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TITLE: Diagnosis of Compartment Syndrome Based on Tissue Oxygenation

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The diagnosis of acute compartment syndrome (CS) remains problematic due to difficulty in diagnosis. 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 after fasciotomy and biochemical measurements of tissue viability. Under general anesthesia, CS was induced in the anterolateral compartment of one hind limb via Hespan infusion with predetermined goal pressures 30 mmHg, 10 mmHg, and 0 mmHg above diastolic blood pressure. Polarographic oxygen probes were placed percutaneously into the anterolateral compartment. PmO2 was recorded every 30 seconds. After approximately 7 hours of compartment syndrome, fasciotomy was performed. Animals were euthanized 2 weeks postoperatively at which point muscle biopsies were performed. Tissue viability was assessed by histologic analysis (H&E, Masson’s Trichrome, and Cytochrome C Oxidase stains) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

In the ΔP < -30 mmHg group, the averaged mean PmO2 before the infusion-induced CS was 35.63 mmHg (range 15.22-53.65 mmHg). During induced CS, PmO2 decreased to an average of 2.54 mmHg (range 0.19-4.92 mmHg, p<0.05 Wilcoxon rank sum test). Following fasciotomy, PmO2 recovered in half of the animals to an averaged mean of 40.11 mmHg (range 22.33-57.89 mmHg), whereas in the other half, PmO2 did not recover and remained at an averaged mean of 4.07 mmHg (range 0.85-7.29 mmHg). In the ΔP = -10 mmHg group, the averaged mean PmO2 before CS was 36.44 mmHg (range 21.79-48.5 mmHg). During induced CS, PmO2 decreased to an average of 3.45 mmHg (range 0.26-5.04 mmHg, p<0.05). Following fasciotomy, PmO2 recovered to an averaged mean of 47.24 mmHg (range 23.15-87.55 mmHg). In the ΔP = 0 mmHg group, mean PmO2 before CS was 22.59 mmHg. During induced CS, PmO2 decreased to 7.03 mmHg (p<0.05). Following fasciotomy, PmO2 recovered to a mean of 93.1 mmHg. The animals with persistently low PmO2 had substantially more signs of necrosis on histologic analysis and lower viability index at 2 weeks. The PmO2 values following fasciotomy appear to reflect underlying muscle viability as confirmed by histologic methods with use of a previously suggested threshold PmO2. This is an important finding if PmO2 is to be used to guide the treatment of CS. 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.

15. SUBJECT TERMS: Anterolateral compartment, compartment syndrome, diagnosis, muscle tissue oxygenation, muscle tissue viability, tourniquet ischemia

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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.4,6

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.7 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.3,8 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 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 on 9 terminal studies, we had established a reproducible dog model for continuous intramuscular tissue oxygenation monitoring during a controlled induction of compartment syndrome, and a reliable anesthetic regimen for hemodynamic stability during the procedure. The measurement of tissue oxygenation with polarographic oxygen probes proved to be highly sensitive to pressure-induced ischemia with high translational potential.
**Animal model for PmO2 measurement:**
While under general anesthesia, compartment syndrome was induced in the anterolateral compartment of the right hind legs (CS limb) by infusing colloid fluid (Hextend) through an intramuscular angiocatheter. One of the four following compartment pressures was maintained, as measured by an arterial line, for approximately 7 hours, followed by fasciotomy:
1) High (N=4): $\Delta P = -30$ mmHg, i.e., compartment pressure is 30 mmHg higher than the diastolic blood pressure.
2) Medium-High (N=4): $\Delta P = -10$ mmHg, i.e., compartment pressure is 10 mmHg higher than the diastolic blood pressure.
3) Medium (N=4): $\Delta P = 0$ mmHg, i.e., compartment pressure is equal to the diastolic blood pressure.
4) Low (N=4): $\Delta P = 10$ mmHg, i.e., compartment pressure is 10 mmHg lower than the diastolic blood pressure.

In the contralateral leg, a tourniquet was applied over the upper leg (TI limb) and the pressure elevated to 300 mmHg to establish a floor value of 0 mmHg for one of the four following durations:
1) High (N=4): 8 hours
2) Medium-High (N=4): 6 hours
3) Medium (N=4): 4 hours
4) Low (N=4): 2 hours

Polarographic oxygen probes were placed percutaneously into the anterolateral compartment of both legs to measure the tissue oxygenation. A 16 gauge IV catheter is inserted obliquely into the muscle substance of the anterolateral compartment. The probe is placed through the IV, and the IV is withdrawn. The probe is then secured to the skin using dressing tape. PmO2 was recorded every 30 seconds on the CS limb. After approximately 7 hours of compartment syndrome and previously stated durations of tourniquet-induced ischemia, fasciotomy was performed and the tourniquet was deflated on respective legs.

