

Contractor/Company Name: 81 MSGS/SGCQ

Address:

81 MSGS/SGCQ
301 Fisher St. Rm BA 144
Keesler AFB, MS 39534

Contract Number: N/A

Title of the Report: Injected Omental Lipids for treatment of Soft Tissue Injury

Date of the Report: 2 June 2011

Principal Investigator: Capt. Andrew Hall, 228-376-4901, Fax: 228-376-0128, andrew.hall.2@us.af.mil

Sponsoring Organization:

81 MDSS/SGSE
Clinical Research Lab
301 Fisher St. Rm BA 144
Keesler AFB, MS 39534

DISTRIBUTION A. Approved for public release: distribution unlimited.

Pages: 15

Words: 1575

Figures/Tables: 4

Photos: 1

References: 3

Contact: Andrew B. Hall

Email: andrew.hall.2@us.af.mil

Telephone: 228-376-4901

FAX: 228-376-0128

Guarantor: Andrew B. Hall

Injected Omental Lipids for treatment of Soft Tissue Injury

Capt. Andrew B. Hall, M.D., USAF MC*

Capt. Charles T. Woodham, M.D., USAF MC*

*81st Medical Group

Department of Surgery

301 Fisher Street

Keesler AFB, MS 39534

Abbreviated Title: Injected Omental Lipids

Supplementary Key Words: Omentum, Lipid, Frostbite, Military

ABSTRACT:

OBJECTIVES: Omental lipids have been shown to be potent angiogenic compounds. In several models, these lipids have improved healing. This investigation was to determine if a single injected dose could improve militarily relevant injuries – frostbite and soft tissue injury.

MATERIALS AND METHODS: Six pigs (*Sus scrofa domestica*) were given standardized elliptical wounds and 2 mL of purified omental lipid extract were injected intracutaneously on either side of one wound post wound closer. In two weeks scars were excised and the resulting scar tensile strengths were measured with a tensometer. Three rabbits were given cold injuries to both ears, with the right ears treated with 2 mL of omental lipid. Weekly physical exams and percent tissue loss comparisons were conducted for three weeks.

RESULTS: There was no statistical difference between control scar tensile strength and the tensile strength of wound scars treated with omental lipids ($p=0.762$). Frostbitten ears showed no positive difference from injection in physical exam findings and tissue loss for the three week exam intervals.

CONCLUSIONS: As a single injection at time of injury, omental lipids lack an identifiable effect on outcomes.

INTRODUCTION:

Omental lipids have been shown to be potent agents for neovascularization and useful in the treatment of ischemic and traumatic models.¹⁻³ The military, along with civilian trauma, frequently deals with ischemic (thermal injury) and mechanical trauma. In this study we aimed to determine if a single injection of omental lipid to the site of injury could improve outcomes in both cold injury and minor soft tissue trauma.

MATERIAL AND METHODS:

The study was conducted with approval of the 81st Medical Group Institutional Animal Care and Use Committee and conducted in conformity with the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals.

Omentum Preparation

The omental lipid fraction was prepared based on methods described in prior literature with some modification. Omentum was homogenized in a 2:1 chloroform to methanol mixture. Following homogenization, the mixture was centrifuged for 20 minutes with supernatant removed. The supernatant was then purified via use of a rotary evaporator. The purified lipid extract was again centrifuged with the white lipid layer extracted for use. In cases where usage had to be delayed, lipid extracts were stored in sealed containers at -20° C. In prior studies, phosphate-buffered saline was included in the purification process. This was eliminated because the saline and lipids are immiscible, and the water, naturally present in the tissue, served as an ample bulking agent for ease of purification processes.

For frostbite injury experiments, the rabbit's own omentum was utilized for each individual. For experiments on soft tissue injury, pig omentum acquired via tissue-sharing policies was utilized.

