Supplemental Data IV

New Synthetic Bile Acids FXR and TGR5 Agonists: Physicochemical Properties, Pharmacokinetics and Metabolism in Rat

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Hepatic metabolism and biliary secretion in bile fistula rats

The study was performed in 60 male Winstar-Han rats (220-250g bw) (Charles River Laboratories, Calco, Italy). All experiments were conducted according to relevant National and International Guidelines according to Public Health Service Policy on Humane Care and Use of Laboratory Animals and approved by the Ethical Committee of the University of Bologna, Italy (PR 22.03.10). The animals were kept at constant temperature, light/dark cycle. at least 1 week before the experiment and given full access to water and food. Twelve hours before the experiment food was withdrawn and water was allowed ad libitum. On the day of the experiment the animals were anesthetized with an intraperitoneal injection of Zoletil 50®(Virbac Laboratories, France) (5 mg/Kg bw); a booster of anesthesia was administrated when necessary during the experiment. A median laparotomy was performed in order to have a full access to the abdominal cavity. The bile duct was cannulated with a PE 50, 10 cm long, siliconated catheter(Clay Adams, Becton Dickinson, Parsippany, NJ, USA) and secured with a 7/0 Vycril suture, in order to collect the bile.

The BA were delivered either iv or per gavage id. For the intravenous delivery, the right femoral vein was isolated, cannulated with an Abbocath TI.V. catheter (21 gauge) and secured with a 4/0 monofilament. The femoral access was then connected to a syringe pump and 2.5 ml of 3%$_{w/v}$ bovine serum albumin (Sigma-Aldrich, St.Louis, MO, USA) in saline solution was infused over 1 hour in order to prevent BA-related hemolysis., After 1 hour steady state, the bile acid infusion was started: 2.5 ml of each BA at a dose of 1 µmol/min/kg bw (either INT-767, or INT-747 or INT-777) as sodium salt was infused for 1 hour at 2.5 ml/h in saline solution with 3%$_{w/v}$ albumin as above. Bile was collected throughout the infusion at 15 min time intervals and for two hours after the infusion was over.

For the intraduodenal administration a button-ended stainless steel curved gavage feeding tube (16ga x 76mm) (Instech Solomon, Plymouth Meeting, PA USA) was inserted in the duodenum from a section on the body of the stomach and ligated with a running suture on the stomach. This way, the infused solution was delivered directly into the duodenum, bypassing the stomach and avoiding delay of the gastric emptying. The gavage needle was then collected to a syringe pump and after 1 h baseline steady-state the infusion of 2.5 ml (at a dose of 1 µmol/min/kg bw in saline solution of each BA was started for 1 hour at 2.5 ml/h. The right or left femoral vein was then isolated, cannulated with an Abbocath TI.V. catheter (21 gauge) and secured with a 4/0 monofilament: 300µl heparinized blood

was withdrawn at 30 time intervals before the BA infusion and throughout the study in order to measure the BA plasma levels only for the intraduodenal infusion study CA, CDCA and TCDCA were used as controls in parallel experiments. Each BA was infused in a single animal, for a total of six animals for each BA.

The bile flow was measured gravimetrically taking the density of bile as one; the concentrations of the administered BA and its main metabolites were measured in bile and plasma samples with the HPLC-ES-MS/MS as above reported.

BA secretion, calculated from the volume of secreted bile and from the BA concentration in each sample, was expressed as μmol/min/kg. From the plot of biliary secretion versus time, the following parameters were calculated: mean ± SD maximum secretion rate SB_{max}, the time of the maximum value T_{max} and the area under the curve (AUC) of the biliary secretion rate values over the three hours bile collection.