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14. ABSTRACT The diagnosis of acute compartment syndrome (CS) remains problematic due to difficulty in diagnosis. Current treatment for acute extremity symptomatic CS is fasciotomy. However, surgical treatment has associated morbidity and may delay the recovery of the patients. Continuous measurement of intramuscular tissue oxygenation (PmO2) of the leg has been shown to be feasible in humans and highly responsive to induced compartment syndrome and fasciotomy in a dog model. Using the same model, we investigated the relationship between PmO2 and tissue viability. We further tested the feasibility of non-surgical treatment of compartment syndrome using phenylephrine and dobutamine in the dog model. Under general anesthesia, CS was induced in the anterolateral compartment of one hindlimb via Hespan infusion. Polarographic oxygen probes were placed percutaneously into the anterolateral compartment. Compartment pressure, diastolic blood pressure and tissue oxygenation (PmO2) was recorded every 30 seconds. In the treated group, pharmacological treatments begin at 1 hour after the compartment syndrome is induced. Infusion of intravenous phenylephrine was initiated at 25mcg/min and titrated up to 100mcg/min as needed to increase the diastolic blood pressure 30mmHg above the baseline. Intravenous dobutamine at 60mcg/min was initiated 2 hours later. Six to seven hours after treatment, fasciotomy was performed on one leg. Animals were euthanized 2 weeks postoperatively at which point muscle biopsies were performed. Tissue viability was assessed MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Pharmacological treatment significantly increased PmO2 in the anterior compartment muscle. Two weeks after surgery, there was no significant difference between pharmacological treated and pharmacological plus fasciotomy treated groups. However, either treated group has a significantly higher tissue viability compared to the non-treated group (P<0.01). This result suggests that keeping the blood pressure at a high level using pharmacological agents may serve as an alternative to surgical treatment for acute compartment syndrome.					
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INTRODUCTION

Acute compartment syndrome (CS) describes the elevation of pressure in the muscle compartment of the extremity within the unyielding fascia, leading to a pressure-induced decrease in circulation, lack of oxygen, and ultimately muscle and nerve death. Delays in diagnosis or treatment have potentially catastrophic consequences, including amputation and death. CS remains a challenge in orthopaedic trauma due to difficulty of diagnosis. The current standard diagnosis of CS is primarily based on a combination of a high index of suspicion and interpretation of clinical symptoms and a needle measurement of the pressure within the affected compartment. However, clinical diagnosis lacks definite, objective criteria, which becomes problematic in obtunded or polytrauma patients. Controversy also exists regarding the use of absolute compartment pressure vs. the difference between diastolic blood pressure and compartment pressure (ΔP) as an objective diagnostic test, due to poor specificity, potentially leading to an unacceptably high rate of fasciotomy.^{1,2}

Thus, the development of a minimally invasive, physiologic, and reliable method of diagnosing CS would represent a substantial advance in orthopaedic trauma care. Because the pathophysiology of CS is pressure-induced ischemia of the muscle tissue, monitoring muscle tissue oxygenation as a novel approach for diagnosing CS is logical. While pressure measurement reflects the mechanism, tissue oxygenation measurement directly indicates the actual pathophysiology of muscle ischemia and necrosis. Recently, tissue oxygen tension measured with microprobes has been shown to be highly correlated with tissue oxygenation and the extent of ischemia reperfusion injury.³ Near-infrared spectroscopy (NIRS), which uses light absorption through the skin, has been proposed as a potential mechanism to continuously measure changes in muscle oxygen saturation after trauma as diagnosis of lower extremity CS.^{4,5} However, definite, objective tissue oxygenation thresholds for CS still have not been determined.

In this study, we propose using a minimally invasive polarographic electrode probe that measures the current generated when in contact with oxygen in order to monitor tissue oxygenation. The goals of this study is the continuous measurement of intramuscular tissue oxygenation (PmO₂) of the leg during controlled induction of CS in a canine model and the development of warning criteria for irreversible muscle death due to pressure-induced ischemia based on PmO₂. In Phase 1, CS of known severity will be induced based on previous authors' intracompartmental pressure criteria (ΔP) to establish the threshold duration and values of PmO₂ of irreversible ischemia. In Phase 2, CS of varying severity will be induced based on PmO₂ and correlated with the degree of necrosis to validate the use of measuring PmO₂ as a diagnostic marker for irreversible muscle damage. In Phase 3, PmO₂ measurements will be used to investigate nonsurgical treatment consisting of oxygen and inotropic and vasoactive drugs to enhance tissue perfusion.

