Acid-Stabilized Plasmin as a Novel Direct-Acting Thrombolytic

Valery Novokhatny* and Steve Petteway

Research and Development
Milestone in Thrombolytic Therapy

1933 Streptokinase is discovered

1945 Plasminogen is discovered

1952 Streptokinase IV (Lederle Labs)

1953 Plasminogen is purified

Plasmin, rather than Sk is considered for thrombolysis

1953 Actase (Ortho) Thrombolysin (Merk), Pig Plasmin (Novo)

1965 Urokinase (Abbott)

1981 Eminase Sk-Pg (SKB)

1984 Rec. tPA (Genentech)

80-90’s New PA: Reteplase, TNK-tPA, Desmoteplase, Pro-urokinase, Staphylokinase

1984 New generation of direct thrombolytics: Plasmin (Talecris), Micro-plasmin (Thrombogenics), Alfimeprase (Nuvelo)

Realization of PA’s problems: bleeding complications and stroke

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

Sk was found to be an active moiety in Plasmin preparations

Attempts to improve PA’s with Lys-Plasminogen (Immuno, NovoNordisk)
Plasmin is a Key Component of the Fibrinolytic System

- Plasminogen Activators (PAs) act indirectly, and thus are dependent on availability of plasminogen
- **Plasmin acts directly on the fibrin clot**
- High fibrin-binding affinity allows Plasmin to bind to the clot, facilitating effective clot lysis
- Unbound Plasmin is quickly inhibited by alpha-2-antiplasmin, which is present in excess of therapeutic dose, enabling a strong safety profile
Schematic Mechanism of Action (Plasmin versus tPA)

- Administered locally, tPA dissolves thrombus.
- tPA circulates and binds to hemostatic plugs at sites of vascular trauma.
- Hemostatic plugs dissolved.
- Bleeding follows.

- Local plasmin administration dissolves thrombus.
- In the circulation, plasmin neutralized by antiplasmin.
- Plasmin does not reach hemostatic plugs.
- No bleeding.

(Marder et al. Thromb Haemost 2001; 86: 739-45)
Preclinical Studies to Assess Efficacy and Safety: Benefit Ratio

- **Working hypothesis:**
  - Plasmin dissolves clot effectively when delivered locally by catheter, without causing bleeding.

- **Experimental plan:**
  - Determine efficacy of plasmin on vitro and in relevant animal thrombosis models.
    - Compare with tPA.
  - Determine hemostatic safety in the ear puncture model of hemorrhage.
    - Compare with tPA.
  - Conduct a series of formal GLP Toxicolgy studies.
Efficacy of Plasmin vs tPA in *In Vitro* Model of Local Thrombolysis

Insertion of the catheter into the clot

Catheter

Retracted Clot

Clot Weight Reduction, %

<table>
<thead>
<tr>
<th>Dose, mg</th>
<th>Plasmin</th>
<th>t-PA</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Preclinical *In Vivo* Thrombolytic Efficacy Study: Rabbit MCA Stroke model

- Model: 2-hour, thrombin-induced middle cerebral artery occlusion using angiographic documentation of vascular patency and recanalization
- Plasmin, delivered by catheter over a median duration of 10 minutes.
- Plasmin induced early recanalization in all animals (3 per group) within 10 minutes after discontinuation of a 3, 2, or 1 mg infusion.
- Control saline infusion failed to induce recanalization in all tested animals.
Preclinical Safety: Ear Puncture Bleeding Model

- General anesthesia (isoflurane).
- Cut down internal jugular vein for double lumen catheter.
- Randomized, blinded infusion of tPA or plasmin.
- Primary bleeding times prior to, during and after infusions.
- Bleeding sites observed for rebleeding.
- Blood for coagulation assays from jugular vein.

<table>
<thead>
<tr>
<th>Plasmin or TPA Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>-30 0 30 60 90 120 150 180</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bleeding Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blood Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑</td>
</tr>
</tbody>
</table>
Representative Results in the Ear Puncture Model

Plasmin (2 mg/kg)
Normal Bleeding Time
No rebleeding

BT long (post 60 min).
Rebleeding (4 sites).

tPA (1 mg/kg)
Rabbit Re-bleeding Safety Model Suggests Plasmin Safety Margin

• In an animal model of fibrinolytic hemorrhage, re-bleeding of previously stable puncture sites was observed in rabbits treated with tPA
  – Occurred even at sub-therapeutic dose of 0.25 mg/kg

• With Plasmin, prolongation of bleeding time is the main adverse outcome
  – At 3-4X the therapeutically effective dose


GLP Safety Pharmacology and Toxicology Studies

- Studies were conducted in healthy, naïve Sprague-Dawley rats and Beagle dogs

- Safety Pharmacology
  - cardiovascular system (rats and dogs)
  - nervous system (dogs)
  - pulmonary function (rats)
  - hematological function was integrated into the acute- and repeat-dose toxicity studies (rat, dog)
  - behavioral assessment (for CNS function) and renal function were also monitored as standard assessments

- Toxicology
  - repeat-dose (3-day daily) toxicity studies (rats and dogs)
  - toxicokinetic studies (dogs)
GLP Toxicology and Safety Pharmacology Studies

- All dose levels (up to 16 mg/kg) were well tolerated, and none caused severe hemorrhage or treatment-related histopathological changes.

