Annexin V-6L15, A Novel Injury-Site Directed Anticoagulant Ameliorates Ischemic-Reperfusion Injury and Promotes Survival of Ischemic Rat Abdominal Fasciocutaneous Flaps

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Background

• Imperfect technique, inappropriate anticoagulation or perfusion facilitation, extended ischemia time are key reasons for partial flap failure

• Current anticoagulants: aspirin, warfarin, heparin, LMWH, direct thrombin inhibitors → systemic effects may lead to bleeding risk
Design of Annexin V-6L15

• Fusion protein: Annexin V (ANV) + 6L15

• Acts as Tissue factor-VIIa pathway inhibitor

Image sources: Chen HH, et al. *Blood* (2005); Wikimedia Commons
Mechanism of action

- Annexin V: targets phosphatidylserine (PS) binding sites, which are expressed in apoptotic/injured endothelial cells
- 6L15: aprotinin mutant (KPI domain) with inhibitory activity towards VIIa-TF complex

Abdominal Fasciocutaneous Flap Model

- Based on unilateral superficial inferior epigastric artery (SIEA)
- Pedicle extension to proximal femoral artery/vein
- Artery clamped, No anastomosis

Suprapubic Line

xiphoid process
mid-axillary line
1 cm from inguinal crease
Treatment Protocols- Flap model

- Drug administered through penile vein (n=6)
  1. Dosage: 200µg/kg, total 4 dosages

A. Pretreatment
- Flap elevation
- Ischemia
- Reperfusion
- Data Collection
- 45 min.
- 10 hrs
- 12 hrs
- 12 hrs

ANV-6L15 (1/2 dose)
ANV-6L15 (1/2 dose)
ANV-6L15
ANV-6L15

B. Ischemia Control
- Flap elevation
- Ischemia
- Reperfusion
- Data Collection
- 45 min.
- 10 hrs
- 12 hrs
- 12 hrs
- 12 hrs

ANV-6L15
ANV-6L15
ANV-6L15
ANV-6L15

C. Post-treatment
- Flap elevation
- Ischemia
- Reperfusion
- Data Collection
- 45 min.
- 10 hrs
- 12 hrs
- 24 hrs
- 12 hrs

ANV-6L15
ANV-6L15
ANV-6L15
ANV-6L15

D. Sham
- Flap elevation
- Reperfusion
- Data Collection
- 45 min.
- 10 hrs
- 12 hrs
- 12 hrs
- 12 hrs
- 12 hrs

ANV-6L15
ANV-6L15
ANV-6L15
ANV-6L15
Sham

Day 5 Survival

84.7±4.6%

Post-Rx

61.2±6.9%

ANOVA: p<0.0001

Post-hoc test:
All vs. Sham
Post vs. Control
Pre vs. Control
p<0.05

Control

48%±8.7%

Pre-Rx

59.3±6.9%
Near-IR perfusion imaging with novel imaging agent at 36 hours post op
*Dark zones are poorly perfused
PT/APE Assay

- Samples: Day 2 (n=5~6)
- Positive control: unfractionated heparin (300u/kg)
- Blood samples taken 5 min. post Rx
- The Ceveron alpha (Technoclone GmbH, Austria) automated blood coagulation analyzer was used.
  Reagents: Technoplastin® HIS for PT and Siron LS for aPTT.
Cremaster model

- Drug administered through right JV cannula
- Dosage: 200µg/kg
- Groups: Treatment vs. Ischemia vs. ANV

1. Post stabilization baseline taken

Intravital microscope observations (n=5)

Treatment protocol
Leukocyte-endothelial Interaction

Baseline

No Treatment

Increased number of leukocytes

Treatment

No increase
Tissue viability assay

skeletal muscle flap was incubated in 2,3,5-triphenyl-tetrazolium chloride solution (Sigma-Aldrich, USA) at 37°C for 30 minutes: non-viable tissue (white staining) vs viable tissue (brick-red)

<table>
<thead>
<tr>
<th>TCC Assay</th>
<th>No Treatment</th>
<th>Treatment</th>
<th>Annexin V</th>
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<tbody>
<tr>
<td>Treatment</td>
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<tr>
<th>2,3,5 TTC assay for cremaster island flap survival (n=5)</th>
<th>Control</th>
<th>Treatment</th>
<th>Annexin V</th>
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<tbody>
<tr>
<td>Viable flap (%)</td>
<td>68.5±9.79</td>
<td>84.7±5.14</td>
<td>70.9±8.61</td>
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* p<0.05
Distribution and Excretion

1. Instantaneous distribution of ANV-6l15 into cremaster circulation
2. Fluorescence begins to fade by 10 minutes
3. Excretion of ANV-6l15 by 2 hrs (penis)

Near-IR fluorescent probe conjugated ANV-6l15 study
Specific Binding & Duration

Near-IR fluorescent probe conjugated ANV-6l15 study

- Specific binding at SIEA vessel trauma site (white arrows)
  1. Binding for up to 24 hours
Conclusion & Significance

• ANV-6L15, a site-targeted, novel anticoagulant demonstrates effects in ameliorating ischemic injury within flaps

• May work through promoting perfusion, preventing thrombosis, and amelioration of ischemic-reperfusion injury related leukocyte adhesion and capillary dysfunction

• ANV-6L15: new class of anticoagulants with no effects on PT/APTT at dosages that promote flap survival