

SDC, Materials and Methods

First Report of siRNA Uptake During Ex Vivo Hypothermic and Normothermic Liver Machine Perfusion

Methods: All studies were approved by the Umass Medical School IACUC. Healthy male Wistar Furth rats (~300g) were housed in standard conditions per institutional regulations with free access to fresh water and plain rodent chow. An n=5 was used for each temperature of perfusion and controls. Anesthesia was induced via inhalation of 5% isoflurane and analgesia was maintained via IP injection with ketamine (60mg/kg) + xylazine (8mg/kg). A single dose of 100u heparin was delivered via tail vein injection. Animals were exsanguinated via angiocatheterization of the aortic bifurcation marking the beginning of warm ischemic time. Both the splenic vein and inferior mesenteric vein were identified, ligated with 8-0 silk ties, and divided. A venotomy was performed on the anterior portal vein, which was cannulated via modified 18g angiocath secured with a 4-0 silk tie. The infrahepatic vena cava was divided and the liver was flushed via PV cannula with 10cc cold saline + 100u heparin. Hepatectomy was performed in standard fashion preserving some diaphragmatic tissue. Livers were immediately perfused on a continuous closed loop circuit by roller pump, warmed (to 37C) or cooled (to 4-7C). Portal vein perfusion pressure was maintained electronically at 10mmHg. Warm ischemia time ended upon initiation of machine perfusion and was on average 25 minutes. A standard perfusate was made by combining 99mL Williams E media (ThermoFisher) with 10U insulin. Invivofectamine lipid nanoparticles (ThermoFisher) were complexed via manufacturer protocol to FAS siRNA modified at 3' with AlexaFluor-555 (Qiagen), diluted to 1 mL in saline, then mixed with perfusate to a final concentration of 50nM siRNA. Control livers were perfused with perfusate plus invivofectamine alone in the same volume. Liver biopsies were obtained before perfusion and after 4 hours of perfusion, fixed in formalin, and paraffinized. Sections were rehydrated in standard fashion in xylene, ethanol and water. Parenchyma was stained with wheat germ agglutinin conjugated to AlexaFluor-488 (ThermoFisher) and DAPI (VectorShield). Slides were imaged on a Nikon A1 confocal microscope and images edited with ImageJ software.