Potential for better thrombolytic therapy with Plasmin – a novel plasma product

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Plasmin Basics
Diseases of Thrombosis

Clot formation in blood vessels represent the mechanism of mortality in some of the most prevalent, debilitating diseases.

- **Thrombosis is primary mechanism in:**
  - Myocardial Infarction
  - Ischemic Stroke
  - Pulmonary Embolism
  - Acute Peripheral Arterial Occlusion
  - Deep Vein Thrombosis

- **Fibrin-related conditions:**
  - Occlusion of grafts and catheters
  - Scar formation- Surgical adhesions
  - Wound healing
Fibrinolytic System

Plasminogen Activators

PAI-1
(inhibits tPA, present in blood in miniscule amount)

Cleave plasminogen and render it active

Plasminogen → Plasmin

Tissue Plasminogen Activator (tPA)
Urokinase, Streptokinase
Staphylokinase, Vampire Bat PA

α2-antiplasmin
(physiological inhibitor of plasmin present in blood in concentration 6-8X exceeding therapeutic dose of plasmin)

Fibrin

Degrades fibrin clot, core of thrombus

Fibrin Degradation Products
Milestones in Thrombolytic Therapy

1933 Streptokinase is discovered

1953 Plasminogen is purified

Attempts to improve PA’s with Lys-Plasminogen (Immuno, NovoNordisk)

1945 Plasminogen is discovered

Plasmin, (IV) rather than Sk is considered for thrombolysis

1942 Plasmin, (IV) rather than Sk is considered for thrombolysis

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Actase (Ortho) Thrombolysin (Merck), Pig Plasmin (Novo)

1952 Streptokinase IV (Lederle Labs)

1965 Urokinase (Abbott)

1981 Eminase Sk-Pg (SKB)

1984 Rec. tPA (Genentech)

80-90’s New PA for MI: Reteplase, TNK-tPA, Vampire Bat PA, Pro-urokinase, Staphylokinase

Realization of PA problems: bleeding complications and stroke
Plasmin Structure

714 amino acids, 2 chain, disulfide linked
Heavy or Binding Chain: 5 “kringle” domains
Light or Catalytic Chain: catalytic site

Plasminogen concentration in pooled plasma: 0.14 mg/mL

Activators:
tPA, Urokinase
Streptokinase, Staphylokinase

24 disulfide bonds

Pre-activation Peptide

Plasminogen
Plasmin

Heavy Light

Catalytic Chain

2 glycosylation forms at 1:2 ratio:
Thr^{346} O-linked
Thr^{346}/Ser^{248} O-linked & Asn^{289} N-linked

Glycosylation sites - N^{289} and T^{346}
Plasmin Use

Plasmin is for use for local, catheter-assisted administration
Plasmin Manufacturing
Purification of Plasmin from II+III Paste

Part 1: Purification of Plasminogen

Part 2: Activation of plasminogen and removal of impurities

Part 3: Formulation, Fill and Freeze-Dry
Plasmin formulation

Low pH, low buffering capacity formulation is the key for production of the stable and clean material. This formulation is compatible with parenteral administration (does not require neutralization prior to infusion).

- US Patent #6,355,243: “Method of thrombolysis by local delivery of active plasmin, plasmin formulation, and process of producing”
- US Patent #6,964,764: “Method of thrombolysis by local delivery of reversibly inactivated acidified plasmin”
  Claims methods involving therapeutic administration of acidified serine proteases (and plasmin specifically) to subjects having thrombotic occlusions.
Viral validation data demonstrate that the Plasmin manufacturing process is capable of removing and inactivating significant levels of both enveloped and non-enveloped viruses. Overall the Plasmin manufacturing process yielded reductions of $\geq 18.5 \log_{10} \text{ HIV-1}$, $\geq 14.2 \log_{10} \text{ BVDV}$, $\geq 17.4 \log_{10} \text{ PRV}$, $\geq 13.0 \log_{10} \text{ Reo3}$, $\geq 9.5 \log_{10} \text{ HAV}$, and $7.9 \log_{10}$ of Parvoviridae models for B19V.