Soft Tissue Injury

Six pigs received two 3 cm x 1 cm elliptical excisions of skin made at approximately the exact same distance from the head and from the spinal column on the dorsal surface of the back using a premade template. Prior to excision, pigs were anesthetized using Ketamine (20 mg/kg IM) plus Xylazine (2 mg/kg IM) or another appropriate anesthetic regimen approved by the attending veterinarian. Post surgery, Buprenorphine every 6 – 12 hours under the attending veterinarian's supervision was given for pain relief. Excisions consisted of skin and subcutaneous tissue down to muscle fascia. The wounds were closed using 2-0 prolene suture in identical fashion on both sides. After closure, one wound was injected with 2 mL of omental lipid evenly on both sides of the wound closure. On day 13 or 14, post euthanasia (due to utilization in training protocols), the area of each wound was excised down to the muscle fascia. A 2 cm by 4 cm section of tissue through the center of the scar was placed in a tensometer to determine tensile strength. Results were compared using paired T-test.

Cold Injury

Following twelve hours of fasting, three rabbits were anesthetized with an intramuscular dose of Ketamine (30 mg/kg) and Xylazine (5 mg/kg) followed in 5 minutes with a single 1-second application (200 mg) of cetacaine to permit placement of the laryngeal mask airway. After placement, a surgical plane of anesthesia was maintained using an appropriate dose of isoflurane inhalant anesthetic (1% to 5%) in 100% oxygen, which was delivered through an anesthesia machine. Following sterile preparation, a midline laparotomy incision was made. The greater omentum was taken in entirety and homogenized.

The distal 3 cm tips of each ear were bathed in a mixture of ethylene glycol, isopropyl and ethyl alcohol at -12 to -19° Celsius for 30 minutes. Post cold insult, the ears were rewarmed to body temperature in 39° C water.

Following re-warming, 2 mL of lipid extract were injected in a uniform manner in the right ear of the rabbit. Analgesics (Buprenorphine every 6 – 12 hours or carprofen 2.2 mg/kg PO every 12 hours) were administered as dictated by animal distress. Over 21 days, ears were compared on a weekly basis with physical findings such as hair loss, erythema, and necrosis measured and photographed. Final observations were conducted on post-op day #21.

Rabbit ears post operatively were observed weekly for tissue loss, hair loss, and sensation. Hair loss was judged subjectively based on a spectrum between light hair loss being thinning to heavy being near complete absence; tissue loss was based on amount of necrotic tissue or absence of tissue; and sensation measured as present or absent based on needle prick.

RESULTS:

Soft Tissue Injury

A total of 6 pigs were utilized with 1 pig disqualified due to self-destruction of the wound during animal activity. The sections of scar were tested on a tensometer with total mass load of untreated wounds averaging 10.25 kg versus 10.50 kg ($p=0.762$) for omental lipid-treated wounds (Table 1). The average difference between untreated versus treated wounds was 2.75 kg. There was no trend of consistent outperformance of omental lipid-treated wound repairs.

Cold Injury

Three rabbits underwent the procedure with one rabbit perishing on post-operative day #3 secondary to gastric perforation. The result of the experimentation resulted in skin changes and loss of hair equivalently on both surviving rabbits (Figure 1). This occurred equally in both the treated and untreated ear. Sensation was tested with a sterile needle pricking at various parts of the tested areas

detecting any movement of the ear in response. Sensation remained absent but did reappear in the 1st rabbit's treated ear. (Table 2)

In the demised rabbit, the treated and untreated ears were examined histiologically. The treated ear was notable for having many areas of hollow space which may indicate a substantial amount of free lipid remained at post-operative day #3 or represents separation of tissue secondary to injection (figure 2).

DISCUSSION:

Omental lipids have shown results in prior studies that prove angiogenetic properties. Those results make it a topic of research in an effort to find a simple and effective means to improve outcomes in trauma and frostbite. This study was aimed to look at the feasibility of injecting omental lipids with their angiogenic properties into both thermal and excisional wounds to assist in wound healing.

In the soft tissue injury section of our study, we looked to test at 30 days the tensile strength of injected wounds. Our hope was that the angiogenic properties of the lipids infused would provide evidence of improved wound healing. Although our study did not support this hypothesis, there are possible explanations for our results. By injecting our solution into the wound, we introduced a space-occupying solution and thus increased the size of tissue disturbance within the wound. This in turn created a larger area that had to be healed by each test subject. In addition to the space-occupying effects, the injected material was of a higher viscosity and had a different osmotic characteristic than normal tissue and substrates which could have impeded or counteracted the intended effects. Finally, there was an amount of every injection that oozed directly out of the site of needle puncture.

In our frostbite injury study we looked to determine if the omental injections would improve healing or tissue salvage in extreme cold injury. Again, this study did not actually show improved wound healing, however, it did give possible histologic evidence that our administered omental lipids were actually

retained at the point of injection (Figure 2). The possible presence of some lipids, but lack of response, may be due to the effect of ischemia and cold temperatures. Some blood flow to the site may be required to initiate vascular proliferation, and in a frostbite injury there isn't enough remaining circulation to promote angiogenesis. The same weaknesses of the soft tissue injury study are again present in this portion of our study.

A prolonged exposure to the omental lipids and a substrate to contain it at the point where it is needed may be necessary. In the study performed by Nottebaert et al, the authors showed that a continuous infusion both increased the regional blood perfusion and increased bone density, fusion rate and strength.³ In a study by Takada et al, ischemic skin flap survival was improved using a topical omental lipid layer suspended in fibrin glue.² The omental lipid group had an increased flap survival area and showed vasodilatation of the wound with increased vascularization between the flap and recipient bed.

While a single site injection may not be feasible, there may be benefit in future trials. One theory is that a topical administration similar to the study by Takeda et al may be used with a coated dressing or bandage with a long release and could help localize the effects to a wound.² Another improvement would be to attempt to find a different medium that might be more homogenous with the native wound milieu of fluids. The fine line that has to be walked in studies of this nature is obtaining the desired effect of increased wound healing with the angiogenic properties of the omental lipids without causing more damage by the administration that has to be healed by the lipids.

Battlefield trauma response and treatment is of utmost importance in military medicine, but research in this field offers benefits to civilian medicine as well. Omental lipids may be a cheap and effective tool, but more research is required to produce a medically beneficial product.

CONCLUSIONS:

At the moment of trauma, a simple, cheap, beneficial medical aid to improve outcomes would be indispensable. Although omental lipids are tantalizing for this purpose, they do not appear effective as a one-time site injection. A means of prolonged delivery or formulations with better tissue diffusion may allow their use in soft tissue and thermal injuries in the future.

Acknowledgements and Notice of Grant Support:

The work reported herein was performed under USAF Surgeon General-approved research investigation FKE20100031A and FKE20080003A along with a \$21,000 grant from the USAF Surgeon General's Office.

The opinions and/or assertions contained herein are solely those of the authors and should not be construed as reflecting those of the US Air Force, Department of Defense, or government. All authors are without conflict of interest.

REFERENCES:

1. Goldsmith H, Griffith A, Kupferman A, Catsimpoalas N. Lipid Angiogenic Factor From Omentum. JAMA 1984;252:2034-2036
2. Takada, T., Y. Kamei, T. Iwata, et al. Effect of Omental Lipid Fraction on Enhancement of Skin Flap Survival. Ann Plast Surg 1998;41:70-74
3. Nottebaert M, Lane JM, Juhn A, Burstein A, Schneider R, Klein C, Sinn RS, Downling C, Cornell C, Catsimpoalas N. Omental Angiogenic Lipid Fraction and Bone Repair. An Experimental Study in the Rat. Journal of Orthopaedic Research 1989;7:157-169

Table 1: Comparison of tensile strength of scar tissue between treated and untreated wounds.

Pig #	Maximum Load (Kg)	
	Untreated	Treated
1	9.98	10.08
2	13.11	9.97
3	10.64	13.68
4	10.81	7.71
6	6.72	11.07

Table 2: Comparative table of observations of both rabbits’ ears. Omental lipid was injected in the treated arm.

	Week #	Hair Loss			Sensation			Tissue Loss		
		1	2	3	1	2	3	1	2	3
Rabbit 1	Treated	Light	Moderate	Moderate	Insensate	Insensate	Insensate	0%	0%	0%
	Control	Light	Moderate	Moderate	Insensate	Insensate	Insensate	0%	0%	0%
Rabbit 2	Treated	Heavy	Heavy	Heavy	Insensate	Insensate	Insensate	0%	0%	>90%
	Control	Heavy	Heavy	Heavy	Insensate	Insensate	Insensate	0%	0%	>75%

Figure 1: Weekly photos of rabbit #2's ears over course of experiment. Omental lipids were injected into the treated group. The week 3 necrotic tip in the treated group fell off the day after official evaluation ceased.

Figure 2: Representative H&E stained slide of treated rabbit ear.