BODY

Previously reported findings:

In the previous report, we reported that in Phase 1, polarographic oxygen probe monitoring was responsive and sensitive to changes in muscle tissue oxygenation, and PmO₂ appears to correlate reasonably with tissue viability.

The PmO₂ values following fasciotomy appear to reflect the underlying muscle viability as confirmed by histologic methods with use of a previously suggested threshold PmO₂ of 10 mmHg. Measurement of intramuscular tissue oxygenation detects pressure-induced ischemia and may also predict irreversible necrosis in an animal model with high translational potential. It may represent a minimally invasive, physiologic, and continuous method for diagnosing compartment syndrome. This work has been presented on the 59th Annual Orthopaedic Research Society Meeting during Jan 26-29, 2013 in San Antonio, TX. (Kang H, Mok J, Hansen E, Kandemir U, Rollins M, Liu X, Kim H. Relationship between Intramuscular Tissue Oxygenation and Viability in a Compartment Syndrome Model. Poster No: 1180). In a pilot

study, found that a combination of phenylephrine and dobutamine can significantly improve muscle tissue oxygenation and viability during and after compartment syndrome.

Work accomplished in current period:

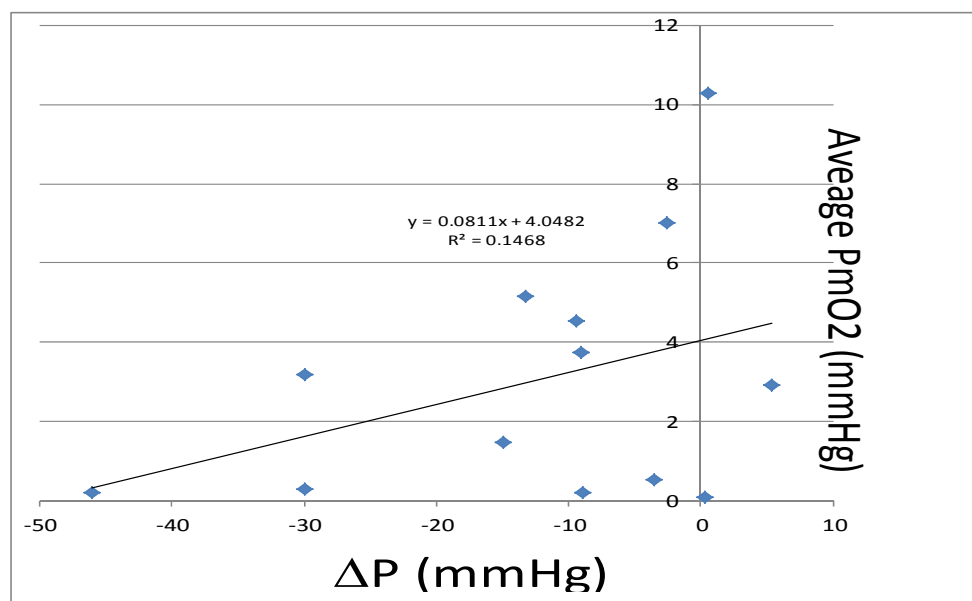
Phase I: We have accomplished all animal surgeries in this phase.

- 1) Ischemia time inversely correlates to muscle viability. In our tourniquet ischemia group, we found that 2 hours ischemia has minimal effect on muscle viability. However, 4 hours or longer time of ischemia significantly reduced muscle viability.

Tourniquet Time (hour)	PmO2 before tourniquet	PmO2 during ischemia	PmO2 after tourniquet removal	Tissue viability of TA/quadriceps
2h	33.9875	1.38	32.8675	92.52%
4h	44.07	0.31125	63.9675	86.44%
6h	37.775	0.615	62.825	54.59%
8h	29.48	0.5785	11.9875	68.61%