**Current and future experimental set-up:**
Currently, 10 chronic experiments of Phase 1 have been completed:

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The remaining 6 chronic experiments of Phase 1 are planned:

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**Tissue Oxygenation (PmO2) data for CS limb:**
Graph axes and legends: The y-axis represents PmO2 or differential pressure ($\Delta P$) in mmHg. The x-axis represents time. Blue markers indicate PmO2 and green markers indicate $\Delta P$. First arrow indicates time of probe insertion; second arrow indicates start of infusion-induced CS; third arrow indicates time of fasciotomy; and fourth arrow indicates time of probe removal.
Notes: The oxygen probe is calibrated to room air (154 mmHg) before insertion. Upon insertion, PmO2 equilibrates within the muscle. After the start of infusion-induced CS, there is a rapid decrease in PmO2 and a corresponding decrease in ΔP. Following the removal of the probe, PmO2 returned to room air values.

Group A) CS Experiments #1-4: High severity compartment syndrome (ΔP < -30 mmHg)

Figure 1

Figure 2
Group B) CS Experiments #5-8: Medium-high severity compartment syndrome ($\Delta P = -10$ mmHg)
Group C) CS Experiments #9-10: Medium severity compartment syndrome ($\Delta P = 0$ mmHg)
Across 4 animals in CS Group A, where ΔP < -30 mmHg, the averaged mean PmO2 before the infusion-induced CS was 35.63 mmHg (range 15.22-53.65 mmHg). During induced CS, PmO2 decreased to an average of 2.54 mmHg (range 0.19-4.92 mmHg, p<0.05 Wilcoxon rank sum test). Following fasciotomy, PmO2 recovered in CS Experiments 2 and 3 to an averaged mean of 40.11 mmHg (range 22.33-57.89 mmHg), whereas in CS Experiments 1 and 4, PmO2 did not recover and remained at an averaged mean of 4.07 mmHg (range 0.85-7.29 mmHg).

In CS Group B, where ΔP = -10 mmHg, the averaged mean PmO2 before CS was 36.44 mmHg (range 21.79-48.5 mmHg). During induced CS, PmO2 decreased to an average of 3.45 mmHg (range 0.26-5.04 mmHg, p<0.05). Following fasciotomy, PmO2 recovered to an averaged mean of 47.24 mmHg (range 23.15-87.55 mmHg).

In CS Experiment #9, where ΔP = 0 mmHg, mean PmO2 before CS was 22.59 mmHg. During induced CS, PmO2 decreased to 7.03 mmHg (p<0.05). Following fasciotomy, PmO2 recovered to a mean of 93.1 mmHg.

*Tissue Oxygenation data for TI limb:*
Graph axes and legends: The y-axis represents PmO2 in mmHg. The x-axis represents time. Blue markers indicate PmO2.

Notes: The oxygen probe is calibrated to room air (154 mmHg) before insertion. Upon insertion, PmO2 equilibrates within the muscle. After the application of a tourniquet, there is a rapid decline in PmO2. With release of tourniquet, there is a rapid increase of PmO2 towards pre-tourniquet values. Following the removal of the probe, PmO2 returned to room air values.

Group A) TI Experiments #1-4: 4 hours of tourniquet-ischemia
Figure 10

TI Experiment #1

Figure 11

TI Experiment #2
Group B) TI Experiments #5-8: 6 hours of tourniquet-ischemia
Figure 14

TI Experiment #5

Figure 15

TI Experiment #6
Figure 16

TI Experiment #7

Figure 17

TI Experiment #8

Group C) TI Experiments #9-10: 2 hours of tourniquet-ischemia
Muscle tissue viability assessment – MTT:

Affected anterolateral muscle tissue from the CS and TI limbs were biopsied two weeks after injury. Tissue viability was assessed with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) according to Bonheur et al.\textsuperscript{2} The assay quantifies the reduction of a tetrazolium salt (MTT) to water-insoluble colored formazan crystals by mitochondrial enzymes of viable tissue. The absorbance of the tetrazolium solution is read at 570nm and normalized to the dry weight of the muscle sample. The tissue viability index was represented as the percentage of the normalized absorbance of affected tissue to that of the negative control quadriceps tissue.
Figure 20. MTT muscle viability two weeks after CS injury. Group A: CS#1-4 ($\Delta P < -30$ mmHg), Group B: CS#5-8 ($\Delta P = -10$ mmHg), Group C: CS#9-10 ($\Delta P = 0$ mmHg, CS#10 data pending). The viability indices of Group A was significantly lower than that of Group B ($p<0.05$, Student’s T-test). Within Group A, the viability indices from experiments 1 and 4, in which PmO2 did not recover after fasciotomy, were substantially lower than those from experiments 2 and 3, in which PmO2 recovered after fasciotomy.

