Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Murine model of long-term obstructive jaundice

Hiroaki Aoki, MD, PhD,[a,b] Masayo Aoki, MD, PhD,[a,b] Jing Yang, BS,[c] Eriko Katsuta, MD, PhD,[a,b] Partha Mukhopadhyay, PhD,[a,b] Rajesh Ramanathan, MD,[a] Ingrid A. Woelfel, BS,[a,b] Xuan Wang,[c] Sarah Spiegel, PhD,[b] Huiping Zhou, PhD,[c] and Kazuaki Takabe, MD, PhD, FACS[a,b,d,]*

[a] *Division of Surgical Oncology, Department of Surgery, Virginia Commonwealth University School of Medicine, Richmond, Virginia*

[b] *Department of Biochemistry and Molecular Biology, Virginia Commonwealth University School of Medicine, Richmond, Virginia*

[c] *Department of Microbiology and Immunology, Virginia Commonwealth University School of Medicine, Richmond, Virginia*

[d] *Division of Breast Surgery, Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, New York*

ARTICLE INFO

Article history:
Received 5 February 2016
Received in revised form
16 June 2016
Accepted 8 July 2016
Available online 18 July 2016

Keywords:
Obstructive jaundice
Murine model
Bile acid
Mouse
bile acid
Bile duct
Cholestasis

ABSTRACT

Background: With the recent emergence of conjugated bile acids as signaling molecules in cancer, a murine model of obstructive jaundice by cholestasis with long-term survival is in need. Here, we investigated the characteristics of three murine models of obstructive jaundice.

Methods: C57BL/6J mice were used for total ligation of the common bile duct (tCL), partial common bile duct ligation (pCL), and ligation of left and median hepatic bile duct with gallbladder removal (LMHL) models. Survival was assessed by Kaplan–Meier method. Fibrotic change was determined by Masson-Trichrome staining and Collagen expression.

Results: Overall, 70% (7 of 10) of tCL mice died by day 7, whereas majority 67% (10 of 15) of pCL mice survived with loss of jaundice. A total of 19% (3 of 16) of LMHL mice died; however, jaundice continued beyond day 14, with survival of more than a month. Compensatory enlargement of the right lobe was observed in both pCL and LMHL models. The pCL model demonstrated acute inflammation due to obstructive jaundice 3 d after ligation but jaundice rapidly decreased by day 7. The LHML group developed portal hypertension and severe fibrosis by day 14 in addition to prolonged jaundice.

Conclusions: The standard tCL model is too unstable with high mortality for long-term studies. pCL may be an appropriate model for acute inflammation with obstructive jaundice, but long-term survivors are no longer jaundiced. The LHML model was identified to be the most feasible model to study the effect of long-term obstructive jaundice.

This manuscript was presented at the 11th Annual Academic Surgical Congress at Jacksonville, Florida.

* *Corresponding author*. Breast Surgery, Roswell Park Cancer Institute, Elm & Carlton Streets, Buffalo NY 14263. Tel.: +1 716 845 5128; fax: +1 716 845 5705.

E-mail address: Kazuaki.takabe@roswellpark.org (K. Takabe).

0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2016.07.020

Introduction

Obstructive jaundice is often one of the clinical signs of blockage of biliary tract. For instance, advanced cholangiocarcinoma, cancer of the bile duct, is commonly associated with obstructive jaundice after development of bile duct obstruction. Obstruction of the biliary tract results in elevated levels of primary and conjugated bile acids (CBAs), which have recently been shown to promote cholangiocarcinoma progression.[1-3]

During the last decade, bile acids have been recognized not only as detergents but also as important signaling molecules involved in the regulation of metabolism.[4-6] Recently, we found that CBAs activate the cell proliferation and survival signaling pathways primarily through binding to sphingosine-1-phosphate receptor 2 (S1PR2) in hepatocytes.[5] We further discovered that CBAs activate S1PR2 and upregulate expression of sphingosine kinase 2 in the nucleus of hepatocytes that epigenetically regulate lipid and sterol metabolism in the liver.[6] Thus, indicating that bile acid signaling via S1PR2 and SphK2 plays pivotal roles in the liver. After our results, CBAs were also found to promote the growth and invasion of cholangiocarcinoma cells via activation of S1PR2 *in vitro*.[2] This is possibly due to induction of cyclooxygenase-2 expression.[1]