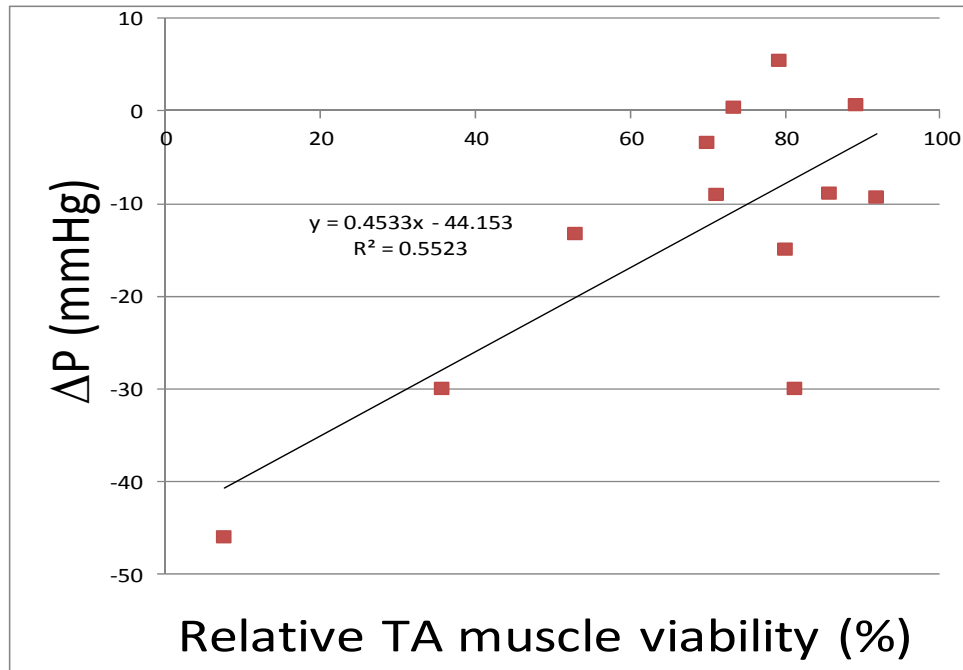
- 2) Correlation between ΔP and PmO2. In our CS group, we tested the relationship between ΔP and PmO2. We found there is a trend that ΔP and average PmO2 are correlated. Overall, the lower ΔP (higher compartment pressure compared to diastolic blood pressure), the lower PmO2 in the muscle (Fig.1)

Figure 1. Correlation between ΔP and PmO2 in Phase I.



- 3) Correlation between ΔP and muscle viability. In our CS branch, we further tested the relationship between ΔP and muscle viability. We used MTT assay to evaluate the viability of Tibialis Anterior (TA) muscle (a muscle in the anterior compartment, which is effected by CS) and Quadriceps muscle (a muscle is not effected directly by CS, thus serves as an internal control). We used the relative TA muscle viability (viability of TA muscle /viability of Quadriceps muscle) as a parameter to evaluate the muscle viability reduction in CS. We found that ΔP and relative TA muscle viability are positively correlated ($R^2=0.55$). The lower ΔP (higher compartment pressure compared to diastolic blood pressure), the lower relative TA muscle viability (more severe muscle damage) in our model (Fig 2).

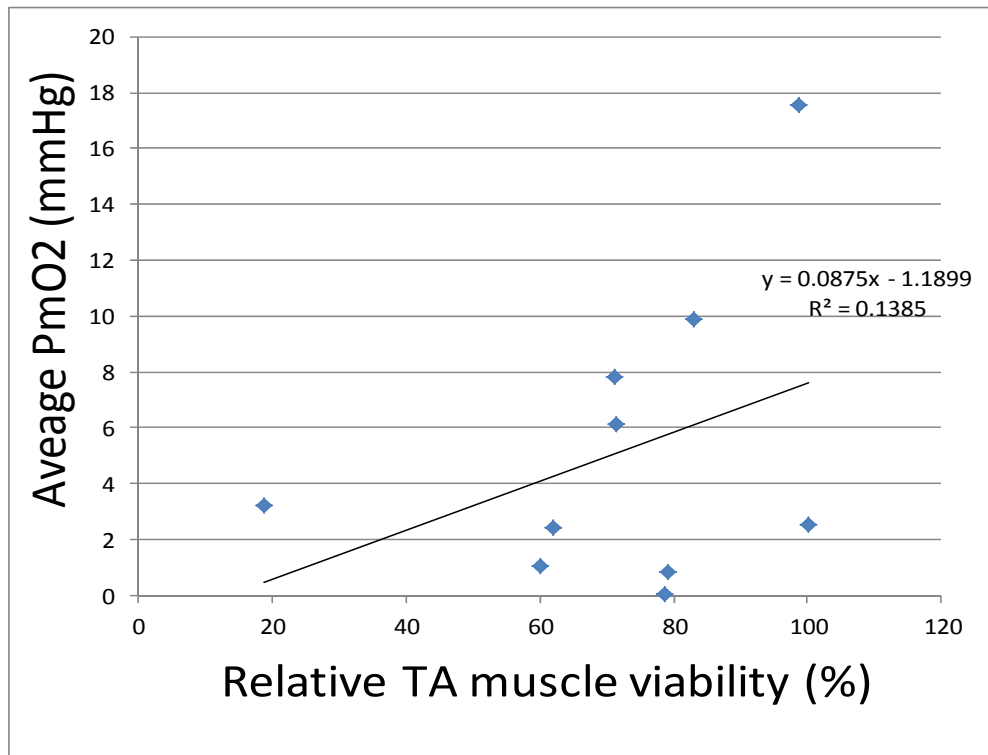
Figure 2. Correlation between ΔP and relative TA muscle viability in Phase I.



