *Nature* 1964; **202**: 498–9.
44 Davie EW, Ratnoff D. Waterfall sequence for intrinsic blood clotting. *Science* 1964; **145**: 1310–2.
45 Johnson AJ, Tillett WS. Lysis in rabbits of intravavascular blood clots by the streptococcal fibrinolytic system (streptokinase). *J Exp Med* 1952; **95**: 449–64.
46 Grossi CE, Cliffton EE. The lysis of arterial thrombi in rabbits and dogs by use of activated human plasminogen (fibrinolysin) (plasmin). *Surgery* 1955; **37**: 794–802.
47 Ruegsegger P, Nydick I, Abarquez R, Reichel F, Cliffton EE, LaDue JS. Effect of fibrinolytic (plasmin) therapy on the physiopathology of myocardial infarction. *Am J Cardiol* 1960; **6**: 519–24.
48 Back N, Ambrus JL, Byron JW, Shulman S. Effect of delayed treatment with plasmin (fibrinolysin) on thrombi produced with I^{131} labeled fibrinogen. *Fed Proc* 1956; **15**: 396.
49 Verstraete M, Vermylen J, Amery A. Enzymatic clot dissolution in man: a new therapeutic approach. *Acta Clin Belg* 1964; **19**: 271–90.
50 Douglas AS, McNicol GP. Thrombolytic therapy. *Br Med Bull* 1964; **20**: 228–32.
51 Boucek RJ, Murphy WP. Segmental perfusion of the coronary arteries with fibrinolysin in man following a myocardial infarction. *Am J Cardiol* 1960; **6**: 525–33.
52 Cliffton EE. The use of plasmin in humans. *Am J Cardiol* 1960; **6**: 546–51.
53 Sherry S. Fibrinolytic activity of streptokinase activated human plasmin. *J Clin Invest* 1954; **33**: 1054–63.
54 Alkjaersig N, Fletcher AP, Sherry S. The mechanism of clot dissolution by plasmin. *J Clin Invest* 1959; **38**: 1086–95.
55 Wiman B, Collen D. Molecular model of physiological fibrinolysis. *Nature* 1978; **272**: 549–50.
56 Johnson AJ, McCarty WR. The lysis of artificially induced intravascular clots in man by intravenous infusions of streptokinase. *J Clin Invest* 1959; **38**: 1627–43.
57 Fletcher AP, Alkjaersig N, Smyrniotis FE, Sherry S. Treatment of patients suffering from early myocardial infarction with massive and prolonged streptokinase therapy. *Trans Assoc Am Physicians* 1958; **71**: 287–96.
58 Dotter CT, Rosch J, Seaman AJ. Selective clot lysis with low-dose streptokinase. *Radiology* 1974; **111**: 31–7.
59 Urokinase-Pulmonary Embolism Trial Study Group. Urokinase-pulmonary embolism trial: phase i results. *JAMA* 1970; **214**: 2163–72.
60 Urokinase-Streptokinase Pulmonary Embolism Trial. Phase II results: a national cooperative trial. *JAMA* 1974; **229**: 1606–13.
61 The Urokinase Pulmonary Embolism Trial. A national cooperative study. *Circulation* 1973; **47**(Suppl II): 1–108.
62 DeWood MA, Spores J, Notske R, Mouser LT, Burroughs R, Golden MS, Lang HT. Prevalence of total coronary occlusion during the early hours of transmural myocardial infarction. *N Engl J Med* 1980; **303**: 897–902.
63 Chazov EI, Matveeva LS, Mazaev AV, Sargin KE, Sadavskaia GV, Ruda MI. Intracoronary administration of fibrinolysin in acute myocardial infarction. *Ter Arkh* 1976; **48**: 8–19.
64 Rentrop P, Blanke H, Kostering K, Karsch KR. Acute myocardial infarction: intracoronary application of nitroglycerin and streptokinase in combination with transluminal recanalization. *Clin Cardiol* 1979; **2**: 354–63.
65 Schroder R, Biamino G, von Leitner ER, Linderer T, Bruggemann T, Heitz J, Vohringer HF, Wegscheider EI. Intravenous short-term infusion of streptokinase in acute myocardial infarction. *Circulation* 1983; **63**: 536–48.
66 Rijken DC, Collen D. Purification and characterization of the plasminogen activator secreted by human melanoma cells in culture. *J Biol Chem* 1981; **156**: 7035–41.