<table>
<thead>
<tr>
<th>Purification Step</th>
<th>Enveloped viruses</th>
<th>Non-enveloped viruses</th>
<th>Model for B19V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV</td>
<td>BVDV</td>
<td>PRV</td>
</tr>
<tr>
<td>Caprylate Cake Extraction / PEG Precipitation &amp; Depth Filtration</td>
<td>3.6</td>
<td>2.1</td>
<td>4.3</td>
</tr>
<tr>
<td>Caprylate Incubation</td>
<td>$\geq 5.7$</td>
<td>$\geq 4.3$</td>
<td>$\geq 4.5$</td>
</tr>
<tr>
<td>Benzamidine Sepharose Chromatography</td>
<td>2.9</td>
<td>2.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Nanofiltration</td>
<td>$\geq 6.3$</td>
<td>$\geq 5.3$</td>
<td>$\geq 4.6$</td>
</tr>
<tr>
<td>Global reduction factor</td>
<td>$\geq 18.5$</td>
<td>$\geq 14.2$</td>
<td>$\geq 17.4$</td>
</tr>
</tbody>
</table>
Comparison of Plasmin to tPA for Local Thrombolysis
(direct versus indirect thrombolysis)
Different Mechanisms of Action for tPA (indirect thrombolytic) and Plasmin (direct thrombolytic)

**Coronary thrombosis**
- Small clots, plasminogen available
- tPA
- Plasminogen (Pg)
- Thrombolysis
- Systemic tPA works

**Peripheral thrombosis**
- (Peripheral Arterial Occlusion - PAO)
- (Deep Venous Thrombosis - DVT)
- Very large clots, plasminogen not readily available
- Local Plasmin works
- Catheter
Plasmin vs tPA in an *In Vitro* Model of Local Thrombolysis
Plasmin is superior to tPA and urokinase in animal models of arterial and venous thrombosis.

### Rabbit Arterial Occlusive Model

<table>
<thead>
<tr>
<th>Restoration of Flow</th>
<th>Partial</th>
<th>Complete</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0</td>
<td>0</td>
<td>0/2</td>
</tr>
<tr>
<td>TPA</td>
<td>0</td>
<td>0</td>
<td>0/4</td>
</tr>
<tr>
<td>Urokinase</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Plasmin</td>
<td>1</td>
<td>3</td>
<td>4/4</td>
</tr>
</tbody>
</table>

- **Clot Weight, mg**
- **catheter-delivery, 10 ml, 2 x 30 min each**
  - 2 x 1 mg/kg tPA
  - 2 x 20,000 IU Urokinase
  - 2 x 2 mg/kg Plasmin

* p < .05

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**Notes:**
- **Activase**
- **tPA**
- **Urokinase**
- **Plasmin**

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**Image Descriptions:**
- **Flow Probe**
- **Catheter In Mesenteric Artery**
- **Arterial Clot**
- **Direction Of Flow**
- **Occluder**
Plasmin could be safer than tPA because it does not cause uncontrolled bleeding in a rabbit re-bleeding safety model (V. Marder, UCLA).

In this animal model of fibrinolytic hemorrhage, rebleeding of previously stable puncture sites was observed in rabbits treated with tPA (even at sub-therapeutic dose of 0.25 mg/kg) but not with Plasmin, even at doses up to 6 mg/kg. (therapeutically effective dose is ~ 1mg/kg)

**tPA vs plasmin: hemorrhage dose-dependence**

<table>
<thead>
<tr>
<th></th>
<th>tPA</th>
<th>Plasmin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombolytic dose</td>
<td>1 mg/kg</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td>Dose at which bleeding occurred</td>
<td>0.25 mg/kg</td>
<td>6 mg/kg</td>
</tr>
<tr>
<td>Safety margin</td>
<td>None</td>
<td>Significant (6-fold)</td>
</tr>
</tbody>
</table>

