18 MAR 2016

MEMORANDUM FOR SGVT
ATTN: LT COL MICHAEL R DAVIS

FROM: 59 MDW/SGVU

SUBJECT: Professional Presentation Approval

1. Your paper, entitled *Hydrogen Sulfide Delays Onset of Acute Rejection in a Porcine VCA Model* presented at 2016 Society of Military Surgeons, Boston, MA, 17-19 March 2016 with MDWI 41-108, and has been assigned local file #16136.

2. Pertinent biographic information (name of author(s), title, etc.) has been entered into our computer file. Please advise us (by phone or mail) that your presentation was given. At that time, we will need the date (month, day and year) along with the location of your presentation. It is important to update this information so that we can provide quality support for you, your department, and the Medical Center commander. This information is used to document the scholarly activities of our professional staff and students, which is an essential component of Wilford Hall Ambulatory Surgical Center (WHASC) internship and residency programs.

3. Please know that if you are a Graduate Health Sciences Education student and your department has told you they cannot fund your publication, the 59th Clinical Research Division may pay for your basic journal publishing charges (to include costs for tables and black and white photos). We cannot pay for reprints. If you are 59 MDW staff member, we can forward your request for funds to the designated wing POC.

4. Congratulations, and thank you for your efforts and time. Your contributions are vital to the medical mission. We look forward to assisting you in your future publication/presentation efforts.

[Signature]

LINDA STEEL-GOODWIN, Col, USAF, BSC
Director, Clinical Investigations & Research Support

*Partners in a high-performance health system, dedicated to excellence in global care*
Vascularized Composite Allotransplantation (VCA) in Swine (Sus scrofa) for Optimization of Reconstruction of Battlefield Injuries Using the Hydrogen Sulfide Delays Onset of Acute Rejection in a Porcine VCA Model
| Slide presentation includes the disclaimer required by the Joint Ethics Regulation. There are no ethics issues with making this presentation at the 2016 Society for Military Surgeons meeting. |
Hydrogen Sulfide Delays Onset of Acute Rejection in a Porcine VCA Model

CA Fries, SD Lawson, LC Wang, CY Villamaria
Vijay Gorantla, MR Davis

Kevin Wu, MD
Research Fellow, US Army Institute of Surgical Research

RESTOR™ Program, 59th Medical Wing, JBSA Lackland AFB

SAGES 2016 Military Surgical Symposium
Disclaimer

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of Defense.

The experiments reported herein were conducted according to the principles set forth in the National Institute of Health Publication No. 80-23, Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966, as amended
Introduction

Surviving soldiers from battelfield injuries are presenting with more complex wound patterns in the extremities and face.

The demand for an advanced method of reconstruction, such as Vascularized composite allotransplantation (VCA), is increasing.
Introduction
New rung on the reconstructive ladder

Vascularized composite allograft

Free tissue transfer
e.g., latissimus dorsi flap

Regional flaps
e.g., posterior interosseous

Local flap
e.g., rotational/transposition

Skin graft

Secondary closure

Primary closure
Current limitations to vascularized composite allotransplantation (VCA)

- Requires systemic immunosuppression
- Skin is a primary target for rejection
- Limited donor pool
- Few indications
- Shorter period for ischemia time
Ischemia-reperfusion injury (IRI)

- Exacerbation of cellular/tissue injury after an ischemic insult with re-establishment of blood flow
  - Generation of oxidative stress
  - Microvascular obstruction/thrombosis
  - Neutrophil activation
  - Complement activation
  - Release of anaphylotoxins

- Increasing severity of IRI at time of transplant in solid organs associated with increased rates and severity of acute rejection

Effects of Ischemia and Reperfusion Injury on Long-Term Graft Function

L.R. Requião-Moura, M. de Souza Durão, E.J. Tonato, A.C. Carvalho Matos, K.S. Ozaki, N.O.S. Câmara, and A. Pacheco-Silva
Hydrogen Sulfide

H$_2$S Induces a Suspended Animation-Like State in Mice

Eric Black Stone, Mike Morrison, Mark B. Roth

Science 22 Apr 2005:
Vol. 308, Issue 5721, pp. 518
DOI: 10.1126/science.1108581

- Reversibly reduce the metabolic rate of mice exposed to 80 ppm of hydrogen sulfide
- Core temperature reduced as much as 11 degrees
- Metabolic rate as judged by carbon dioxide production and oxygen consumption dropped 10-fold
- Animals in this state for 6 hours and they recover completely
Role of \( \text{H}_2\text{S} \) in Large Animals

- Porcine organ transplant model:

  Original Article
  Hydrogen sulphide ameliorates ischaemia–reperfusion injury in an experimental model of non-heart-beating donor kidney transplantation
  S. A. Hosgood and M. L. Nicholson

- In porcine composite flap model:

  Transplantation Surgery and Research
  Hydrogen Sulfide Mitigates Reperfusion Injury in a Porcine Model of Vascularized Composite Autotransplantation
  Carole Y. Villamaria, MD, C. Anton Fries, MA, MRCS, Jerry R. Spencer, BS, Mark Roth, MD, and Michael R. Davis, MD, FACS
Allotransplantation
Gracilis myocutaneous VCA model
Gracilis myocutaneous VCA model

Artery

Vein
Gracilis myocutaneous VCA model

- Nerve
- IJ Vein
- External Carotid Artery
Methods

• Single swine leukocyte antigen (SLA) mismatched recipients
• Two groups of 8 animals
  • Group 1 flaps received no additional treatment before transplantation
  • Group 2 flaps received ex-vivo intra-arterial infusion with H$_2$S solution before transplantation

• Post-operative period
  • 4-mm punch biopsy every 1-3 days for 14 days
  • Blinded histologic examination using Banff working classification
Results

Grade 4

Grade 0
### Results

<table>
<thead>
<tr>
<th>Grade 1</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>(time in days to acute rejection)</td>
<td>(time in days to acute rejection)</td>
</tr>
<tr>
<td>Mean: 6.4</td>
<td>Mean: 10.5</td>
</tr>
<tr>
<td>SD: 0.52</td>
<td>SD: 2.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Controls</th>
<th>H$_2$S treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean: 8.9</td>
<td>Mean: &gt;14 days</td>
</tr>
<tr>
<td>SD: 2.3</td>
<td>p-value = 0.0095</td>
</tr>
</tbody>
</table>
Results

14 day Rejection by Treatment and Grade

Days to Rejection

Rejection Free Percent

- Control-G1 (p=0.0003)
- Control-G4
- H₂S-G1 (p=0.0001)
- H₂S-G4
Conclusions

- Swine gracilis myocutaneous flap is a reliable and consistent animal model for studying VCA rejection

- $\text{H}_2\text{S}$ may play a role in mitigating onset of acute rejection in porcine VCA model in the absence of immunosuppression

- Potential use for graft preservation strategies in a clinical setting that may require prolonged ischemic periods
Future research directions

- Repeat applications of $\text{H}_2\text{S}$ and $\text{H}_2\text{Se}$ in limb preservation for Non-human primates (NHP)

- Combination of local immunomodulatory methods, like drug-eluting microparticles or drug-releasing hydrogels

- Application to swine forelimb allotransplantation model
Thank You
References


