

Validation Of a Bile Duct Cannulation Rat Model

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Introduction

One of the liver's principal functions is the formation of bile, which is requisite for the digestion of fat and the elimination of drugs and metabolites. Hepatic metabolism is an important determinant of drug clearance and bioavailability. Hepatic drug disposition depends upon organ perfusion rate, protein binding and drug metabolizing activity. Rats are frequently the first species used to characterize drug clearance and bioavailability and, because rats lack gallbladders, are ideal models for examining biliary drug disposition. A variety of chronic bile loop models in rats have been described and the advantages and disadvantages reported¹. The major variation of the models is where the bile returning catheter is placed, i.e., – either in the distal bile duct or the duodenum. This study was designed to validate a bile loop model, which involves ligating the bile duct and shunting bile directly into the duodenum by evaluating biliary flow rates and hematologic indicators of hepatic function post- surgery.

Material and Methods

Animals

Eight adult male CD® rats (*Rattus norvegicus*) (CrI:CD(1SD)), weighing between 225 and 250 grams and, produced by Charles River, were used. The study was approved by the institution's Animal Care and Use Committee. The animals were maintained in polycarbonate cages in a dedicated rodent surgical complex that was kept at 21 ± 2°C with a relative humidity of 60 ± 5% and a 12/12 hour light/dark cycle. Commercially produced, sterilized feed, bedding and water were provided *ad libitum*. All conditions of animal preparation and use were in accordance with recommendations set forth in the *Guide of the Care and Use of Laboratory Animals*. The animals were of VAF® (specific pathogen free) health status, which was verified by an in-room sentinel system.

Surgical Procedures

The eight animals were surgically implanted with jugular vein and bile duct catheters according to the following approved surgical procedures. Both catheters were made with 3 French polyurethane tubing materials.

Bile duct Catheterization

The rats were anesthetized with ketamine (43 mg/kg BW) and xylazine (8.7 mg/kg BW). After surgical skin preparation, two catheters (intestinal and biliary) were passed subcutaneously from the dorsal scapular region into the abdomen. Via ventral midline abdominal incision, the bile duct was isolated and ligated and the biliary catheter was inserted into the bile duct and secured. The intestinal catheter tip was placed into the duodenum just distal to the entry point of the bile duct and secured by placement of a purse string suture. The two catheters were exteriorized at the scapular incision and joined via use of a rigid cannula to create an uninterrupted loop, allowing bile flow from the liver to the intestinal lumen.

Jugular Vein Catheterization

The skin overlying the scapula and over the right jugular vein were shaved and skin aseptically prepared, followed by catheterization of the jugular vein. After fixation of the catheter, the distal end of the catheter was then tunneled subcutaneously to the dorsal scapular region, where it was exteriorized. Catheter patency was verified and the catheter locked with 50% heparinized dextrose solution. The exteriorized end of the catheter was sealed with a stainless steel plug.

Sample Collections

A 0.5 ml blood sample was collected via the jugular vein catheter immediately after the catheterization to serve as a control. Once every other day, a 0.5 ml blood sample was collected from each rat via the jugular catheter for liver

function panel analysis. Every other day, bile samples were collected twice daily at 6:00 am and 3:00 pm by temporarily disconnecting the external loop and the collection of free flowing bile and volume was determined. After each collecting session, the bile duct end and the duodenal end of the catheter were reconnected with a metal connector so that the bile enterohepatic circulation was reestablished. The amount of bile collected in 15 minutes was used to calculate the bile flow rate.

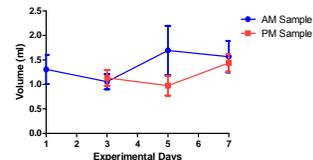
Statistical Analysis

Data of bile flow rate and biochemistry values were analyzed using a two-tailed t-test and a one-way ANOVA test. *p* values less than 0.05 were considered significant.

Results

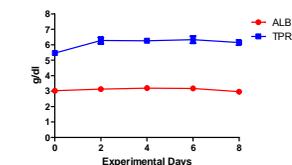
The average bile flow rate was 1.41 ml/hr (9.4 µl/min/100 g) and 1.18 ml/hr (7.9 µl/min/100 g) for the AM sampling and PM sampling, respectively (Figure 1).

Fig. 1: Bile Duct Catheterization, Male CD Rats Bile Flow Rates (ml/hr)



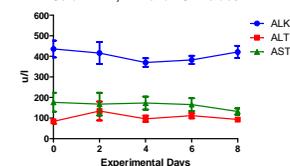
The differences between AM and PM flow rates were not statistically significant (*P* = 0.3228). No significant changes were noted in either albumin (ALB) or total protein (TPR) during the study period, with mean values of 3.1 ± 0.1 g/dl and 6.1 ± 0.4 g/dl, respectively (Figure 2).

Fig. 2: Bile Duct Catheterization, Male CD Rats Serum ALB and TPR Values



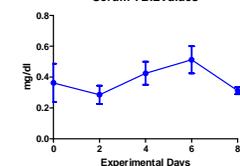
The values of alanine transaminase (ALT) remained consistent throughout the eight days study period, with a mean value of 104 ± 19.7 µl. There were no significant changes in alkaline phosphatase (ALP), with the mean value of 405.2 ± 27.7 µl. The level of aspartate transaminase (AST) remained consistent during the eight days post-surgery, with a mean value of 163.1 ± 18 µl (Figure 3).

Fig. 3: Bile Duct Catheterization, Male CD Rats Serum ALK, ALT and AST Values



Total bilirubin values did not vary significantly throughout the eight days study period, with a mean value of 0.38 ± 0.1 mg/dl (Figure 4).

Fig. 4: Bile Duct Catheterization, Male CD Rats Serum TBIL Values



Discussion

Although bile duct cannulation is performed primarily to assess hepatic metabolism of xenobiotics, the procedure carries a potential to interfere with hepatobiliary function. Thus, assessment of liver health and function, including analytes such as bilirubin, AST, ALT, ALK, ALB and TPR, is important.

Bilirubin is useful for measuring liver dysfunction because it is metabolized and eliminated by the liver. As a liver-specific enzyme, ALT is a generally considered a good indicator of liver health since it is released from hepatocytes into the systemic circulation upon cellular damage. The stable values of ALT, ALB, AST, TPR and TBIL throughout the study period supports normal hepatocyte function, i.e., the surgery did not cause hepatocellular injury.

The bile flow rate presented by this model were comparable to the literature reports^{1,2}. A typical single pharmacokinetics of enterohepatic circulation study requires time that is equivalent to three emptyings of the gallbladder in human (about a 36-hour period) and three half-lives of the drug (up to two days per half life), bringing the total to about 8 days¹. The results of this study indicate that this bile duct catheterization model works well for a typical single PK study.

References

1. Wang, Y.C and Reuning, R.H (1994). A comparison of two surgical techniques for preparation of rats with chronic bile duct cannulae for the investigation of enterohepatic circulation. *Laboratory Animal Science*. **44** (5) 479 -485 (1994).
2. Rolf Jr., L., Bartels, KE., Nelson, EC. & Berlin, K. Chronic bile duct cannulation in laboratory rats. *Laboratory Animal Science*. **41** (5) 486 – 492 (1991).