Iodide reduces intramuscular inflammation following hind limb ischemia in mice

Poster 9-08

Michael A. Insko, Faraday Pharmaceuticals, Seattle, WA (USA)
Mark B. Roth, Ph.D. Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA (USA)

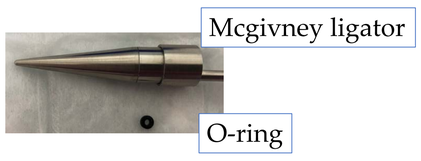
Introduction: Faraday Pharmaceuticals is focused on the research and development of elemental reducing agents (ERAs). These agents have potential applications for the treatment of critical care diseases. Inflammation and damage induced by reactive oxygen species (ROS) are well known to impair muscle leading to wasting. In this study we investigated the utility of FDY-5301 (sodium iodide) to reduce intramuscular inflammation in the mouse hind limb following ischemia.
Methods: Male C57BL/6 mice were anesthetized and subject to 2.5 hours of bilateral hind limb ischemia using an O-ring placed above the thigh. Five minutes prior to O-ring removal a 1 mg/kg intravenous bolus of FDY-5301 was given retro-orbitally. The next day (24 hours post O-ring removal), the gastrocnemius muscle was removed and snap frozen in liquid nitrogen. Following homogenization the levels of various cytokines were assessed using a MILLIPLEX® MAP magnetic bead-based analysis on a Luminex MAGPIX® system. The following biomarkers were measured: Interferon gamma (INFγ), Interleukin 1 beta (IL-1ß), IL-2, IL-6, IL-10, KC (a.k.a. CXCL1), Lipopolysaccharide-induced CXC chemokine (LIX), macrophage inflammatory protein-2 (MIP-2, a.k.a.CXCL2), and tumor necrosis factor alpha (TNFα).
Results: Administration of FDY-5301 lead to a statistically significant reduction in muscle levels of: IL-6, IL-10, KC, and MIP-2, and also reduced LIX and TNF-α. A significant reduction in systemic IL-6 in the plasma was also observed.
Conclusions: Iodide administration reduces intramuscular inflammation following bilateral hind limb ischemia.

Background

Faraday Pharmaceuticals is a clinical stage pharmaceutical company focused on the development of elemental reducing agents (ERAs) for the potential treatment of critical care diseases such as: ST segment elevation myocardial infarction (STEMI), tourniquet induced sarcopenia, and intensive care unit acquired weakness (ICUAW). There is mounting evidence to suggest that ICUAW organ and muscle injury are mediated, at least in part, by reactive oxygen species (ROS) [1] and subsequent systemic inflammation [2]. Faraday has shown that FDY-5301 (sodium iodide) reduces cardiac muscle damage in animal models of acute myocardial infarction [3, 4]. The mechanism is multifactorial but includes the ability of FDY-5301 to catalytically destroy peroxide [4] and reduce inflammation [3-5]. In this study we investigated the utility of FDY-5301 to reduce intramuscular inflammation in the mouse hind limb following ischemia.

Materials & Methods

- Male C57BL/6 mice (7-10 weeks old) were acquired from Charles River Laboratories (Hollister, CA) and housed in individually ventilated cages (IVC) from Innovive (San Diego, CA) with corncob bedding, enrichment, InnoDome™/InnoWheel™ and Nestlets™ from Anacare (Bellmore, NY). The vivarium was controlled for temperature (20-26 °C) and humidity (30-70%), with a 12 hour light dark cycle, 6 am to 6 pm. Animals had ad lib access food, LabDiet 5001 (St.Louis, MO) and water Aquavive® (Innovive). All animals acclimatized for >96 hours before study initiation.
- The mice were anesthetized with an intraperitoneal (i.p.) bolus of ketamine and xylazine.
- A Mcgivney hemorrhoidal ligator and O-rings were used to induce bilateral hind limb ischemia (HLI) by pulling the leg of the mouse through the ligator and releasing the O-ring close to the groin above the thigh (see photos).



