Transfusion Medicine Reviews 29 (2015) 102–109

Contents lists available at ScienceDirect

Transfusion Medicine Reviews

journal homepage: www.tmreviews.com

The Fibrinolytic System—More Than Fibrinolysis?

Dominik F. Draxler, Robert L. Medcalf *

Australian Centre for Blood Diseases, Monash University, Melbourne, Victoria, Australia

ARTICLE INFO

Available online 18 December 2014

Keywords:
t-PA
Plasmin
Fibrinolysis
Tranexamic acid
Plasminogen activation

ABSTRACT

The fibrinolytic system, known for its ability to regulate the activation of the zymogen plasminogen into active plasmin, has been primarily associated with the removal of fibrin and blood clots. Tissue-type plasminogen activator, the most well-recognized plasminogen activator, was harnessed for therapeutic benefit against thromboembolic disorders more than 30 years ago, whereas inhibition of this system has been proven effective for certain bleeding disorders. However, in recent years, new and unexpected functional roles for this system have been identified mostly in relation to the central nervous system that are both unrelated and independent of fibrin degradation and clot removal. Hence, it seems reasonable to ask whether agents used to modify components or activities of the fibrinolytic system have any clinical consequences unrelated to their intended use in hemostasis. This review will provide an overview of these new features of the fibrinolytic system and will also focus on prospective considerations in the use of fibrinolytic and antifibrinolytic agents.

Contents

Blood coagulation is initiated by platelet activation and fibrin formation following injury or tissue damage [1]. On the other side of the coin, a cascade of serine proteases collectively referred to as the fibrinolytic system, counteracts this by initiating fibrin degradation and clot dissolution. The final step in this process of fibrinolysis is the conversion of the circulating zymogen plasminogen into its active form plasmin. In

Abbreviations: AIS, acute ischemic stroke; aPC, activated protein C; ATC, acute traumatic coagulopathy; BBB, blood-brain barrier; CPB, cardiopulmonary bypass; DAMP, damage-associated molecular pattern; DIC, disseminated intravascular coagulation; FDP, fibrin/fibrinogen degradation products; HIF-1α, hypoxia-inducible factor 1α; ICH, intracerebral hemorrhage; IL, interleukin; INR, international normalized ratio; aPTT, activated partial thromboplastin time; LDL receptor, low-density lipoprotein receptor; LRP, LDL receptor-related protein; MMP, matrix metalloproteinase; NCC, nucleocytoplasmic coagulation; NF-κB, nuclear factor-κB; NMDA receptor, *N*-methyl-D-aspartate receptor; PAI-1, plasminogen activator inhibitor 1; PAI-2, plasminogen activator inhibitor 2; PARs, protease-activated receptors; PDGF, platelet-derived growth factor; PDGFR-α, PDGF receptor α; ROTEM, rotational thromboelastometry; rt-PA, recombinant t-PA; TAFI, thrombin activatable fibrinolysis inhibitor; TBI, traumatic brain injury; TEG, thromboelastography; TF, tissue factor; TIMP1, tissue inhibitor of matrix metalloproteinase 1; TNF-α, tumor necrosis factor α; t-PA, tissue-type plasminogen activator; TXA, tranexamic acid; u-PA, urokinase-type plasminogen activator; uPAR, urokinase-type plasminogen activator receptor.

Conflict of interest statement: The authors declare that they have no conflict of interest with regard to the present manuscript.

* Corresponding author. Robert L. Medcalf, PhD, National Health and Medical Research Council (NHMRC) Principal Research Fellow, Australian Centre for Blood Diseases, Monash University, Level 6, Burnet Building, 89 Commercial Rd, Melbourne 3004, Victoria, Australia.

E-mail address: robert.medcalf@monash.edu (R.L. Medcalf).

http://dx.doi.org/10.1016/j.tmrv.2014.09.006
0887-7963/

the circulation, this is mainly achieved by tissue-type plasminogen activator (t-PA). This catalysis is highly dependent on the presence of the very protein that is ultimately being targeted by plasmin, namely fibrin, as binding of both plasminogen and t-PA to fibrin increases plasmin generation by more than two orders of magnitude [1]. Mechanistically, plasminogen binds to exposed lysine residues formed in fibrin and these binding sites increase in number during fibrin cleavage allowing more plasminogen binding to occur, thereby amplifying the process allowing more plasmin to be generated.

Plasmin is a potent protease, and it is critically important that plasmin formation occurs at the appropriate rate and location to minimize indiscriminate proteolysis. Although the lysine-dependent binding to fibrin is key to the targeted localization of plasmin, naturally occurring plasma inhibitors (α2 antiplasmin, plasminogen activator inhibitor [PAI]-1 and PAI-2) also exist to limit plasmin activity *or* its generation in the circulation. The most potent of these is α2 antiplasmin, a highly selective plasmin inhibitor that is present in the plasma at a very high concentration (~1 μM). It is important to add that plasmin is largely protected from α2 antiplasmin while bound to fibrin, allowing fibrin cleavage to occur. Other key regulatory steps occur at the level of the plasminogen activators, as the activity of t-PA and also urokinase-type plasminogen activator (u-PA), the second important endogenous plasminogen activator, are both regulated by PAI-1 and PAI-2 [1,2]. The most recently described mechanism that limits the fibrinolytic system is via "thrombin activatable fibrinolysis inhibitor" [3]. Thrombin activatable fibrinolysis inhibitor is a carboxypeptidase that has no effect on plasmin activity, but rather specifically removes exposed lysine residues from fibrin (and other substrates), thereby removing the ability of plasminogen and t-PA to dock onto lysine binding sites in fibrin. Being activated by thrombin, thrombin activatable fibrinolysis inhibitor appears to be engaged as a direct consequence of coagulation to stabilize and protect clots from premature removal by the fibrinolytic system. Taken together, many regulatory steps are on hand to limit plasmin formation or activity in vivo.

Fibrinolysis and Thrombolysis

A great effort was initiated in the 1980s and 1990s to explore the therapeutic potential of t-PA [4,5]. In 1983, the first study evaluating t-PA administration in patients undergoing acute myocardial infarction was performed (reviewed in Ref. [4]). These trials were positive and t-PA–induced thrombolysis enjoyed a robust period into the early 1990s prior to the introduction of coronary angioplastic approaches. The suitability of t-PA–induced thrombolysis for patients with acute ischemic stroke (AIS) took a longer path, mostly due to fears surrounding the risk of cerebral bleeding. Nonetheless, the use of t-PA in cerebrovascular thromboembolic disorders was confirmed, following the National Institute of Neurological Disorders and Stroke (NINDS) trial in 1995 that reported a significantly improved neurologic outcome after the administration of t-PA within 3 hours after stroke onset. However, t-PA treatment was associated with an increased risk of symptomatic intracerebral hemorrhage [6], which still today limits the effectiveness of t-PA in patients with AIS [7].

Plasmin-Independent Fibrinolysis

Data accumulated in recent years suggested not only that fibrinolysis could proceed in the absence of plasmin but also that the plasminogen activating system participates in processes unrelated to fibrinolysis [1,2,5]. Both t-PA and u-PA were among the first genes to be deleted from the mouse genome. It was anticipated at the time that mice deficient in plasminogen activation would have severe changes in hemostasis including a propensity for thrombosis. The generation of these mice revealed that neither t-PA nor u-PA was essential for embryonic development. Although there was attenuated thrombolytic capacity in t-PA deficient mice, the life span was only reduced in animals deficient for both proteases [8,9]. Spontaneous thrombosis in t-PA–deficient mice occurred only when challenged with endotoxin injection [9]. Combined deficiency, however, was associated with spontaneous fibrin deposits in various tissues, cachexia, reduced fertility, and rectal prolapse [8,9]. Interestingly, patients with homozygous plasminogen deficiency acquire ligneous conjunctivitis but, surprisingly, are not at increased risk for thrombotic events [10]. Similarly, there is no clear evidence of increased thrombotic risk due to decreased t-PA levels [10]. Collectively, these findings intimate that physiological fibrinolysis can proceed in a manner independent of plasmin by being substituted by other proteases. Indeed, plasmin-independent fibrinolysis has been reported in earlier studies using plasminogen-deficient mice [11,12].

Nonfibrinolytic Roles of t-PA and Plasmin: The Central Nervous System

Notwithstanding the importance of the fibrinolytic system in fibrin removal, it had been long suspected that this system also participated in key events related to brain function. Plasminogen activators (initially unidentified but likely to be t-PA) were first detected in the brain in 1981 [13,14], whereas enhanced t-PA expression in the brain was subsequently observed in rat models of seizure, experimental epilepsy, and long-term potentiation [15]. However, the most compelling findings linking brain t-PA to central nervous system (CNS) function were revealed by Tsirka et al [16] in 1995. In a series of landmark studies from this group, t-PA was found not only to mediate excitotoxic neuronal degradation via its proteolytic activity but also to activate microglia in a manner independent of its proteolytic function [17], indicating that t-PA can act as a ligand and promote intracellular signalling. Tissue-type plasminogen activator deficient mice were also found to display retarded migration of granule cells into the internal granule cell layer of the cerebellum [18], thereby associating t-PA with motor function.

A plethora of data has been produced in recent years further cementing t-PA and related proteins in neurobiology. It is now clear that t-PA is involved not only in physiological events such as synaptic plasticity, learning, and behavior, but also pathological events such as seizures, dementia, multiple sclerosis, and cerebral ischemia [19]. Tissue-type plasminogen activator has also been implicated in ethanol withdrawal seizure [20] and drug addiction (see Ref. [21] for review).

How does t-PA modulate neuronal function? Most early reports pointed to a direct interaction with *N*-methyl-D-aspartate (NMDA) receptor activation. Indeed, t-PA was described to enhance glutamatergic neurotransmission, resulting in increased calcium influx and neuronal cell death by its proteolytic activity on NMDA receptors [22,23], although this is an area of controversy [24]. Although the mechanisms underlying t-PA–mediated neurotoxicity are still being pursued, other studies, contrastingly, have suggested neuroprotective effects of t-PA by preventing zinc toxicity, by its interaction with other cellular targets including hypoxia-inducible factor 1 [25,26] and by promoting glucose uptake via the GLUT3 glucose transporter [27]. Further neuroprotective effects of t-PA and rt-PA in cerebral ischemia have been described via induction of neuronal tumor necrosis factor α [25] as well as interaction with NMDA receptors [25,26]. These contrasting effects of t-PA seem to be explained by the different approaches used by various researchers and also the concentration at which t-PA was used, because neuroprotective effects were usually observed only at very low concentrations and damaging effects seen with high concentrations. Nonetheless, the original observation that t-PA–deficient mice were protected from excitotoxic injury directly implicated t-PA in this neurotoxic pathway.

A key question is whether the ability of t-PA to modulate neuronal function has any bearing on the clinical use of t-PA in patients with AIS; can blockade of any neurotoxic effect of t-PA result in an improved outcome, or alternatively can potentiation of protective effects be considered? In this context, a beneficial effect of antibodies that blocked the binding of t-PA to NMDA receptors, reducing neurotoxicity was seen in a rodent model of ischemic stroke [28], giving some support to this notion, although this is still an area of controversy.