Phase II: We have accomplished all animal surgeries in this phase.

Correlation between PmO₂ and muscle viability. We analyzed the correlation between the PmO₂ and relative TA muscle viability in 10 of the 16 legs in this phase. We found there is a trend that average PmO₂ level is correlated to the relative TA muscle viability. Overall, the higher PmO₂, the higher relative TA muscle viability (the less muscle damage).

Figure 3. Correlation between PmO₂ and relative TA muscle viability in Phase II.



Phase III: We have complicated all experiments in this phase.

In this phase, we tested the feasibility of non-surgical treatment for CS using the combination of phenylephrine and dobutamine. After 1 hour of induction of severe CS ($\Delta P = -30\text{mmHg}$), infusion of intravenous phenylephrine was initiated at 25mcg/min and titrated up to 100mcg/min as needed to increase the diastolic blood pressure 30mmHg above the baseline ($\Delta P = 0\text{mmHg}$). Intravenous dobutamine at 60mcg/min was initiated 2 hours later. Six to seven hours after treatment, fasciotomy was performed on one leg of the animals and the skin was closed 1 hour later.

- 1) Non-surgical treatment significantly improved the muscle tissue oxygen in compartment syndrome.

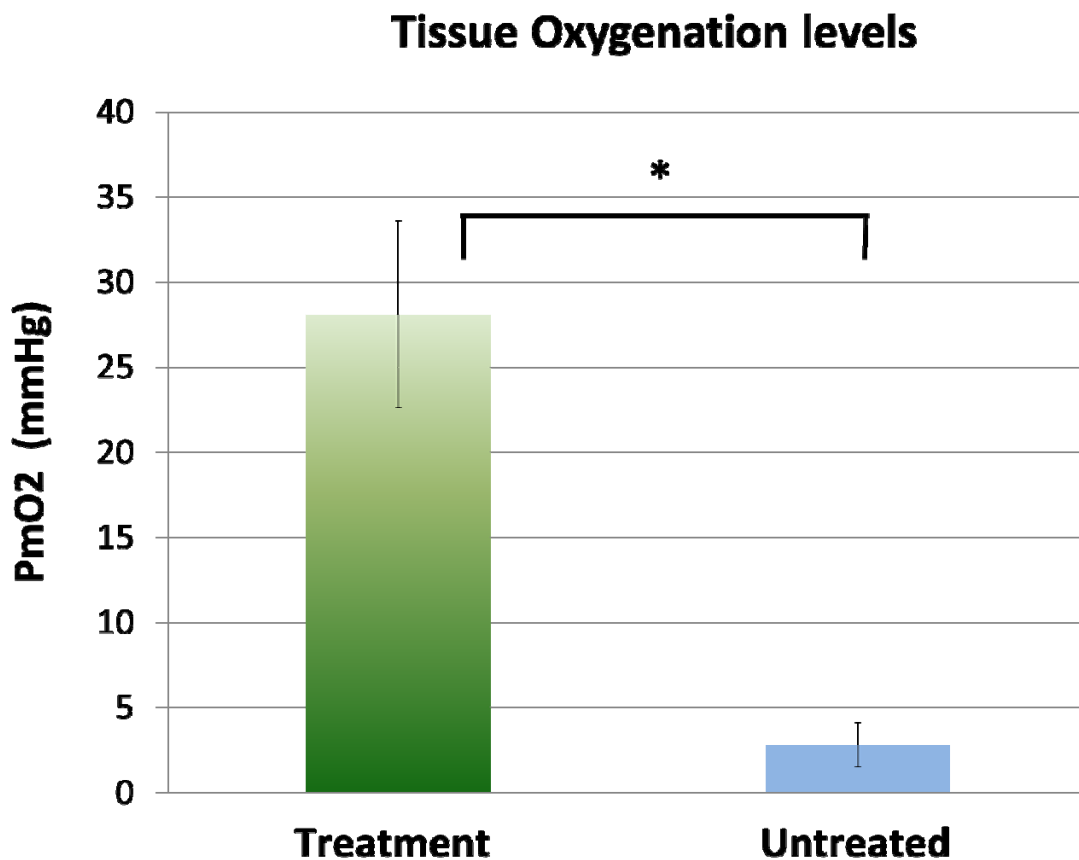


Figure 4: Tissue oxygenation levels were significantly higher in treated animals ($n=12$) compared to untreated controls ($n=6$). Treated animals had an average $\text{PmO}_2 = 28.1 \pm 5.6 \text{ mmHg}$ over the 7 hours of CS and untreated had $\text{PmO}_2 = 2.82 \pm 1.3$ ($*=p < 0.01$). There was no difference in PmO_2 between the two treatment groups.

- 2) Non-surgical treatment significantly improved the muscle viability after compartment syndrome

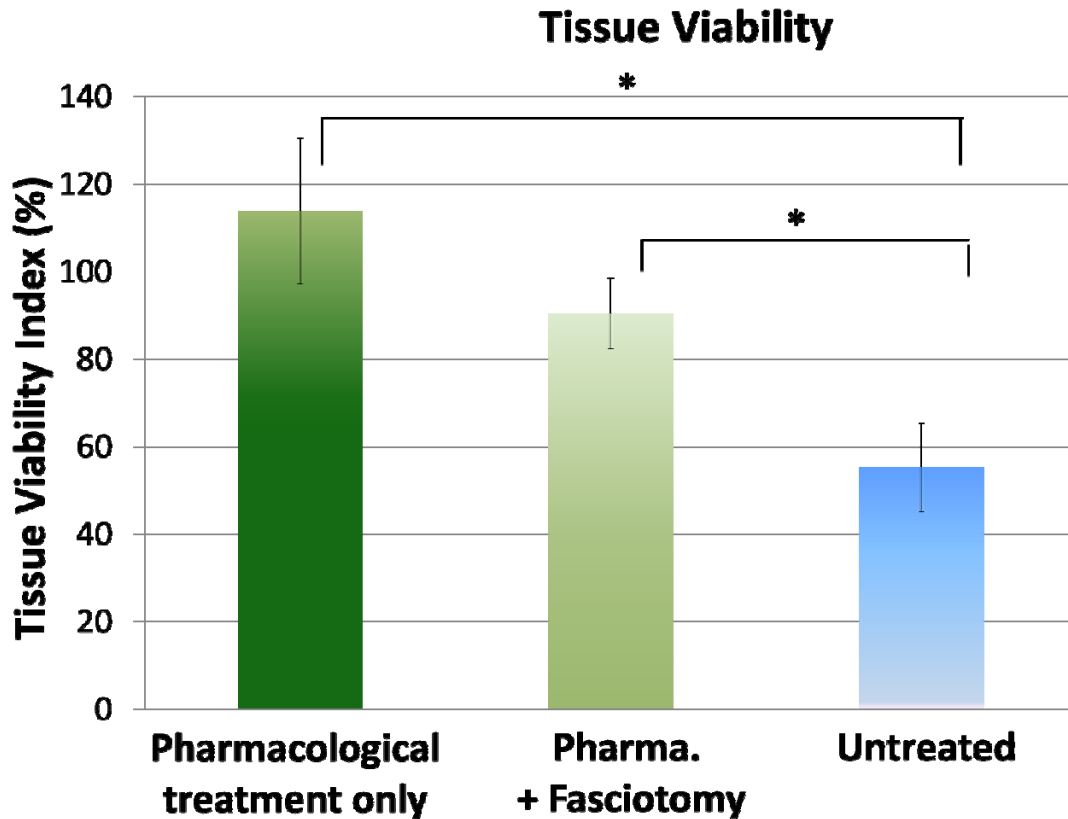


Figure 5 : Muscle viability after treatment with drugs-only and drugs +fasciotomy was compared with untreated controls. There was no significant difference in tissue viability between the two treatment groups (113 +/- 16.7% for drugs-only and 90 +/- 8.1% for drugs + fasciotomy). Both treated groups showed increased viability compared to the untreated controls (* = $p < 0.03$).