- Sedation during the 2.5 hours of ischemia was maintained by keeping the mice in an induction chamber filled with isoflurane (1.5 %, 0.5 L/min oxygen). Temperature was maintained by keeping the induction chamber inside of an incubator (Panasonic, model MIR-154) set to 28.5°C.
- 5-minutes prior to reperfusion a 1 mg/kg i.v. bolus of FDY-5301 or vehicle (saline) was delivered retro orbitally (n=25/group)
- Reperfusion was achieved by cutting the o-ring off the hindlimb. The mice were kept at 28.5 °C for an additional 3 hours (no isoflurane) before being returned to standard IVC housing until their sacrifice at 24 hours.
- At sacrifice, whole blood was taken and kept on ice in a MiniCollect® plasma separator tube with lithium heparin until centrifugation, plasma was stored at -80C. The gastrocnemius muscle was removed and snap frozen in liquid nitrogen and stored at -80C.
- Homogenization buffer [6] containing: 1x TRIS, 1% IGEPAL CA-630 and cOmplete™ protease inhibitors was used for tissue processing using a 4-Place Mini Bead Mill (VWR®) and Hard Tissue Homogenizing Mix, with 2.8 mm Ceramic Beads (Omni International). ~70-100 mg of tissue was placed inside the homogenization tube and 10 µL of ice cold buffer was added for every 1 mg of muscle. The tissue was homogenized using speed 5 for 90 seconds, and further processed by centrifugation for 20 minutes at 15,000 xG at 4°C. The supernatant was diluted 1:10 in buffer prior to analysis.
- The following cytokines were assessed using a MILLIPLEX® MAP magnetic bead-based analysis on a Luminex MAGPIX® system according to the manufacturers instructions:
  - Interferon gamma (INFγ)
  - Interleukin (IL) 1 beta (IL-1ß),
  - IL-2
  - IL-6
  - IL-10
  - KC (a.k.a. CXCL1)
  - Lipopolysaccharide-induced CXC chemokine (LIX)
  - macrophage inflammatory protein-2 (MIP-2, a.k.a.CXCL2)
  - tumor necrosis factor alpha (TNFα)
- GraphPad Prism version 8.1 was used to generate graphs and perform statistical analysis. If the cytokine value was below lower limit of quantitation (LLOQ) then a value of the LLOQ divided by the square root of 2 was used for graphing and statistics [7].
- Differences were considered significant if $p<0.05$ using an unpaired t-test.
- In a separate study, mice were subject to the same model of injury. An i.v. bolus of vehicle or FDY-5301 was delivered 5 minutes prior to reperfusion at: 0.5, 1, 2, or 4 mg/kg (n=26/group). After 24 hours of reperfusion, the gastrocnemius muscle was removed and weighed so that muscle loss could be determined.

- A pilot study evaluating 1.5 or 2.5 hr of ischemia with reperfusion times of: 3 hr, 1 day, 2 days, and 3 days was performed (n=5 animals/group) (data not shown), and the final experimental conditions of 2.5 hr ischemia and 24 hr reperfusion were chosen. The results for the 5 mice in the pilot study treated the same as the main study are included in the data sets shown.

Results

- Administration of FDY-5301 lead to a statistically significant reduction in muscle levels of: KC, MIP-2, IL-6, and IL-10 (Figure 1).
- A significant reduction in systemic IL-6 in the plasma was also observed (Figure 2).
- In general, there is reduced intramuscular and systemic inflammation (Figure 3).
- Administration of 2-4 mg/kg FDY-5301 in this model significantly preserves muscle (Figure 4).

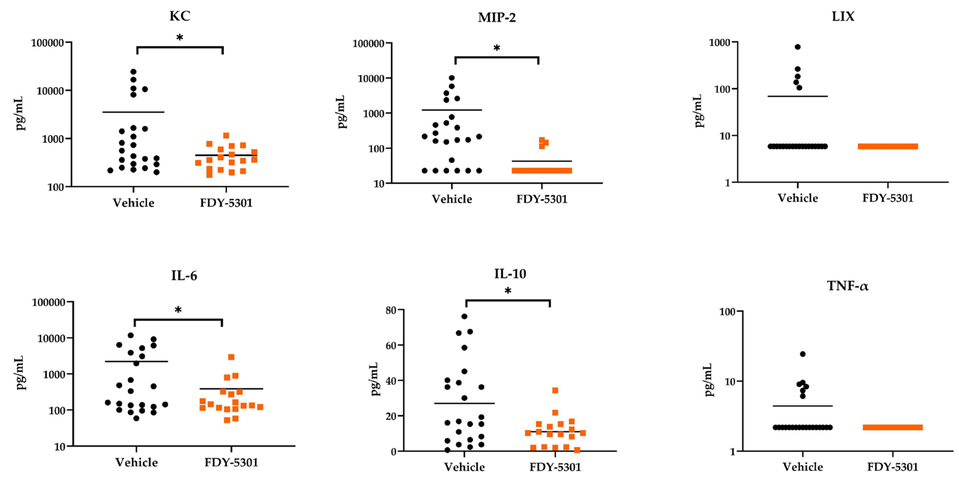


Figure 1: Iodide decreases intramuscular inflammation. Intramuscular cytokine levels were measured 24 hours following reperfusion in a mouse model of bilateral HLI. A 1 mg/kg i.v. bolus of FDY-5301 (sodium iodide) given 5 min prior to reperfusion significantly reduced: KC, MIP-2, IL-6, and IL-10. * $p< 0.05$ using t-test. LIX and TNF-α were reduced but the majority of the values were below the limit of quantitation of the assay. The final group sizes were: vehicle (n=23) & FDY-5301 (n=18).

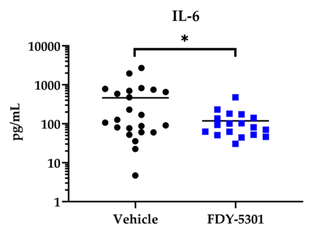


Figure 2: Iodide decreases systemic inflammation. Circulating levels of cytokines were measured in the plasma 24 hours following reperfusion in a mouse model of bilateral HLI. A 1 mg/kg i.v. bolus of FDY-5301 (sodium iodide) given 5 min prior to reperfusion significantly reduced: IL-6. * $p< 0.05$ using t-test. The final group sizes were: vehicle (n=23) & FDY-5301 (n=18).

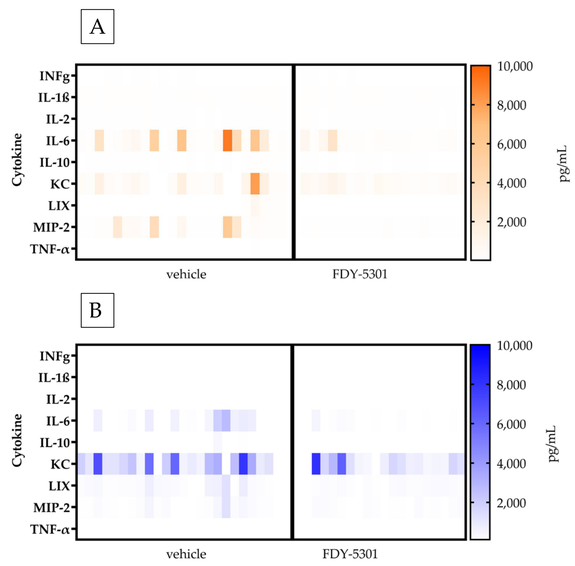


Figure 3: Iodide decreases intramuscular and systemic inflammation. These heat maps show the entire panel of cytokines measured in muscle (A) or plasma (B) 24 hours following treatment with either vehicle or FDY-5301.

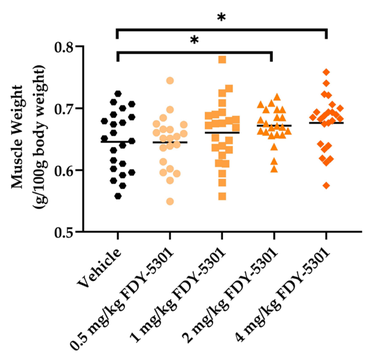
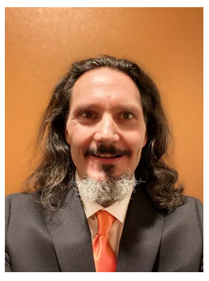


Figure 4: Iodide preserves muscle mass. The weight of the gastrocnemius muscle was determined 24 hours following reperfusion in a mouse model of bilateral HLI. A 2 or 4 mg/kg i.v. bolus of FDY-5301 (sodium iodide) given 5 minutes prior to reperfusion significantly preserved muscle weight. * $p<0.05$ using t-test comparing FDY-5301 to vehicle. n=21-24/group.

Conclusions

- This model demonstrates the usefulness of using bilateral HLI to induce intramuscular and systemic inflammation.
- Intramuscular inflammation is associate with impaired anabolic recovery (8) and the ability of FDY-5301 to decrease inflammation and preserve muscle indicates it may be beneficial in muscle wasting diseases, including ICUAW.



Michael A. Insko

References

1. Abrigo, J., et al., Role of Oxidative Stress as Key Regulator of Muscle Wasting during Cachexia. Oxid Med Cell Longev, 2018. 2018: p. 2063179.
2. Friedrich, O., et al., The Sick and the Weak: Neuropathies/Myopathies in the Critically Ill. Physiol Rev, 2015. 95(3): p. 1025-109.
3. Iwata, A., M.L. Morrison, and M.B. Roth, Iodide protects heart tissue from reperfusion injury. PLoS One, 2014. 9(11): p. e112458.
4. Morrison, M.L., et al., Iodide Improves Outcome After Acute Myocardial Infarction in Rats and Pigs. Crit Care Med, 2018. 46(11): p. e1063-e1069.
5. Gamon, L.F., et al., Iodide modulates protein damage induced by the inflammation-associated heme enzyme myeloperoxidase. Redox Biol, 2019. 28: p. 101331.
6. Roche, J.A., et al., Myofiber damage precedes macrophage infiltration after in vivo injury in dysferlin-deficient A/J mouse skeletal muscle. Am J Pathol, 2015. 185(6): p. 1686-98.
7. Croghan, C.a.E., P., Methods of dealing with values below the limit of detection using SAS, in Southeastern SAS User Group. 2003: St. Petersburg, FL.
8. Puthucheary, Z.A., et al., Metabolic phenotype of skeletal muscle in early critical illness. Thorax, 2018. 73(10): p. 926-935.