

IACUC statement

Animal care. The animal protocol was designed to minimize pain or discomfort to the animals. The animals were acclimatized to laboratory conditions (23°C, 12h/12h light/dark, 50% humidity, *ad libitum* access to food and water) for two weeks prior to experimentation. Intragastric gavage administration was carried out with conscious animals, using straight gavage needles appropriate for the animal size (15-17 g body weight: 22 gauge, 1 inch length, 1.25 mm ball diameter). Angion's Animal Welfare assurance # A4532-01. Animal experiments were performed at the Angion animal facility, a modern, fully equipped facility with full surgical support. Animals were cared for on a daily basis by our research staff. Angion contracts with Dr Thomas Donnelly, a board certified laboratory animal veterinarian. SOPs are in place for compound administration procedures and recognizing signs of pain or distress in order to institute the appropriate care. Angion's approved protocols # are as follows. All procedures involving animals were reviewed and approved by our institutional animal use and care committee.

1. Protocol # 2015-010 entitled KCa3.1 Inhibitor as Therapy for Primary Biliary Cirrhosis.
2. Protocol # 2015-019. KCa3.1 inhibitor liver fibrosis.
(for diet induced liver fibrosis in mouse models)
3. Protocol #. 2016-001 entitled "KCa3.1 inhibitor for liver fibrosis in TAA rats.

Procedures to minimize distress. Animals will be anesthetized with ketamine (100 mg/kg) + xylazine (10 mg/kg) or isoflurane prior to intravenous administration, surgery and sacrifice. Appropriate level of anesthesia will be assured by using the standard toe-pinch reflex. If the animals are in undue distress or pain (as evidenced by our SOP for recognition of signs of pain & distress in rodents) at any point during the experimental protocol, analgesics not described/anticipated in the animal protocol will be administered in consultation with the veterinarian. If necessary, animals will be euthanized.

Methods of euthanasia. Animals were anesthetized with Ketamine (100 mg/Kg, IP) and Xylazine (10 mg/Kg, IP) for surgical and terminal procedures for tissue collection. .

At the times indicated in the study protocol or at times specified under pain or lethargic conditions were euthanized by American Veterinary Medical Foundation (AVMF), compliant CO2 euthanasia guidelines (Animals sacrificed by CO₂ asphyxiation. 2013 edition (Leary et al.; Schaumburg, IL: American Veterinary Medical Association, 2013; 102). CO₂ overdose for large rat cage used at a flow rate of 4 Liters/Minute and mice cages, used a flow rate of 1.2 L/min.