- Administration of Plasmin (Human) up to 4 mg/kg is devoid of clinically relevant adverse effects and was the typical NOAEL in toxicology and safety pharmacology studies.

- Toxicology studies showed that an infusion of approximately 8 mg/kg Plasmin was required to see any systemic effects on coagulation.
  - Typical findings included prolongation of coagulation times and rebleeding from hemostatically stable remote incisions.
  - Biomarkers such as fibrinogen and A2AP were depressed but not depleted.

- Drug-interaction studies did not identify synergistic effects upon cotreatment with therapeutically relevant doses of aspirin, heparin or both.

- No adverse findings in cardiovascular, respiratory, neurological safety pharmacology or brain histopathology studies.

- Nonclinical data generated in multiple models with a wide range of doses strongly support the safety of Plasmin (Human).
Plasmin: Efficacy/Safety Relation

**Efficacy**
- HGO Phase I: Efficacious dissolving 4-5g clots at ~0.3 mg/kg
- Highest dose in Stroke Phase I (60 mg/~1 mg/kg)
  - Clot Lysis in Rabbit venous occlusion model (2-4 mg/kg)
- Restoration of Flow in Rabbit arterial occlusion model

**Safety**
- NOAEL—Single dose, dogs
  - Appearance of free Pm activity in plasma (in vitro study)
- Bleeding in dogs in repeat dose tox study (single dose – at 16 mg/kg)
- α2AP Inhibiton
- α2-Macroglobulin

**Therapeutic Index**
- Depletion of plasma inhibitors
- Prolongation of PBT in Rabbit ear bleed model
Challenges of working with Plasmin
- Active, broad specificity protease prone to autodegradation
- Precipitation/Aggregation during pH swings
- Protection from autolysis

Challenges specific to Processing
- Minor component in IGIV-C Caprylate Cake
- Poor solubility
- Activation of Plasminogen to Plasmin & removal of activator (Sk) and impurities
- Incorporation of Viral inactivation steps

Challenges specific to Testing
- Maintenance of Plasmin Activity
- Interference with many standard assays techniques (e.g., endotoxin, prion bioassay)
- Administration of known amount of active drug during lengthy clinical procedures
Low pH 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; # 6,964,764; # 6,969,515: “Method of thrombolysis by local delivery of active plasmin, plasmin formulation, and process of producing”
  These patents claim methods involving therapeutic administration of acidified serine proteases (and plasmin specifically) to subjects having thrombotic occlusions. As well as the process of plasmin production.
- Sister patents are granted in EU including the patent on Plasmin formulation.
Part 1: Purification of Plasminogen
- IGIV-C Caprylate Cake Suspension
- Depth Filtration Viral/TSE reduction
- Dowex CIEX / Lysine Affinity Chromatography
- Plasminogen

Part 2: Purification of Plasmin and Removal of Impurities
- Caprylate Incubation Viral inactivation
- Streptokinase Activation
- Benzamidine Affinity Chromatography Viral removal
- Octyl-Sepharose Hydrophobic Chromatography
- UF/DF Nanofiltration Viral/TSE removal

Part 3: Formulation, Fill and Freeze-Dry
- Formulated Bulk
- Sterile Filter
- Freeze Dry
Pathogen Safety

• Viral validation data demonstrate that the Plasmin manufacturing process is capable of removing and inactivating significant levels of both enveloped and non-enveloped viruses.
• In addition, effective removal of prion proteins, which have been associated with transmissible spongiform encephalopathies. The caprylate cake suspension/PEG precipitation/depth filtration and the nanofiltration steps were shown to provide significant removal of these proteins should they be present in the starting material.

<table>
<thead>
<tr>
<th>Purification Step</th>
<th>Enveloped viruses</th>
<th>Non-enveloped viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV</td>
<td>BVDV</td>
</tr>
<tr>
<td>Caprylate Cake Extraction / PEG Precipitation &amp; Depth Filtration</td>
<td>3.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Caprylate Incubation</td>
<td>≥ 5.7</td>
<td>≥ 4.3</td>
</tr>
<tr>
<td>Benzamidine Sepharose Chromatography</td>
<td>2.9</td>
<td>2.5</td>
</tr>
<tr>
<td>Nanofiltration</td>
<td>≥ 6.3</td>
<td>≥ 5.3</td>
</tr>
<tr>
<td>Global reduction factor</td>
<td>≥ 18.5</td>
<td>≥ 14.2</td>
</tr>
</tbody>
</table>
Plasmin Product Characterization

A. Plasminogen process

B. Plasmin process

Panel A
1. Caprylate Cake suspension
2. CUNO filtrate
3. DOWEX column load
4. Lysine-Sepharose FT
5. Lysine-Sepharose eluate

Panel B
1. Thawed Plasminogen
2. Post Streptokinase activation
3. Benzamidine-Seph load
4. Benzamidine-Seph FT
5. Benzamidine-Seph eluate
6. HIC column load
7. HIC column FT
8. HIC column FT (hold)
9. UF/DF concentrate
10. Formulated bulk Plasmin.

