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TITLE: Investigating Novel Approaches to Block Inflammation and Prevent Ischemia Reperfusion Injury During VCA Transplantation

PRINCIPAL INVESTIGATOR: Christene A. Huang, PhD

CONTRACTING ORGANIZATION: REGENTS OF THE UNIVERSITY OF COLORADO, Aurora, Colorado

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14. ABSTRACT

Ischemia reperfusion injury (IRI) contributes to inflammation, acute rejection, and negatively impacts vascularized composite allograft (VCA) function and survival. Strategies to prevent IRI could decrease rejection episodes, improve VCA outcomes and facilitate immune tolerance induction strategies. Galectin-3 (Gal3), a beta-galactoside binding lectin, contributes to acute inflammation in response to tissue hypoxia, an unavoidable consequence of organ harvest and VCA transplantation. Blocking extracellular Gal3 using known Gal3 inhibitors including modified citrus pectin (MCP) has been shown in animal models to reduce inflammation and fibrosis. This proposal will elucidate the role of Gal3 in VCA IRI. We hypothesize that Gal3 significantly contributes to VCA IRI and that blocking extracellular Gal3 function using MCP can serve as a novel therapeutic approach to prevent or reduce IRI. We also hypothesize that circulating Gal3 levels can serve as a predictive biomarker of IRI and VCA function post transplantation.

15. SUBJECT TERMS
Vascular Composite Allograft (VCA) Transplantation; Ischemia Reperfusion Injury (IRI)

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1. Introduction

Over half of all combat related casualties in the US military since 2006 were sustained in IED related incidences. Up to 80% of those involved sustained wounds to the hands and or face. Victims of such attacks are often left permanently disfigured and in some cases with extremities or portions of their face missing.The reconstructive procedures used for major tissue loss are inadequate to reconstruct a lost hand or to treat significant injuries to the extremities or face. One solution for complex reconstruction is the use of hand and face vascularized composite allografts (VCA). VCA are potential surgical solutions for patients with traumatic or disfiguring injuries but are highly immunogenic and require elevated levels of immunosuppression. Although clinical VCA transplantation has resulted in successful outcomes, the high rates of acute rejection and increased requirements for immunosuppression have led to significant long-term complications. Because of their significant muscle components, VCAs are vulnerable to ischemia reperfusion injury (IRI). IRI is known to contribute to inflammation, acute rejection and poor VCA outcomes. Approaches that reduce inflammation associated with ischemic injury during VCA transplantation may permit reduction in immunosuppression with improved function. This study investigates a novel approach to reduce ischemia reperfusion injury (IRI) and the concomitant acute inflammatory responses associated with VCA transplantation. VCA are comprised of different tissue types and have a significant skeletal muscle component which makes them particularly susceptible to ischemic injury. IRI negatively impacts Vascularized Composite Allograft (VCA) function and survival. Strategies to prevent IRI could decrease rejection episodes, improve VCA outcomes and facilitate immune tolerance induction strategies. It is known that inflammation plays an important role in the pathogenesis of IRI. Reducing IRI may improve graft function and reduce the risk of chronic rejection. In addition, numerous approaches have been hypothesized to improve the graft survival by IRI reducing the inflammation.

Galectin-3 is a β-galactoside binding lectin found in the nucleus, cytoplasm, mitochondrion, cell surface, and extracellular space. Pleiotropic biological functions of Gal3 have been reported depending on its subcellular or extracellular location. Numerous reports have demonstrated a strong association with high circulating levels of extracellular Gal3 and a transition to chronic inflammation and fibrosis in a wide range of inflammatory diseases including arthritis, cardiovascular disease, cancer and autoimmune diseases. Blocking Gal3 using known Gal3 inhibitors including modified citrus pectin (MCP) has been shown in animal models to reduce inflammation and fibrosis. The mechanism of action of MCP is through the binding of pectin-

derived galactose to Gal3 in the extracellular space. In this proposal we will elucidate the role of Gal3 in VCA IRI. We hypothesize that Gal3 significantly contributes to VCA IRI and that blocking Gal3 function using MCP can serve as a novel therapeutic approach to prevent or reduce IRI. We also hypothesize that circulating Gal3 levels can be used as a predictive biomarker of IRI and VCA function post transplantation.

Numerous reports have demonstrated a strong association with high circulating levels of extracellular galectin-3 (Gal3) and a transition to chronic inflammation and fibrosis in a wide range of inflammatory diseases. Blocking Gal3 using known Gal3 inhibitors including modified citrus pectin (MCP) has been shown in animal models to reduce inflammation and fibrosis. In this study we will elucidate the role of Gal3 in VCA IRI using two approaches. Gal3 blockade: Transplant recipients will be treated with MCP in the drinking water to block the carbohydrate binding domain of Gal3. Gal3 genetic depletion: A novel Gal3 knockout (KO) rat was established through a contract with GenOway, France to accomplish this goal. Gal3 KO rats will be used as VCA donors and/or recipients. We hypothesize that Gal3 significantly contributes to VCA IRI and that blocking Gal3 function can serve as a novel therapeutic approach to prevent or reduce IRI. We also hypothesize that circulating Gal3 levels can be used as a predictive biomarker of IRI and VCA function post transplantation.

2. Keywords

Ischemia reperfusion injury; Vascular Composite Allograft; Hind limb transplant; Inflammation; Galectin-3

3. Accomplishments

o What were the major goals of the project?

Specific Aim 1: Determine the role of donor and recipient galectin-3 in VCA ischemia reperfusion injury (IRI)

Major Task 1.1: Establish the optimal timing and temperature of hind limb ischemia to assess IRI

Major Task 1.2: Elucidate the role of donor and recipient galectin-3 in IRI and VCA function in an established rat hind limb VCA model with prolonged cold ischemia

Specific Aim 2: Determine whether blocking circulating galectin-3 function reduces graft failure following prolonged ischemia in the rat hind limb VCA model.

Major Task 2.1: Block galectin-3 function in recipients using modified citrus pectin

Specific Aim 3: Assess circulating levels of galectin-3 in response to IRI as a predictive biomarker of VCA graft outcome.

Major Task 3.1: Determine whether high levels of circulating galectin-3 correlate with graft failure following prolonged ischemia in the rat hind limb VCA model.

o What was accomplished under these goals?

Specific Aim 1: Determine the role of donor and recipient galectin-3 in VCA ischemia reperfusion injury (IRI)

Methods: The ischemia reperfusion injury model establishment, surgical process and post operation care was described in 2020 technical report. In this report period, we compared with 6 hours and 24 hours cold ischemia reperfusion injury, and we optimized the cold ischemia time to 24 hours.

Results: In this report period, we compared with 6 hours and 24 hours cold ischemia reperfusion injury, and we optimized the cold ischemia time to 24 hours.

Specific Aim 2: Determine whether blocking circulating galectin-3 function reduces graft failure following prolonged ischemia in the rat hind limb VCA model.

Methods: The recipient animals were provided with either Gal3 blocker, modified citrus pectin (MCP) in drinking water or control untreated water since Day -7. Water intake and animal care was described in 2020 technical report. Skin and muscle were obtained at post-operative day (POD) 6 and examined histologically using H&E to quantify injury and inflammation.

Results: All transplanted limb grafts exposed to 6 or 24 hours cold ischemia showed dermal and interstitial edema, myocyte necrosis and myofiber disorganization on POD6. In non-MCP recipients, neutrophil and macrophage infiltration was also observed by POD 6 consistent with reperfusion injury. In contrast, in the MCP recipients, inflammation was greatly reduced (figure 1).

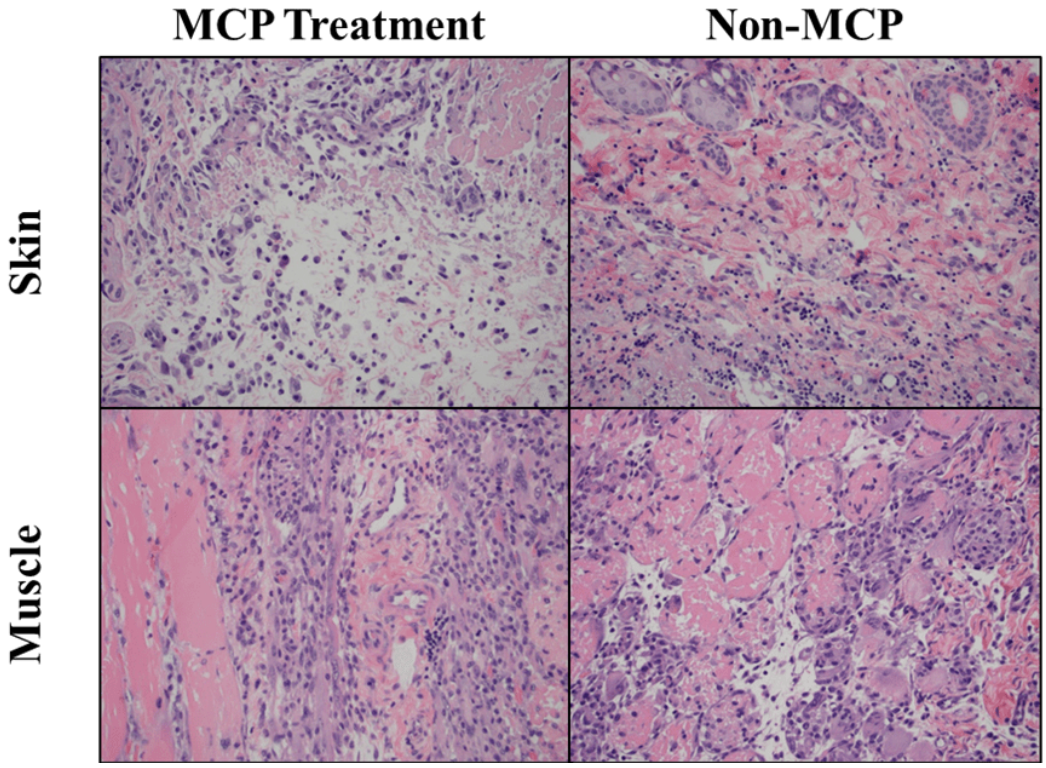


Figure 1: H&E staining for skin and muscle from the graft limb from MCP or non-MCP recipient, with 24 hours cold ischemia

Conclusion and discussion: In this model 6 hours or 24 hours cold ischemia consistently yields extensive inflammation throughout the dermis and endomysium on POD 6. Blocking Gal3 using MCP in drinking water significantly reduces inflammation. The graft injury assessment was quantitatively described below (table 2 and figure 2).

Accomplishment: Established a quantitative method to evaluate tissue injury caused by ischemia reperfusion.

Methods: Muscle injury was evaluated with five parameters: muscle inflammation, myofiber disorganization, myocyte nuclei, Interstitial edema, and vessels injury. Meanwhile, skin injury was evaluated with six parameters: epidermis injury, demal edema, dermal inflammation, vessel injury, erector pili, and appendages. Each parament will be graded by 0, 1, 2, 3 based on the severity of injury (table 1).

Table 1. Quantitative score for pathology descriptions

Muscle			Skin		
Parameter	Description assessment	Score	Parameter	Description assessment	Score
Muscle inflammation	None	0	Epidermis	Intact	0
	Focal/mild - few interstitial cells	1		Damaged (Thickening, thinning, etc.)	1
	Moderate - abundant interstitial cells	2			
	Severe - sheetlike, overriding, or destructive infiltrate	3			
Myofiber disorganization	None - sarcomeres intact	0	Demal edema	None	0
	Minimal - sarcomeres blurred or absent	1		Trace/mild/deep dermal only	1
	Mild/focal - myofibers splaying, mild	2		Moderate	2
	Moderate - myofiber splaying, moderate	3		Severe	3
	Severe/Diffuse - myofiber disarray or necrosis	4			

myocyte nuclei	Intact	0	Dermal inflammation	None	0
	Focal karyolysis/proliferative	1		Trace	1
	Moderate/multifocal	2		Mild	2
	Diffuse/Absent	3		Moderate	3
				Severe	4
Interstitial edema	None	0	Vessel injury	Intact/normal	0
	Trace/Mild	1		Abnormal	1
	Moderate	2			
	Severe	3			
Vessel injury	Intact	0	Erector pili	Intact	0
	Focal hemorrhage/extravasation or intact with hemorrhage	1		Moderate myofiber disarray	1
				Necrosis	2
	Multifocal extravasation, hemorrhage, sloughing, thrombi, or congestion	2		Obliterated	3
			Appendages	Intact	0
				Focal damage / inflammatory	1

Results: muscle inflammation score of MCP group (n=5) is significantly lower than the score of Non-MCP group (n=6) using two tailed t test (P= 0.0063). However, the MCP group has a comparable assessment score with Non-MCP group in myofiber disorganization, myocyte nuclei, Interstitial edema, and vessels injury as well as skin injury including epidermis injury, demal edema, dermal inflammation, vessel injury, erector pili, and appendages (figure 2).