Figure 21. MTT muscle viability two weeks after TI injury. Group A: TI#1-4 (4 hours of ischemia), Group B: TI#5-8 (6 hours of ischemia), Group C: TI#9-10 (2 hours of ischemia, TI#10 data pending).

Muscle tissue viability assessment – Histology:

CS histology:
The histology data show that in the high CS severity group, muscle has been largely replaced by fibrotic tissues, represented by the increased uptake of blue staining in the Masson’s trichrome stain. Small irregular round fibers are indicative of degeneration and regeneration of muscle fibers; infiltration of inflammatory cells (e.g. neutrophils) is evident; enlarged interstitial spaces between neighboring muscle fibers suggest intercellular edema; and white, unstained circles represent fatty infiltration. The degree of these pathological findings seems at least qualitatively to be mitigated in the medium-high CS severity group, corresponding to the trend in the biochemical MTT viability data.

Hematoxylin & Eosin (H&E) Stain: H&E is the gold standard structural stain, useful for observing morphologic changes. Hematoxylin stains the nuclei deep blue-purple color; eosin stains the cytoplasm and extracellular proteins pink. (Figures 22-24)

Figure 22. CS Group A) CS Experiments #1-4: High severity compartment syndrome (ΔP < -30 mmHg)
Masson’s Trichrome Stain: Masson’s Trichrome stain is used to distinguish collagen from muscle tissue and to identify an increase in collagenous tissue. Collagen is stained blue and muscle is stained red. (Figures 25 and 26)
Figure 25. CS Group A) CS Experiments #1-4: High severity compartment syndrome ($\Delta P < -30$ mmHg)
Figure 26. CS Group B) CS Experiments #5-8: Medium-high severity compartment syndrome (ΔP = -10 mmHg)

[Histology data pending for CS Group C) CS Experiments #9-10: Medium severity compartment syndrome (ΔP = 0 mmHg)]

Cytochrome C Oxidase (COX) Stain: COX activity is an indicator of oxidative phosphorylation and mitochondrial viability. Cells with normal mitochondrial COX activity is stained dark brown, whereas necrotic cells with mitochondrial defects do not stain. (Figure 27)
Figure 27. CS Group A) CS Experiments #1-4: High severity compartment syndrome ($\Delta P < -30$ mmHg)

[Histology data pending for CS Group B) CS Experiments #5-8: Medium-high severity compartment syndrome ($\Delta P = -10$ mmHg) and for CS Group C) CS Experiments #9-10: Medium severity compartment syndrome ($\Delta P = 0$ mmHg)]

**TI histology:**
The first histology picture in each group is stained with H&E and the second histology picture in each group is stained with Masson’s Trichrome. TI Group C (2 hours of tourniquet ischemia) shows virtually no damage and well-preserved structural integrity, comparable to control tissues. TI Group A (4 hours of tourniquet ischemia) shows minor signs of fatty infiltration. TI Group B (6 hours of tourniquet ischemia) shows fatty infiltration and degeneration of muscle fibers, evidenced by small round irregularly sized fibers. Blaisdell\(^1\) has reported that in general, muscle appears tolerant of ischemia for up to 4 hours, and after 6 hours of ischemia, irreversible damage of muscle occurs. Our TI histology data support similar parameters of critical muscle tissue ischemic times.

Figure 28. TI Group A) TI Experiments #1-4: 4 hours of tourniquet-ischemia
Figure 29. TI Group B) TI Experiments #5-8: 6 hours of tourniquet-ischemia

Figure 30. TI Group C) TI Experiments #9-10: 2 hours of tourniquet-ischemia (Histology pending for TI Experiment #10)

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Table 1: Average PmO2 values before induction of CS, during CS, and after fasciotomy and muscle viability at two weeks after CS.
Challenges and limitations:
The main limitation of the study is that in a large animal model, injury is not homogenous across the entire muscles of the anterolateral compartment being studied. Thus, partial biopsies may fail to capture the global representation of the injury, leading to some bias. We have tried to mitigate the bias by taking multiple biopsies per group over the entire tissue being studied.