<table>
<thead>
<tr>
<th>TEST</th>
<th>REQUIREMENTS</th>
<th>RESULTS (N=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>average</td>
<td>s.d.</td>
</tr>
<tr>
<td>Plasmin Potency*</td>
<td>NLT 0.8 mg/mL</td>
<td>1.08</td>
</tr>
<tr>
<td>Specific Activity</td>
<td>NLT 0.7 mg/mg</td>
<td>0.96</td>
</tr>
<tr>
<td>Purity by SEC-HPLC (% Monomer)</td>
<td>NLT 90%</td>
<td>99.21</td>
</tr>
<tr>
<td>Protein Purity by Electrophoresis, reduced (Bioanalyzer)</td>
<td>Report % (establish for BLA)</td>
<td>89.6</td>
</tr>
</tbody>
</table>
Clinical Experience

Plasmin is for local, catheter-delivered administration
In a Phase I, first in man study in Hemodialysis Graft Occlusion, Plasmin was well tolerated and demonstrated a dose response trend in clot lysis

- Lysis observed in 100% of 24 mg dose cohort
  - Dose-dependent increase in number subjects with ≥ 50% lysis by angiography

<table>
<thead>
<tr>
<th></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>

- Plasmin was well tolerated
- No signals of concern for bleeding, thrombosis, allergic reactions
  - No major bleeding events in HGO Phase I
    - minor bleeding, related to heparin, in 5 subjects
  - No identified dose-related increase in adverse events or effect on laboratory parameters, including fibrinogen and alpha-2 antiplasmin.
Peripheral Arterial Occlusion (PAO) Study

- **Phase 1/2** Dose Escalation and Safety Study of **Plasmin (Human)** In Acute Lower Extremity Native Artery or Bypass Graft Occlusion (PRIORITY) has been completed.
- Sequential 10-13 patient cohorts received a single, ~ 5-hour intrathrombus dose of Plasmin (25 to 175 mg) by multi-slit catheter using manual pulse-spray injections followed by continuous infusion.
- Safety was assessed with clinical evaluations and monitoring of plasma concentrations of fibrinogen and α2-antiplasmin inhibitor at the end of the Plasmin treatment and on days 1–2, 7, 14 and 30.
- Of 83 patients enrolled, 19 (22.9%) had serious adverse events (SAEs) and 57 (68.7%) had treatment-emergent (TE) adverse events (AEs). Two patients died of conditions unrelated to Plasmin administration (myocardial infarction, sepsis). Four patients had a major bleeding event and 14 had minor bleeding events.
- **None of the SAEs, deaths or bleeding events were reported by investigators to be Plasmin-related.**
- TEAEs, SAEs, and bleeding events did not increase with Plasmin dose
- Plasmin has a potential thrombolytic benefit in treating aPAO: greater than 50% thrombolysis of the occlusions was demonstrated in half of the subjects.
- **Phase 2** study is to optimize **Plasmin (150 mg)** delivery by comparing 4 different delivery regimens in patients with acute peripheral arterial occlusion is on-going. The study will also compare **Plasmin** to placebo and to plasminogen activators.
Stroke Study

- Acid-stabilized **Plasmin** is being tested in a Phase I, open-label, multi-center, international, sequential dose escalation, safety study in acute ischemic stroke caused by middle cerebral artery occlusion documented by arteriography.

- Acidified **Plasmin** is administered through a catheter into the thrombus within 9 hours of stroke onset.

- A maximum of seventy-five (75) patients will receive **Plasmin**, a maximum of twenty-five (25) patients at each of the 3 dose levels (20 mg, 40 mg, and 60 mg).

- The objectives of this study are to determine the safety and tolerability of **Plasmin**, to select a dose for further testing, and to determine the proportion of patients with partial or full recanalization.
Summary

- Plasmin is being developed by Talecris Biotherapeutics as a direct acting thrombolytic for local, catheter-assisted administration.
- Preclinical models suggest good efficacy and a superior safety profile with respect to bleeding risk with Plasmin compared to tPA.
- Preclinical toxicology and safety pharmacology studies support the safety profile of Plasmin.
- Plasmin is manufactured in highly pure and stable form, double viral inactivated.
- Clinical studies with Plasmin are on-going: good safety and thrombolysis of clots are being seen.
Questions