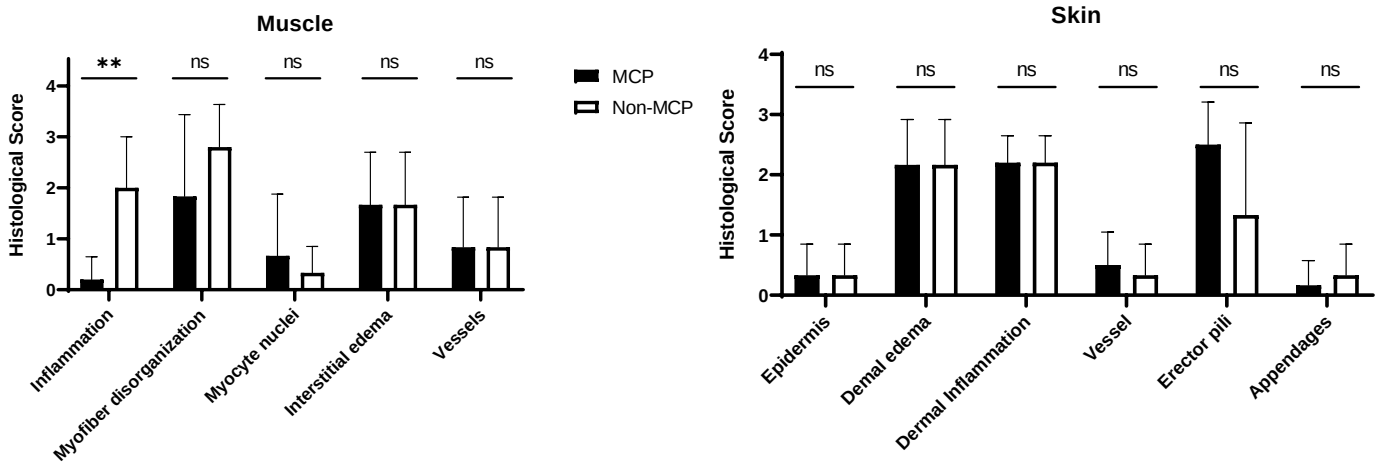


Figure 2: Pathology assessment scores for skin and muscle from the graft limb from MCP (n=5) or non-MCP group (n=5), with 24 hours cold ischemia

Methods: Serum was collected on POD 6 to measure circulating Gal3 with rat gelatin-3 ELISA kit (NBP2-76724) according to manufacturer's instruction.

Results: ELISA data shows, with 6 hours cold ischemia, Gal3 is 8.17±1.53 ng/ml from MCP recipient and 12.86±1.29 ng/ml from Non-MCP recipient, respectively. With 24 hours cold ischemia, Gal3 is 11.09±1.94 ng/ml (MCP) and 18.64±2.04 ng/ml (Non-MCP). Gal3 is significantly decreased (Figure 2) with MCP treatment.

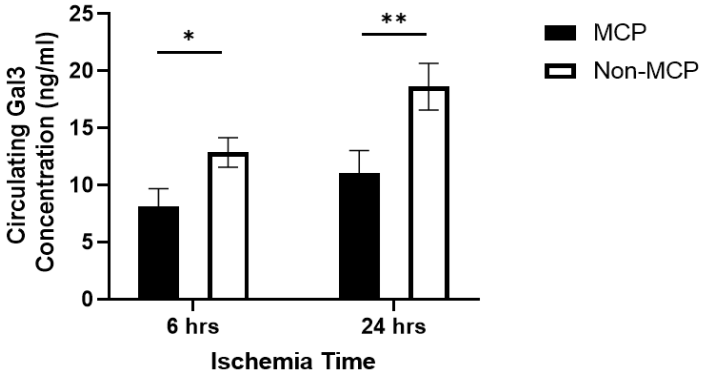


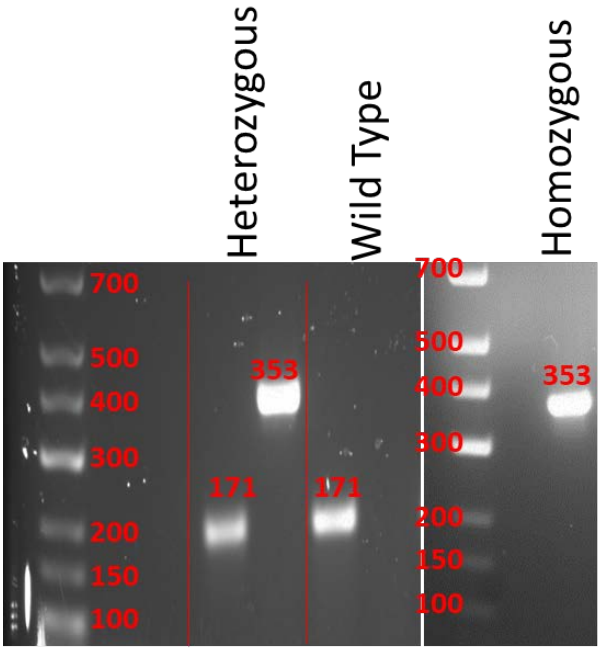
Figure 3: Circulating galectin-3 level on POD 6.

Conclusion: Blocking galectin-3 in this model by providing MCP in the drinking water significantly decreases circulating Gal3 and reduces inflammation.

Accomplishment: Developed galectin-3 knock out Brown Norway rat using CRISPS-Cas9 technique to confirm the role of Galectin 3 in response to IRI.

Methods: The Gal3 knockout (KO) rat was established by GenOway, France to accomplish this goal. CRISPR/Cas9, a nuclease-based approach was used to create a STOP codon in the 3' part of exon 6. Four pars of heterozygous rat were obtained and set as breeding pairs in CU Anschutz animal facility. Two set of primers were designed to detect both wild type (WT) and knock out alle using PCR which was conducted using genomic DNA extracted from tail biopsies or ear snip from the rat offspring. The PCR product was analyzed by 3% agarose electrophoresis, at 120V. Neb OneTaq Hot Start Quick-Load 2X Master Mix (Neb M0488S) was used as PCR polymere in PCR reaction, while GeneRuler Low Range DNA Ladder (Thermo SM1193) was used in electrophoresis as marker.

Results: The ~171 bp band on the agarose gel represents the wild type alle and the ~353 bp band represents the KO alle. The genotype can be determined (figure 4)



	WT alle	KO alle
Wild type	171 bp	//
Heterozygous	171 bp	353 bp
Homozygous	//	353 bp

Conclusion and discussion: Knock out rat was successfully bred in CU Anschutz facility with initially heterozygous + heterozygous. Genotyping protocol was established to detect wild type, heterozygous and homozygous rat. The genotype frequency complies with Mendel's law.

- **What opportunities for training and professional development has the project provided?**

This project has allowed research staff in the laboratory to learn proper animal care of Brown Norway rat, knowledge of ischemia reperfusion injury, inflammation, function of the galectin-3 and basic concept of gene editing with CRISPR-Cas9. In addition, this project provided training in microsurgical skills for medical students and research residents involved in this project. Furthermore, lab trainees have had the opportunity to present this project at research seminars and medical conferences for knowledge development.

This project has provided trainees with a better understanding of the potential for vascularized composite allografts to restore limb function in military veterans; an understanding of the current limitations to vascularized composite allograft transplantation and associated risks of immunosuppression; and an appreciation of the impact of ischemia reperfusion injury on allograft outcomes and potential for galectin-3 blockade to mitigate ischemia reperfusion injury.

- **How were the results disseminated to communities of interest?**

Oral presentation at the 2020 Mountain West Society of Plastic Surgery, Snowmass, CO, Feb 27, 2020

Abstract accepted for the 2020 Virtual American Transplant Congress, May 30, 2020.

