Iodide reduces intramuscular inflammation following hind limb ischemia in mice

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Introduction: Faraday Pharmaceuticals is focused on the research and 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 FDY5301 (sodium iodide) reduces cardiac muscle damage in animal models of acute myocardial infarction [3, 4]. The mechanism is multifactorial but includes the ability of FDY5301 to catalytically destroy peroxide [4] and reduce inflammation [5-7]. In this study we investigated the utility of FDY5301 to reduce intramuscular inflammation in the mouse hind limb following ischemia.

Methods: Male C57BL/6 mice (7-10 weeks old) were acquired from Charles River Laboratories (Holliston, CA) and housed in individually ventilated cages (IVC) from Innovive (San Diego, CA) with corn cob bedding, enrichment, InnDose™/BioNest™ and Neoliters™ from Anacate (Belfort, 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.

Interferon gamma (INFγ), Lymphotoxinβ (LXβ), and tumor necrosis factor alpha (TNFα).

Results: Reduction of intramuscular inflammation in mouse hind limb following ischemia:

- 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.

- Administration of FDY5301 lead to a statistically significant reduction in muscle levels of: IL-6, IL-10, KC, and MIP-2, and also reduced LIX and TNFα. A significant reduction in systemic IL-6 was the plasma also was observed.

Conclusions: Iodide administration reduces intramuscular inflammation following hind limb ischemia.

Materials & Methods:

- Male C57BL/6 mice (7-10 weeks old) were acquired from Charles River Laboratories (Holliston, CA) and housed in individually ventilated cages (IVC) from Innovive (San Diego, CA) with corn cob bedding, enrichment, InnDose™/BioNest™ and Neoliters™ from Anacate (Belfort, 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-ring was used to induce bilateral hind limb ischemia (HBI) 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 in an incubator, (Panasonic, model MJ-154) set to 28.5°C.

- 5-minutes prior to reperfusion a 1 mg/kg i.v. bolus of FDY5301 or vehicle (saline) was delivered retro orbitally (n=25/group).

- Retraction was achieved by cutting the o-ring off the hind limb. The mice were kept at 28.5 °C for an additional 3 hours (no ischemia) 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 -80°C. The gastrocnemius muscle was removed and snap frozen in liquid nitrogen and stored at -80°C.

- Homogenization buffer [6] containing: 1% TRIS, 1% Igepal CA-630 and cOmplete™ protease inhibitors was used for tissue processing using a 4-Place Mini Bore Mill (WWR®) and Hard Tissue Homogenizing Mallet (Seward). A single mallet mashes muscle tissue and cryo-minced into ~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 9 for 90 seconds, and further processed by centrifugation for 20 minutes at 15,000 g 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 the 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 the pilot study, administering FDY5301 did not seem to be a different model of injury. An i.v. bolus of vehicle or FDY5301 was delivered 5 minutes prior to reperfusion at 0.5, 1, 2, or 4 mg/kg (n=6/group). After 24 hours of reperfusion, the gastrocnemius muscle was removed and weighed so that muscle loss could be determined.

- This model demonstrates the usefulness of using bilateral HLI to induce intramuscular and systemic inflammation.

- Intramuscular inflammation is associated with impaired anabolic recovery (9) and the ability of FDY5301 to reduce inflammation and preserve muscle indicates it may be beneficial in muscle wasting diseases, including ICUAW.

References:


