Adenosine mono-phosphate activated kinase (AMPK) activation as a novel analgesic target for chronic low back pain

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Objective: Disc herniation is a cause of radicular pain in humans. About 20% of US adults suffer from chronic pain and one the most common reasons is LBP. Fifty million Americans are suffering from daily chronic pain due to lack of effective pain treatments, and paucity of novel targets. The overarching goal of this project is to identify a non-opioid, druggable target and evaluate the anti-nociceptive efficacy of metformin and O304 that is efficacious in relieving chronic low back pain (LBP).

Methods: Following IACUC approval, young adult C57BL/6J female and male mice underwent surgery under isoflurane anesthesia with 1.5 % isoflurane in oxygen. Mice were allocated into 2 groups: lumbar disc puncture and sham disc exposure. Mice were placed in the dorsolateral position. The peritoneum was separated from the subcutaneous fat and retracted to the right side to expose the space between the hind peritoneum and the left psoas major muscle, using #15-blade scalpel and sterile tongue depressors. The hind peritoneum was gently retracted to the right side to allow visualization and access the spine. The L4/L5 and L5/L6 discs were exposed using sterile cotton tipped applicator. A 27 G hypodermic needle, with a plastic stop limiting depth penetration to 0.5 mm, was used to make a single puncture in each disc. The nucleus pulposus gelatinous tissue was seen inside the needle lumen upon removal. The skin incision was closed with 4-0 nylon suture. Mice were randomized into 4 drug treatment groups: 1) vehicle; 2) metformin 200 mg/kg; 3) O304 200 mg/kg; 4) metformin 100 mg/kg plus O304 100 mg/kg; plus one untreated sham surgery group. Drugs were administered by oral gavage starting 7 days after disc puncture and repeated for 6 more days. Mechanical allodynia in the plantar hindpaw was measured pre-surgery and up to day 28. At 28 days following surgery, mice were perfused transcardially with 4% paraformaldehyde. Ipsilateral lumber L4-L6 DRG were removed and prepared for IHC in paraffin-mixed solutions. Sections (5 μm) were incubated overnight with anti-mouse p-AMPKα antibody, p-mTOR antibody and anti-mouse NeuN antibody. Samples were incubated with secondary antibody with DAPI and cover-slipped. Immunoreactivity was examined using a fluorescence microscope. For each DRG section, the number of immunoreactive neurons was counted using Image J software.

Results: Seven days after disc puncture, female mice had lower von Frey thresholds than male mice, difference -0.46 g, $P<0.001$. Gender adjusted von Frey AUC's between days 7 and 28 for metformin and/or O304 were greater (reduced allodynia) compared to vehicle-treated mice. The difference of mean AUC's were: metformin, 41.1 g*d, O304, 44.7 g*d, drug combination: 33.4 g*d. No gender by treatment interactions were observed. In disc puncture mice, at 4 weeks after disc puncture, the percent of DRG neurons expressing p-AMPKα was decreased by 55.4%; $P<0.001$ and the percent of DRG neurons expressing p-mTOR was increased by 55.9%; $P<0.001$ compared to sham surgery mice.

Conclusions: Lumbar disc puncture in mice produces consistent mechanical allodynia, and post-injury treatment with the AMPK activator drugs metformin and/or O304 reduces the allodynia.