Abstract submitted and accepted for presentation at the 2021 Military Health System Research Symposium. Although the 2020 meeting was cancelled due to the global pandemic, the abstracts was submitted to 2021 meeting.

Abstract accepted for poster presentation at 2021 Virtual American Transplant Congress, June 4, 2021

- **What do you plan to do during the next reporting period to accomplish the goals?**

Repeat more transplants to complete the proposed animal numbers for each group;

H&E and TUNEL staining for the skin and muscle tissue to evaluate injury; meanwhile to determine whether blocking galectin-3 can reduce tissue injury, necrosis or apoptosis.

Complete the transplants using galectin-3 knock out rats to determine whether lack of functional galectin-3 will reduce inflammatory response to ischemic injury post reperfusion.

4. Impact

- **What was the impact on the development of the principal discipline(s) of the project?**

The overall data from Operation Iraqi Freedom (OIF) indicate that there have been of a total 19,511 wounded soldiers. Injuries from improvised explosive devices (IED’s) and other explosive devices differ markedly from those of gunshot wounds. The contamination and soft-tissue injury caused by these explosions require aggressive debridement that result in large complex wounds needing reconstruction. The high rate of

vascular injuries and amputations reflect the substantial degree of severe soft-tissue injury suffered by our warfighters. There were 111 amputations with sixty-four of these amputations involving major limb loss proximal to the ankle or wrist. The clinical application of vascularized composite allograft (VCA) would allow for the reconstruction of the lost tissue with the exact tissue lost with no donor site issues. This could allow for the possibility to return to active duty after what previously were unreconstructible wounds, such as loss of an extremity or a devastating wound to the face.

The use of a VCA to reconstruct soldiers that have sustained severe injuries to their extremity and face would allow for the lost tissue to be replaced by the exact tissue lost. This transplant would enhance the tissue environment for healing by providing the missing bone, soft tissue, muscle and nerves. After the loss of a hand or a complex facial injury with extensive loss of bone, muscle and soft tissue, amputation is often the standard of care. In these cases a VCA could be harvested and enable limb salvage by providing the exact missing tissue and enabling the surgeon to achieve restoration of the bone, nerves and muscles. This could lead not only to a stable, healed wound, as is the goal currently, but a truly functional reconstruction, allowing injured military service members and veterans to continue to play a productive role in the armed services.

The overall scientific goal of this research is to evaluate galectin-3 as a therapeutic target to prevent the acute inflammatory responses associated with ischemia reperfusion injury (IRI). Approaches that reduce inflammation associated with ischemic injury during VCA transplantation may permit reduction in immunosuppression with improved function.

We have now optimized the rat hind limb transplantation model for assessing ischemia reperfusion injury with pharmacological galectin-3 blockade and have successfully engineered a novel galectin-3 knockout rat model in the Brown Norway background to determine the role of galectin-3 in VCA IRI.

- **What was the impact on other disciplines?**

The development of a novel rat galectin-3 knock-out line to accomplish the goals of this study will have a significant impact on research related to galectin-3 biology and how galectin-3 impacts transplant outcomes. Rats are preferred over mouse models for studies involving organ and tissue transplantation due to the vessel size.

- **What was the impact on technology transfer?**

Nothing to Report

5. Changes/Problems

- **Changes in approach and reasons for change**

Nothing to Report

- **Actual or anticipated problems or delays and actions or plans to resolve them**

We faced some problems in establishing the rat hind limb IRI model have now been solved:

1): Several rat recipients chewed the suture/graft after transplant during the initiation of these studies:

Solution: Elizabethan Collar was applied to prevent chewing the graft with a 1 week period of acclimation prior to surgery.

2): Ischemia condition and time optimization:

Solution: we tried different ischemia time and condition including: 1 hour room temperature (minimal ischemic time during transplant surgery), 4 hours at room temperature, 6 hours at room temperature, 6 hours at 4 degrees Celsius, and 24 hours at 4 degrees Celsius. One hour room temperature ischemia will be used as the control for the minimal ischemic time that occurs during the hind limb transplant surgery and static cold storage for 24 hours at 4 degrees Celsius was chosen as the time period that results in consistent histological injury so we can be confident whether our treatment strategies are having a beneficial effect.

3): In order to improve the success rate of the transplant, the recipients were receiving collar and water bottle training since day -7. Daily water intake is monitored to make sure the rat take sufficient hydration or MCP.

4): Post transplant care optimization: antibiotics were given to the recipient to prevent infection; Ringer's solution was given to prevent dehydration and analgesic was given to relief pain and stay comfort.

5): A delay in shipment of the newly established heterozygous Gal3KO rat founders due to Coronavirus shutdown led to an almost 4 month delay in the initiation of the breeding of homozygous rats for experimental purposes. The rats are out of quarantine and currently breeding well. Experiments for Major Task 1.2 will be completed as soon as we have bred enough homozygous Gal3 KO Brown Norway rats to complete this task.

6): COVID19 Colorado state-wide restrictions required the shutdown of on-site research at our institution starting March 16 with a gradual phased-in approach to return to on-site research starting in June. This has resulted in a significant delay in the processing of pathology samples at our pathology research core facility as they were unable to process samples for over 3 months. In addition, given the limited staff allowed to return and the strict physical distancing requirements during this phase, there have been delays with scheduling and completing the rat hind limb surgeries, as only one microsurgeon can be in the animal facility operating room at a time. We are hopeful that COVID19 cases will start to drop in Colorado and that we will be allowed to progress to the next phase of research re-entry soon.

7): limitation of rat reagent and kit: rat antibodies and ELISA kits were limited and not available to all the target markers of interest. Furthermore, some antibodies, reagents and ELISA kits which claims were not full validated: Rat Galectin-3 ELISA kits from Raybioech ELR-Galectin3-1 and Thermo Scientific ERLGALS3, and rat Rat IL-6 ELISA Kit from Thermo Scientific ERA31RB was claimed working with rat, but has neither validation report, nor peer citation in which they were tested with rat. When we purchased these kits, we confirmed that these kits were not working well with rat samples.

Solution: we tried different manufacture and ended up with Rat Galectin-3 ELISA Kit, from Novus NBP2-76724. However, we were still not able to get a reliable rat IL-6 ELISA kit.

8): Compared to the wild type breeding pairs, the heterozygous or homozygous breeding pair has low breeding efficacy and litter number. The homozygous rats grow way slower than wild type rats, which requires longer time to gain enough body weigh to perform transplant surgery. Furthermore, the homozygous offspring rat is 25% from a heterozygous + heterozygous breeding pair, which makes longer process to obtain enough number of homozygous rats. Homozygous + Homozygous or Homozygous + heterozygous breeding strategy will increase the frequency of homozygous offspring. However, the homozygous dam has lower pregnant frequency, litter number and pool pup livability.

Solution: Use university breeding core to provide better breeding management. Try different breeding pattern to optimize breeding efficiency.

- **Changes that had a significant impact on expenditures**

Nothing to Report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to Report

- **Significant changes in use or care of human subjects**

Nothing to Report

- **Significant changes in use or care of vertebrate animals.**

Nothing to Report

- **Significant changes in use of biohazards and/or select agents**

Nothing to Report

6. Products

- **Publications, conference papers, and presentations**
  - **Journal publications**

Nothing to Report

- **Books or other non-periodical, one-time publications**
  Nothing to Report
- **Other publications, conference papers, and presentations**
  Wang Z, Harrant AB, Wang Y, Washington K, Farkash E, Huang CA. Rat Hind Limb Transplant Model to Assess Vascularized Composite Allograft (VCA) Inflammation and Ischemia Reperfusion Injury [abstract]. Am J Transplant. 2020; 20 (suppl 3). https://atcmeetingabstracts.com/abstract/rat-hind-limb-transplant-model-to-assess-vascularized-composite-allograft-vca-inflammation-and-ischemia-reperfusion-injury/. Accessed July 16, 2020.

  Harrant AB*, Wang Z*, Anderson JB, Wang Y, Li B, Johnson AC, Washington K, Navarro-Alvarez N, Farkash EA, Huang CA. Rat Hind Limb Transplant Model to Assess Vascularized Composite Allograft (VCA) Ischemic Reperfusion Injury and Inflammation. Oral Presentation – Mountain West Society of Plastic Surgeons 2020 Annual Meeting, February 27-March 1, 2020; Snowmass Village, CO.
  * Wang Z and Harrant AB contributed equally to this project.