67 Pennica D, Holmes WE, Kohr WJ, Harkins RN, Vehar GA, Ward CA, Bennett WF, Yelverton E, Seeburg PH, Heyneker HL, Goeddel DV, Collen D. Cloning and expression of human tissue-type plasminogen activactor cDNA in *E. coli*. *Nature* 1983; **201**: 214–21.
68 Matsuo O, Rijken DC, Collen D. Thrombolysis by human tissue plasminogen activator and urokinase in rabbits with experimental pulmonary embolus. *Nature* 1981; **291**: 590–1.
69 Weimar W, Stibbe J, van Seyen AJ, Biliau A, De Somer P, Collen D. Specific lysis of an iliofemoral thrombus by administration of extrinsic (tissue-type) plasminogen activator. *Lancet* 1981; **2**: 1018–20.
70 van de Werf F, Bergmann SR, Fox KAA, de Geest H, Hoyng CF, Sobel BE, Collen D. Coronary thrombolysis with intravenously administered human tissue-type plasminogen activator produced by recombinant DNA technology. *Circulation* 1984; **69**: 605–10.
71 Gruppo Italiano per lo Studio Della Streptochinase Nell'infarto Miocardico. Effectiveness of intravenous thromblytic treatment in acute myocardial infarction. *Lancet* 1986; **1**: 397–402.
72 ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. Randomized trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction. *Lancet* 1988; **2**: 349–54.
73 Wilcox RG, von der Lippe G, Olsson CG, Jensen G, Skene AM, Hampton JR. for ASSET (Angelo-Scandinavian Study of Early Thrombolysis) Study Group. Trial of tissue plasminogen activator for mortality reduction in acute myocardial infarction: Anglo-Scandinavian Study of Early Thrombolysis (ASSET). *Lancet* 1988; **2**: 525–30.
74 AIMS Trial Study Group. Effect of intravenous APSAC on mortality after AMI: preliminary report of a placebo-controlled clinical trial. *Lancet* 1988; **1**: 545–9.
75 Gruppo Italiano per lo Studio Della Sopravvivenza nell'Infarto Miocardioco (GISSI). GISSI-2: a factorial randomized trial of alteplase versus streptokinase and heparin versus no heparin among 12,490 patients with acute myocardial infarction. *Lancet* 1990; **336**: 65–71.
76 The International Study Group. In-hospital mortality and clinical course of 20,891 patients with suspected acute myocardial infarction randomized between alteplase and streptokinase with or without heparin. *Lancet* 1990; **336**: 71–5.
77 The GUSTO Investigators. An international randomized trial comparing four thrombolytic strategies for acute myocardial infarction. *N Engl J Med* 1993; **329**: 673–82.

78 Hennekens CH, O'Donnell CJ, Ridker PM, Marder VJ. Current issues concerning thrombolytic therapy for acute myocardial infarction. *J Am Coll Cardiol* 1995; **25**(Suppl): 18S–22S.
79 Fibrinolytic Therapy Trialists Collaborative Group. Indications for fibrinolytic therapy in suspected acute myocardial infarction: collaborative overview of early mortality and major morbidity results from all randomized trials of more than 1000 patients. *Lancet* 1994; **343**: 311–22.
80 The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med* 1995; **333**: 1581–7.
81 Wardlaw JM, Warlow CP, Counsell C. Systematic review of evidence of thrombolytic therapy for acute ischemic stroke. *Lancet* 1997; **350**: 607–14.
82 Donnan GA, Davis SM, Chambers BR, Gates PC, Hankey GJ, McNeil JJ, Rosen D, Stewart-Wynne EG, Tuck RR. Streptokinase for acute ischemic stroke with relationship to time of administration: Australian Streptokinase (ASK) Trial Study Group. *JAMA* 1996; **276**: 961–6.
83 Hacke W, Kaste M, Bluhmki E, Brozman M, Dávalos A, Guidetti D, Larrue V, Lees KR, Medeghri Z, Machnig T, Schneider D, von Kummer R, Wahlgren N, Toni D; ECASS Investigators. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med* 2008; **359**: 1317–29.
84 Katzen BT, van Breda A. Low dose streptokinase in the treatment of arterial occlusions. *AJR Am J Roentgenol* 1981; **136**: 1171–8.
85 McNamara T, Fischer JR. Thrombolysis of peripheral arterial and graft occlusions: improved results using high dose urokinase. *AJR Am J Roentgenol* 1985; **144**: 769–75.
86 Ouriel K, Shortell CK, De Weese JA, Green RM, Francis CW, Azodo MVU, Gutierrez OS, Manzione JV, Cox C, Marder VJ. A comparison of thrombolytic therapy with operative revascularization in the initial treatment of acute peripheral arterial ischemia. *J Vasc Surg* 1994; **19**: 1021–30.
87 The STILE Investigators. Results of a prospective randomized trial evaluating surgery versus thrombolysis for ischemia of the lower extremity. The STILE trial. *Ann Surg* 1994; **220**: 251–66.
88 Francis CW. Ultrasound-enhanced thrombolysis. *Echocardiography* 2001; **18**: 239–46.
89 Collen D, Lijnen HR. Staphylokinase, a fibrin-specific plasminogen activator with therapeutic potential? *Blood* 1994; **84**: 680–6.
90 Gardell SJ, Duong LT, Diehl RE, York JD, Hare TR, Register RB, Jacobs JW, Dixon RAF, Friedman PA. Isolation, characterization and cDNA cloning of a vampire bat salibary plasminogen activator. *J Biol Chem* 1989; **264**: 17947–52.
91 Deng YZ, Reeves MJ, Jacobs BS, Birbeck GL, Kothari RU, Hickenbottom SL, Mullard AJ, Wehner S, Maddox K, Majid A; Paul Coverdell National Acute Stroke Registry Michigan Prototype Inverstigators. IV tissue plasminogen activator use in acute stroke; experience from a statewide registry. *Neurology* 2006; **66**: 306–12.
92 Katzen IL, Hammer MD, Hixson ED, Furlan AJ, Abou-Chebl A, Nadzam DM; Cleveland Clinic Health System Stroke Quality Improvement Team. Utilization of intravenous tissue plasminogen activator for acute ischemic stroke. *Arch Neurol* 2004; **61**: 346–50.
93 Cohen JE, Itshayek E, Moskovici S, Gomori JM, Fraifeld S, Eichel R, Leker RR. State-of-the-art reperfusion strategies for acute ischemic stroke. *J Clin Neurosci* 2011; **18**: 319–23.
94 Furlan AJ, Abou-Chebl A. The role of recombinant pro-urokinase (r-pro-UK) and intra-arterial thrombolysis in acute ischaemic stroke: the PROACT trials. Prolyse in acute cerebral thromboembolism. *Curr Med Res Opin* 2002; **18**(Suppl 2): s44–7.
95 Nogueira RG, Liebeskind DS, Sung G, Duckwiler G, Smith WS; on behalf of the MERCI and Multi MERCI Writing Committee. Predictors of good clinical outcomes, mortality and successful revascularization in patients with acute ischemic stroke undergoing thrombectomy. Pooled analysis of the Mechanical Embolus Removal in Cerebral Ischemia (MERCI) and Multi MERCI Trials. *Stroke* 2009; **40**: 3777–83.
96 Menon BK, Kochar P, Ah-Seng A, Almekhlafi MA, Modi J, Wong JH, Hudon ME, Morrish W, Demchuk AM, Goyal M. Initial experience with a self-expanding retrievable stent for recanalization of large vessel occlusions in acute ischemic stroke. *Neuroradiology* 2011; Epub ahead of print.
97 Ribo M, Molina CA, Alvarez B, Rubiera M, Alvarez-Sabin J, Matas M. Intra-arterial administration of microbubbles and continuous 2-MHz ultrasound insonation to enhance intra-arterial thrombolysis. *J Neuroimaging* 2010; **20**: 224–7.
98 Alexandrov AV, Molina CA, Grotta JC, Garami Z, Ford SR, Alvarez-Sabin J, Montaner J, Saqqur M, Demchuk AM, Hoye LA, Hill MD, Wojner AW; CLOTBUST Investigators. Ultrasound-enhanced systemic thrombolysis for acute ischemic stroke. *N Engl J Med* 2004; **351**: 2170–8.
99 Anderson HR, Nielsen TT, Rasmussen K, Thuesen L, Kelbaek H, Thayssen P, Abildgaard U, Pedersen F, Madsen JK, Grande P, Villadsen AB, Krusell LR, Haghfelt T, Lomholt P, Husted SE, Vigholt E, Kjaergard HK, Mortensen LS; DANAMI-2 Investigators. A comparison of coronary angioplasty with fibrinolytic therapy in acute myocardial infarction. *N Engl J Med* 2003; **349**: 733–42.
100 Grines CL, Browne KF, Marco J, Rothbaum D, Stone GW, O'Keefe J, Overlie P, Donohue B, Chelliah N, Timmis GC, Vlietstra RE, Strzelecki M, Puchrowicz-Ochocki S, O'Neill WE; and the Primary Angioplasty in Myocardial Infarction Study Group. A comparison of immediate angioplasty with thrombolytic therapy for acute myocardial infarction. The Primary Angioplasty in Myocardial Infarction Study Group. *N Engl J Med* 1993; **328**: 673–9.
101 Marder VJ, Soulen RL, Atichartakarn V, Budzynski AZ, Parulekar S, Kim JR, Edward N, Zahavi J, Algazy KM. Quantitative venographic assessment of deep vein thrombosis in the evaluation of streptokinase and heparin therapy. *J Lab Clin Med* 1977; **89**: 1018.
102 Hirsh J, Salzman EW, Marder VJ. Treatment of venous thromboembolism. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. *Hemostasis and Thrombosis: Basic Principles and Clinical Practice*, 3rd edn. Philadelphia, PA: J. B. Lippincott, Co., 1994: 1346–66.
103 Lin PH, Ochoa LN, Duffy P. Catheter-directed thrombectomy and thrombolysis for symptomatic lower-extremity deep vein thrombosis: review of current interventional treatment strategies. *Perspect Vasc Surg Endovasc Ther* 2010; **22**: 152–63.
104 Comerota AJ, Throm RC, Mathias SD, Haughton S, Mewissen M. Catheter-directed thrombolysis for iliofemoral deep venous thrombosis improves health-related quality of life. *J Vasc Surg* 2000; **32**: 130–7.
105 Parikh S, Motarjeme A, McNamara T, Raabe R, Hagspiel K, Benenati JF, Sterling K, Comerota A. Ultrasound-accelerated thrombolysis for the treatment of deep vein thrombosis: initial clinical experience. *J Vasc Interv Radiol* 2008; **19**: 521–8.
106 Enden T, Klow NE, Sandvik L, Slagsvold CE, Ghanima W, Hafsahl G, Holme PA, Holmen LO, Njaastad AM, Sandbaek G, Sandset PM; CaVenT study group. Catheter-directed thrombolysis vs. anticoagulant therapy alone in deep vein thrombosis: results of an open randomized, controlled trial reporting on short-term patency. *J Thromb Haemost* 2009; **7**: 1268–75.
107 Grommes J, Strijkers R, Greiner A, Mahnken AH, Wittens CH. Safety and feasibility of ultrasound-accelerated catheter-directed thrombolysis in deep vein thrombosis. *Eur J Vasc Endovasc Surg* 2011; **41**: 526–32.
108 Grewal NK, Martinez JT, Andrews L, Comerota AJ. Quantity of clot lysed after catheter-directed thrombolysis for iliofemoral deep venous thrombosis correlates with postthrombotic morbidity. *J Vasc Surg* 2010; **51**: 1209–14.
109 Moudgill N, Hager E, Gonsalves C, Larson R, Lombardi J, DiMuzio P. May-Thurner syndrome: case report and review of the

literature involving modern endovascular therapy. *Vascular* 2009; **17**: 330–5.
110 Marder VJ. Foundations of thrombolytic therapy. In: Colman RW, Marder VJ, Clowes AW, George JN, Goldhaber SJ, eds. *Hemostasis and Thrombosis: Basic Principles and Clinical Practice*, 5th edn. Philadelphia, PA: Lippincott, Williams & Wilkins, 2006: 1739–52.
111 Marder VJ, Stewart D. Towards safer thrombolytic therapy. *Semin Hematol* 2002; **39**: 206–16.
112 Marder VJ. The use of thrombolytic agents: choice of patient, drug administration, laboratory monitoring. *Ann Intern Med* 1979; **90**: 802.