![Graph showing rebleeding from stable hemostatic sites vs dose for tPA and Plasmin](image-url)
Bleeding Safety

**tPA**

- Local tPA administration dissolves thrombus
- tPA exceeds the neutralizing capacity of PAI-1 in systemic circulation
- tPA dissolves distant hemostatic plug and causes bleeding

**Plasmin**

- Local plasmin administration dissolves thrombus
- Plasmin is rapidly neutralized by $\alpha_2$-antiplasmin and $\alpha_2$-macroglobulin in systemic circulation
- Hemostatic plug unaffected, preserving hemostasis
Other Direct Thrombolytics in Development

Alfimeprase
Microplasmin
\(\Delta(K2-K5)-\text{Plasmin}\)
## Comparison of Direct Thrombolytic Agents

<table>
<thead>
<tr>
<th>Thrombolytic</th>
<th>Origin</th>
<th>Fibrin Binding</th>
<th>Inhibition In Plasma</th>
<th>Induce Bleeding</th>
<th>Clinical Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD Plasmin</td>
<td>Human (pd)</td>
<td>Yes</td>
<td>Fast</td>
<td>No</td>
<td>PAO on-going</td>
</tr>
<tr>
<td>Delta-plasmin</td>
<td>Human (rec)</td>
<td>Yes</td>
<td>Fast</td>
<td>No</td>
<td>Pre-clinical</td>
</tr>
<tr>
<td>Microplasmin</td>
<td>Human (rec)</td>
<td>No</td>
<td>Slower</td>
<td>Yes</td>
<td>Ophthalmic Ph3</td>
</tr>
<tr>
<td>Alfimeprase</td>
<td>Snake (rec)</td>
<td>No</td>
<td>Slow</td>
<td>Yes</td>
<td>Abandoned</td>
</tr>
</tbody>
</table>

**Plasmin**

**Δ (K2-K5) plasmin**

**Micro-plasmin**

**Alfimeprase**

**rec fibrolase**
pdPlasmin and recPlasmin have similar biological activity

RecPlasmin preserves key functional attributes of plasmin:
- proteolytic catalytic parameters
- rate of inhibition by a2-antiplasmin
- fibrin affinity

Improved bleeding safety
Produced in *E. coli* expression system (yield – up to 3.5 g/L)
Clinical Development

Stroke

aPAO

Vitreal detachment
In a Phase I study in Hemodialysis Graft Occlusion, Plasmin was well tolerated and demonstrated a dose response trend in clot lysis

In this safety oriented clinical trial in a small number of patients, Plasmin appears safe and efficacious

- No major bleeding events in HGO Phase I.
- No identified dose-related increase in adverse events or effect on laboratory parameters, including fibrinogen and alpha-2 anti-plasmin.
- Dose-dependent increase in the proportion of subjects with > 50% lysis by angiography

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>1 mg</th>
<th>2 mg</th>
<th>4 mg</th>
<th>8 mg</th>
<th>12 mg</th>
<th>24 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis (&gt;50%)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Lysis (&gt;75%)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>
Talecris Phase I aPAO trial

- Completed in 2008
- Plasmin in doses of up to 100 mg was safe and well tolerated in PAO patients.
- Good evidence of clot lysis with plasmin (Native artery length average = 26.4 cm; graft length average = 39 cm)
Summary

- Plasmin is being developed by Talecris Biotherapeutics as a direct acting thrombolytic for local, catheter-assisted administration
- Plasmin is produced in highly pure and stable form, double viral inactivated
- Simplified, recombinantly modified plasmin has been created which preserves essential functional characteristics of natural Plasmin
- Plasmin has shown to be efficacious and safe in multiple preclinical models
- Clinical studies are underway. Evidence of Plasmin safety and efficacy are seen
Questions?