  Zhaohui Wang, Yong Wang, Bing Li, Dor Yoeli, Niyati Nakra, Jerry Yang, An-Jey Su, David M. Mathes, Kia Washington1, Evan A. Farkash*, Christene A. Huang. Targeting galectin-3 to block inflammation and prevent ischemia reperfusion injury during VCA transplantation. Accepted for 2021 Military Health System Research Symposium

  Wang Z, Wang Y, Yoeli D, Li B, Su A, Mathes D, Washington K, Farkash E, Huang CA. Galectin-3 Blockade Can Decrease Reperfusion Injury in Response to Ischemia in a Rat Hind Limb Transplant Model [abstract]. Am J Transplant. 2021; 21 (suppl 3). https://atcmeetingabstracts.com/abstract/galectin-3-blockade-can-decrease-reperfusion-injury-in-response-to-ischemia-in-a-rat-hind-limb-transplant-model/. Accessed May 28, 2021.

- **Website(s) or other Internet site(s)**

  https://atcmeetingabstracts.com/abstract/rat-hind-limb-transplant-model-to-assess-vascularized-composite-allograft-vca-inflammation-and-ischemia-reperfusion-injury/

  https://atcmeetingabstracts.com/abstract/galectin-3-blockade-can-decrease-reperfusion-injury-in-response-to-ischemia-in-a-rat-hind-limb-transplant-model/

- **Technologies or techniques**

Established a novel galectin-3 knock-out model to complete these proposed studies

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Nothing to Report

7. Participants & Other Collaborating Organizations

- **What individuals have worked on the project?**

Name:	Christene A. Huang, PhD
Project Role:	Principal Investigator
Researcher Identifier:	ORCID: 0000-0001-9824-5716
Nearest person month worked:	2
Contribute to Projects:	Dr. Huang is the PI in this project and has been responsible for the overall direction of the project and interpretation of the data.

Funding Support:	RT180168

Name:	David W. Mathes, MD
Project Role:	Collaborator
Researcher Identifier:	ORCID: 0000-0003-4388-1373
Nearest person month worked:	1
Contribute to Projects:	Dr. Mathes provides clinical advice on the IRI model
Funding Support:	RT180168/ Department of Surgery Funds

Name:	Kia Washington, MD
Project Role:	Collaborator
Researcher Identifier:	ORCID: 0000-0003-1803-5888
Nearest person month worked:	1
Contribute to Projects:	Dr. Washington provides surgical advice on the rat hind limb transplantation model
Funding Support:	RT180168/ Department of Surgery Funds

Name:	Yong Wang, MD
Project Role:	Research Staff
Researcher Identifier:	ORCID: 0000-0002-7002-6439
Nearest person month worked:	3
Contribute to Projects:	Dr. Wang is the primary surgeon performing the rat hind limb transplantation.
Funding Support:	RT180168

Name:	Zhaohui Wang, DVM
Project Role:	Research Associate
Researcher Identifier:	ORCID: 0000-0002-9035-3867
Nearest person month worked:	4

Contribute to Projects:	Dr. Wang is lead researcher responsible for this project. He is responsible for coordinating and assisting with the rat surgeries, providing post-operative animal care, harvesting tissues, designing assays, and analyzing data. He is also responsible for taking care of galectin-3 knock out rat colony breeding and genotyping
Funding Support:	RT180168 /Dr. Huang's sundry funds

Name:	Evan Farkash, MD, PhD
Project Role:	Research Staff
Researcher Identifier:	ORCID: 0000-0002-5136-079X
Nearest person month worked:	1
Contribute to Projects:	Dr. Farkash serves as pathology consultant and interprets histology
Funding Support:	University of Michigan Department of Pathology

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to Report

- **What other organizations were involved as partners?**

University of Michigan Health System

Dr. Evan Farkash Assistant Professor, Dept. of Pathology, University of Michigan Health System, Ann Arbor, MI reviews the histology for the project. We have an MTA in place to be able to ship slides from UC Denver to University of Michigan for pathological assessment.

8. Special Reporting Requirements

Quad Chart

9. Appendices

Not Applicable