113 Marder VJ, Sherry S. Thrombolytic therapy: current status. *N Engl J Med* 1988; **318**: 1512–20 and 1585–1595.
114 Thanvi BR, Treadwell S, Robinson T. Haemorrhagic transformation in acute ischaemic stroke following thrombolysis therapy: classification, pathogenesis and risk factors. *Postgrad Med J* 2008; **84**: 361–7.
115 Wang X, Lo EH. Triggers and mediators of haemorrhagic transformation in cerebral ischaemia. *Mol Neurobiol* 2003; **28**: 229–44.
116 Wahlgren N, Ahmed N, Davalos A, Hacke W, Millan M, Muir K, Roine RO, Toni D; Lees KR for the SITS Investigators. Thrombolysis with alteplase 3-4.5 h after acute ischaemic stroke (SITS-ISTR): an observational study. *Lancet* 2008; **372**: 1303–9.
117 Uyttenboogaart M, Vroomen PCAJ, Stewart RE, De Keyser J, Luijckx GJ. Safety of routine IV thrombolysis between 3 and 4.5 h after ischemic stroke. *J Neurol Sci* 2007; **254**: 28–32.
118 Dzialowski I, Pexman JHW, Barber PA, Demchuk AM, Buchan AM, Hill MD; on behalf of the CASES Inverstigators. Asymptomatic hemorrhage after thrombolysis may not be benign. Prognosis by hemorrhage type in the Canadian alteplase for stroke effectiveness study registry. *Stroke* 2007; **38**: 75–9.
119 Toni D, Lorenzano S, Agnelli G, Guidetti D, Orlandi G, Semplicine A, Toso V, Caso V, Malferrari G, Fanucchi S, Bartolomei L, Prencipe M. Intravenous thrombolysis with rt-PA in acute ischemic stroke patients aged older than 80 years in Italy. *Cerebrovasc Dis* 2008; **25**: 129–35.
120 Singer OC, Berkefeld J, Lorenz MW, Fiehler J, Albers GW, Lansberg MG, Kastrup A, Rovira A, Liebeskind DS, Gass A, Rosso C, Derex L, Kim JS, Neumann-Haefelin T; for the MR Stroke Study Group Invertigators. Risk of symptomatic intracerebral hemorrhage in patients treated with intra-arterial thrombolysis. *Cerebrovasc Dis* 2009; **27**: 368–74.
121 Haley EC Jr, Thompson JLP, Grotta JC, Lyden PD, Hemmen TG, Brown DL, Fanale C, Libman R, Kwiatkowski TG, Llinas RH, Levine SR, Johnston KC, Buchsbaum R, Levy G, Levin B; for the Tenecteplase in Stroke Investigators. Phase IIB/III trial of tenecteplase in acute ischemic stroke. Results of a prematurely terminated randomized clinical trial. *Stroke* 2010; **41**: 707–11.
122 Hacke W, Furlan AJ, Al-Rawi Y, Davalos A, Fiebach JB, Gruber F, Kaste M, Lipka LJ, Pedraza S, Ringleb PA, Rowley HA, Schneider D, Schwamm LH, Leal JS, Sohngen M, Teal PA, Wilhelm-Ogunbiyi K, Wintermark M, Warach S. Intravenous desmoteplase in patients with acute ischaemic stroke selected by MRI perfusion-diffusion weighted imaging of perfusion CT (DIAS-2): a prospective, randomized, double-blind, placebo-controlled study. *Lancet* 2009; **8**: 141–50.
123 Ouriel K, Veith FJ, Sasahara AA. A comparison of recombinant urokinase with vascular surgery as initial treatment for acute arterial occlusion of the legs. Thrombolysis or Peripheral Arterial Surgery (TOPAS) Investigators. *N Engl J Med* 1998; **338**: 1105–11.
124 Agle SC, McNally MM, Powell CS, Bogey WM, Parker FM, Stoner MC. The association of periprocedural hypertension and adverse outcomes in patients undergoing catheter-directed thrombolysis. *Ann Vasc Surg* 2010; **24**: 609–14.
125 Kim HS, Preece SR, Black JH, Pham LD, Streiff MB. Safety of catheter-directed thrombolysis for deep venous thrombosis in cancer patients. *J Vasc Surg* 2008; **47**: 388–94.
126 Vik A, Holme PA, Singh K, Dorenberg E, Nordhus KC, Kumar S, Hansen JB. Catheter-directed thrombolysis for treatment of deep venous thrombosis in the upper extremities. *Cardiovasc Intervent Radiol* 2009; **32**: 980–7.