The infusion method of inducing compartment syndrome is the best established model in the orthopaedic literature on compartment syndrome, yet not a perfect model in terms of maintaining different steps of constant intramuscular pressure or oxygenation. Especially for milder injury conditions, stably holding the pressure or oxygenation within a tight range has been difficult, leading to larger standard deviation of the mean PmO2 value.

The variability between animals in response to injury also presents a challenge, especially with a minimum sample size. Some animals require more volume of the colloid solution to be infused to reach even lower compartment pressure, leading to more severe injury, compared to animals in higher severity injury group. However, this challenge also seems to reinforce the initial rationale of our study – that pressure is not a good marker for diagnosis of compartment syndrome. In Phase 2, where injury is induced based on PmO2, rather than compartment pressure as in current Phase 1, a more consistently graded injury scale is expected.

KEY RESEARCH ACCOMPLISHMENTS:

- Six hours of tourniquet ischemia appears to be the critical muscle tissue ischemic time, where irreversible changes occur.
- In 2 animals, PmO2 remained below 10 mmHg and did not recover after fasciotomy. These animals with persistently low PmO2 had substantially more extensive signs of necrosis on histological analysis and lower viability index than any other animals at 2 weeks. This PmO2 threshold of 10 mmHg may provide a warning parameter during Phase 2, in which injury is induced based on PmO2.
- The PmO2 values following fasciotomy appear to reflect the underlying muscle viability as confirmed by biochemical and histological methods that target mitochondrial function and evaluate different indications of tissue necrosis/viability.

REPORTABLE OUTCOMES:

See Appendices for full abstract.

CONCLUSION:
To date, 10 out of 16 animal experiments of Phase 1 have been completed. 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. This is an important finding if PmO2 is to be used to guide the diagnosis and treatment of CS. 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.

Next steps include completing the remaining experimental groups of Phase 1 to get a comprehensive picture of pressure-based outcomes of CS injury and systematically correlating PmO2 with tissue viability in Phase 2.

REFERENCES:


APPENDICES:

TITLE: Relationship between Intramuscular Tissue Oxygenation and Viability in a Compartment Syndrome Model

AUTHORS: Kang, Heejae1; Mok, James2; Hansen, Erik3; Kandemir, Utku4; Rollins, Mark4; Liu, Xuhui1,2; Kim, Hubert T.1,2

CURRENT PRIMARY CATEGORY: Trauma - Research Methodologies

ABSTRACT BODY:

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 death. The diagnosis of acute CS remains problematic due to difficulty in diagnosis. Continuous measurement of intramuscular tissue oxygenation of the leg has been shown to be feasible in humans and highly responsive to induced compartment syndrome and fasciotomy in a dog model.1 Using the same model, we investigated the relationship between intramuscular tissue oxygenation after fasciotomy and biochemical measurements of tissue viability.

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 of one leg in 4 animals via Hespan infusion with a goal pressure of 30mmHg above diastolic blood pressure. Polarographic oxygen probes were placed percutaneously into the anterolateral compartment. Intramuscular tissue oxygenation was recorded every 30 seconds. After approximately 7 hours of compartment syndrome, fasciotomy was performed. Animals were euthanized 2 weeks postoperatively at which point muscle biopsies were performed. Tissue viability was assessed by histologic analysis (Hematoxylin & Eosin and Masson's Trichrome) and 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.

Results: The mean duration of induced compartment syndrome was 6.9 hours. The averaged mean intramuscular tissue oxygenation was 35.63mmHg (range 15.22-53.65) and decreased to 2.54mmHg (range 0.19-4.92) during induced CS. Following fasciotomy, 2 animals showed recovery of intramuscular tissue oxygenation exceeding an ischemic threshold of 10mmHg and 2 animals did not. The animals with persistently low intramuscular tissue oxygenation had substantially more fibrosis on histologic analysis (collagen fiber to muscle tissue ratio 45.58% vs. 21.98%, p=0.01, Figure 1) and lower viability index (9.23% vs. 44.41%, p=0.1, Figure 2) at 2 weeks.

Discussion: The intramuscular tissue oxygenation values following fasciotomy appear to reflect the underlying muscle viability as confirmed by histologic methods with the use of a previously suggested threshold oxygenation. This is an important finding if intramuscular tissue oxygenation is to be used to guide the treatment of CS. The measurement of intramuscular tissue oxygenation detects pressure-induced ischemia and may also predict irreversible necrosis in an animal model with high translational potential.

Significance: The measurement of intramuscular tissue oxygenation may represent a minimally invasive, physiologic, and continuous method for diagnosing compartment syndrome.

Acknowledgements: This study was supported by the Department of Defense.