3) Non-surgical treatment significantly reduced muscle fibrosis after compartment syndrome

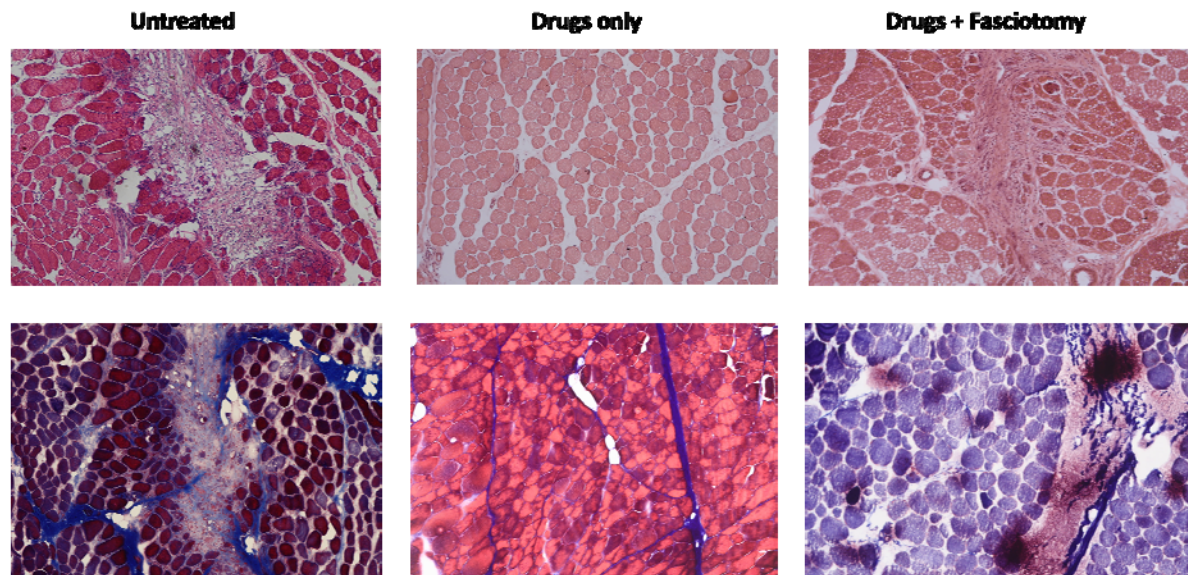


Figure 6: Muscle histology

H & E (top) and Masson's Trichrome (below) showed that untreated dogs had a greater degree of muscle damage and fibrosis than dogs treated with drugs only or drugs + fasciotomy.

Our results showed the feasibility of a non-surgical treatment strategy in treating acute compartment syndrome without fasciotomy. This result is presented on the annual meeting of Orthopedic Research Society in 2014 (abstract is attached in the appendix).

KEY RESEARCH ACCOMPLISHMENTS:

A) Animal surgery accomplishment: We have completed all animal surgeries in this project.

	Phase I	Phase II	Phase III
Number of animals proposed	16	8	8
Number of animals accomplished	16	8	8

B) Phase I: Comparison ΔP to muscle viability in CS.

We found there is a trend that ΔP and relative TA muscle viability are correlated. The lower ΔP (higher compartment pressure compared to diastolic blood pressure), the more severe muscle damage in CS.

C) Phase II: Comparison PmO₂ to muscle viability in CS.

We found there is a trend that average PmO₂ level is correlated to the relative TA muscle viability. The higher tissue PmO₂, the less muscle damage in CS.

D) Phase III: Testing feasibility of a novel non-surgical treatment for CS.

We found that keeping the blood pressure at a high level using pharmacological agents (Phenylephrine/dobutamine combination) may serve as an alternative to surgical treatment for acute compartment syndrome.

REPORTABLE OUTCOMES:

Liu, Xuhui, Mok, James, Kang, Heejae, Jin, Julie, Boehme, Alexandar, Hansen, Erik, Kandemir, Utku, Rollins, Mark, Kim, Hubert, NONOPERATIVE TREATMENT FOR COMPARTMENT SYNDROME WITH PHENYLEPHRINE AND DOBUTAMINE [abstract]. Presented on 60th Annual Orthopaedic Research Society Meeting; 2014 Mar 11-15; New Orleans, LA.