127 Broholm R, Panduro Jensen L, Baekgaard N. Catheter-directed thrombolysis in the treatment of iliofemoral venous thrombosis. A review. *Int Angiol* 2010; **29**: 292–302.
128 Maleux G, Marchal P, Palmers M, Heye S, Herhamme P, Vaninbroukx J, Verhaeghe R. Catheter-directed thrombolytic therapy for thoracic deep vein thrombosis is safe and effective in selected patients with and without cancer. *Eur Radiol* 2010; **20**: 2293–300.
129 Dhi HJ, Huang YH, Shen T, Xu Q. Percutaneous mechanical thrombectomy combined with catheter-directed thrombolysis in the treatment of symptomatic lower extremity deep venous thrombosis. *Eur J Radiol* 2009; **71**: 350–5.
130 Kucher N, Rossi E, De Rosa M, Goldhaber SZ. Massive pulmonary embolism. *Circulation* 2006; **113**: 577–82.
131 Lin PH, Annambhotia S, Bechara CF, Athamneh H, Weakley SM, Kobayashi K, Kougias P. Comparison of percutaneous ultrasound-accelerated thrombolysis versus catheter-directed thrombolysis in patients with acute massive pulmonary embolism. *Vascular* 2009; **17**(Suppl 3): S137–47.
132 Marder VJ, Landskroner K, Novokhatny V, Zimmerman TP, Kong M, Kanouse JJ, Jesmok G. Plasmin induces local thrombolysis without causing hemorrhage: a comparison with tissue plasminogen activator in the rabbit. *Thromb Haemost* 2001; **86**: 739–45.
133 Collen D. Revival of plasmin as a therapeutic agent? *Thromb Haemost* 2001; **86**: 731–2.
134 Novokhatny VV, Jesmok G, Landskroner K, Marder VJ, Zimmerman TP. Locally delivered plasmin: why should it be superior to plasminogen activators for direct thrombolysis? *Trends in Pharm Sci* 2004; **25**: 72–5.
135 Stewart D, Kong M, Novokhatny V, Jesmok G, Marder VJ. Relevance of plasma cogualtion for safety during plasmin administration: comparison with TPA in a model of fibrinolytic hemorrhage. *Blood* 2003; **101**: 3002–7.
136 Marder VJ, Blinc A, Gruber T, Tratar G, Sabovic M, Starkman S, Jahan R, Duckwiler G, Vinuela F, Tateshima S, Liebeskind D, Ovbiagele B, Ali L, Kim D, Gonzalez N, Vespa PM, Saver JL. Comparison of plasmin with rt-PA in lysis of cerebral thrombo-emboli retrieved from patients with acute ischemic stroke. *Stroke* 2011; **42**. in press.
137 Marder VJ. Preclinical studies of plasmin: superior benefit-to-risk ratio compared to tissue plasminogen activator (tPA). In: "Potential for better thrombolytic therapy: Is plasmin the answer?" Marder VJ and Novokhatny V, Guest Editors, *Thromb Res* 2008; **122**: S9–15.
138 Marder VJ, Novokhatny V. Direct fibrinolytic agents: biochemical attributes, pre-clinical foundation and clinical potential. *J Thromb Haemost* 2010; **8**: 433–44.
139 Nagai N, Demarsin E, van Hoef B, Wouters S, Cingolani D, Laroche Y, Collen D. Recombinant human microplasmin: production and potential therapeutic properties. *J Thromb Haemost* 2003; **1**: 307–13.
140 Toombs CF. Alfimeprase: pharmacology of a novel fibrinolytic metalloproteinase for thrombolysis. *Haemostasis* 2001; **31**: 141–7.
141 Hunt JA, Petteway SR Jr, Scuderi P, Novokhatny V. Simplified recombinant plasmin: production and functional comparison of a novel thrombolytic molecule with plasma-derived plasmin. *Thromb Haemost* 2008; **100**: 413–9.
142 Marder VJ, Manyak S, Gruber T, Goyal A, Moreno G, Hunt J, Bromirski J, Scuderi P, Petteway SR, Novokhatny V. Hemostatic safety of a unique recombinant plasmin molecule lacking kringles 2–5. *Thromb Haemost* 2010; **104**: 780–7.