One of the obstacles that hinder the progress of this field of research is the lack of an appropriate animal model of obstructive jaundice. The most commonly used standard murine model is the total ligation of the common bile duct (tCL) model. There have been mixed reports on the survival of animals in this model, with survival rates ranging from around 10% perioperative mortality[7-10] to up to 60 d.[11] In general, however, this model is considered unstable with short survival. The partial common bile duct ligation model (pCL) was established to improve survival so that the long-term effect of obstructive jaundice can be studied.[12] Another model is the left and middle hepatic duct ligation model (LMHL). This model includes hepatic bile duct ligation distal to the merging point of the left and middle hepatic bile duct and proximal to the union of the right and caudate hepatic bile duct, in addition to gallbladder (GB) removal.[3]

To date, there have been no reports to our knowledge that directly compare these models. Here, we investigated the characteristics of the most commonly used tCL model, and pCL and LMHL models to clarify their utility and identify which one will be the most feasible long-term obstructive jaundice model.

Material and methods

Animals

All animal studies were conducted in the Animal Research Core Facility at Virginia Commonwealth University School of Medicine in accordance with institutional guidelines. Surgical procedures were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee, accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Male C57BL/6J (8-26 wk, weight 20-35 g; the Jackson Laboratory, Bar Harbor, ME) were anesthetized with continuous vaporized 2% isoflurane for general anesthesia. The mice were given analgesia (buprenorphine SR or meloxicam SR, Zoopharm, Windsor, CO) for at least 72 h postoperatively and closely monitored throughout the perioperative period. After the procedures, the mice were kept on a warming pad in their cage warmed up by an infrared lamp until the mice were fully awake and active. Any animals appearing to be in significant distress or showing physical signs indicating unlikely survival for an additional 24 h were euthanized as a humane end point based on our Institutional Animal Care and Use Committee protocol. To achieve stable results, we found that 10 operations in each model were necessary to obtain the appropriate surgical expertise. Male mice were chosen following previous publications of obstructive jaundice models. The numbers of mice to be used for each cohort were chosen based on the previous studies.

RNA isolation and quantitative real-time reverse-transcriptase polymerase chain reaction

Total cellular RNA was isolated using Trizol reagent (QIAGEN, Inc, Valencia, CA) and reverse transcribed into first-strand complementary DNA using the High-Capacity complementary DNA Reverse Transcription Kit from Life Technologies. Messenger RNA levels of collagen 1a1 were determined by real-time reverse-transcriptase polymerase chain reaction using iQTM SYBR Green Supermix reagents and normalized glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal control. Sequences of these primers were:

5′-GGC GGT TCA GGT CCA ATG-3’ (collagen 1a1 forward)
5′-GTT CCA GGCAAT CCA CGA-3’ (collagen 1a1 reverse)
5′ GTC GTG GAT CTG ACG TGC C-3’ (GAPDH forward)
5′-GAT GCC TGC TTC ACC ACC TT-3’ (GAPDH reverse)

Bile acid measurement

Mouse Bile Acids Assay Kit (Crystal Chem, Zaandam, Netherlands) was used for measuring the serum concentration of total bile acids.

Histologic analyses

Immediately after sacrifice, liver samples were removed and fixed in 10% neutral buffered formalin. Hematoxylin-Eosin staining or Masson’s-Trichrome staining were performed by the standard manner.

Statistics

All data were expressed as the mean ± standard error. Data were analyzed for statistical significance with unpaired two-tailed Student’s *t* test. Survival analysis was performed using the Kaplan–Meier method, and differences were assessed using the log-rank test with SPSS software (IBM SPSS statistics 22). *P* values < 0.05 were considered statistically significant in all analyses.