See Appendices for full abstract

CONCLUSION:

Continuous tissue oxygenation monitoring is a promising new diagnostic method for acute compartment syndrome. Pharmacological reagents that increase muscle oxygenation may serve as a non-surgical treatment for acute compartment syndrome.

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APPENDICES:

TITLE: NONOPERATIVE TREATMENT FOR COMPARTMENT SYNDROME WITH PHENYLEPHRINE AND DOBUTAMINE

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Introduction: Acute compartment syndrome (CS) of the extremity describes increased pressure within the osseofascial compartment, leading to compromised circulation, hypoxia, and ultimately muscle and nerve necrosis. Current treatment for acute extremity symptomatic CS is fasciotomy. However, surgical treatment has associated morbidity and may delay the recovery of the patients. The goal of this study is to investigate the feasibility of a novel non-surgical

treatment for acute compartment syndrome by increasing blood pressure using a dog CS model.¹ We hypothesize that pharmacological treatment will raise the blood pressure, improve limb perfusion, and increase tissue oxygenation, thus rescuing muscle from CS.

Methods: All procedures were approved by the Institutional Animal Care and Use Committee at ISIS Services. Under general anesthesia, CS was induced in the anterolateral compartment on bilateral legs in 10 animals (4 treated and 6 non-treated) via Hespan infusion with a goal pressure of 30mmHg above diastolic blood pressure ($\Delta P = -30\text{mmHg}$). Polarographic oxygen measurement electrodes were placed percutaneously into the anterolateral compartment. Intramuscular tissue oxygenation, compartment pressure, and blood pressure were recorded every 30 seconds. In the treated group, pharmacological treatments begin at 1 hour after the compartment syndrome is induced. Infusion of intravenous phenylephrine was initiated at 25mcg/min and titrated up to 100mcg/min as needed to increase the diastolic blood pressure 30mmHg above the baseline ($\Delta P = 0\text{mmHg}$). Intravenous dobutamine at 60mcg/min was initiated 2 hours later. Six to seven hours after treatment, fasciotomy was performed on one leg of the animals and the skin was closed 1 hour later. The other leg was kept intact. In the non-treatment group, similar procedures were performed except that neither pharmacological nor fasciotomy was performed. Animals were euthanized 2 weeks postoperatively at which point muscle biopsies were performed. Tissue viability was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay as previously described.^{2,3} This is a validated technique in which the normalized tissue viability index is expressed as a percentage of control (quadriceps muscle).

Results: Pharmacological treatment significantly increased PmO₂ in the anterior compartment muscle. The average PmO₂ in the treatment group was $18.8 \pm 4.3\text{mmHg}$ (mean $\pm$ SE). In contrast, PmO₂ in the non-treated group dropped to 0 mmHg soon after the compartment syndrome was induced. Fasciotomy increased the PmO₂ from $18.8 \pm 6.7\text{mmHg}$ to $35.7 \pm 15\text{mmHg}$. Two weeks after surgery, the muscle viability index in pharmacological treated, pharmacological plus fasciotomy and non-treated groups were $128 \pm 15\%$, $94.3 \pm 8.3\%$, $41.8 \pm 17\%$ (mean $\pm$ SE) respectively. There was no significant difference between pharmacological treated and pharmacological plus fasciotomy groups ($P=0.09$). However, either treated group has a higher tissue viability compared to the non-treated group ($P<0.01$).

Discussion: Our results showed that non-surgical pharmacological treatment significantly increases muscle oxygen and viability and may represent an alternative, less morbid treatment for acute compartment syndrome than fasciotomy. Phenylephrine is often used for trauma patients in the perioperative setting to maintain blood pressure and could serve as initial therapy in patients with possible compartment syndrome. However, in our study, the effect of phenylephrine decreased over time, and a second line drug (dobutamine) was needed after the first few hours. We are currently testing this treatment strategy in more animals. Future works include titrating drug dosing, long-term effect follow up, muscle histology and functional analysis.

Significance: Keeping the blood pressure at a high level using pharmacological agents (Phenylephrine/dobutamine combination) may serve as an alternative to surgical treatment for acute compartment syndrome.

Acknowledgements: This study was supported by the Department of Defense (Grant Number: W81XWH-10-1-1024).

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