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A novel ameliorated rat model of reversible obstructive jaundice

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Obstructive jaundice is a common clinical symptom generally caused by bile duct stones, inflammatory hyperplasia, and tumors. It is characterized by hyperbilirubinemia and may trigger a variety of complications such as hypotension, kidney injury, endotoxemia, multiple organ dysfunction syndrome, and even death (Pavlidis and Pavlidis, 2018; Liu et al., 2021). Relieving bile duct obstruction and providing adequate drainage have been considered as the most effective therapies for obstructive jaundice. However, it has not yet been established whether it is beneficial to treat affected patients by pre-operative biliary drainage (Blacker et al., 2021). Moreover, the pathophysiological changes or mechanisms associated with the reversal of organ function following the relief of bile-duct obstruction are unclear (Huang et al., 2004). Therefore, it is necessary to establish an experimental model of reversible obstructive jaundice to simulate biliary drainage in clinical practice.

Common bile duct ligation (BDL) has been widely used to induce obstructive jaundice (Frissen et al., 2021). Biliary drainages, both internal and external, have been performed to construct reversible models of obstructive jaundice (Li and Chung, 2001). Although reversible obstructive jaundice models have been explored in mice and rats, these procedures have flaws such as the requirement of a second laparotomy, high mortality, and blockage of the drainage tube, which lead to significant direct effects on the results and limit their application (Wu et al., 2013). Thus, new animal models of reversible obstructive jaundice are worth exploring.

In the present study, we successfully developed a rat model of obstructive jaundice by ameliorated BDL (ABDL) rather than BDL. The detailed surgical procedure is shown in Figs. 1 and 2. Reversible obstructive jaundice could be easily achieved based on this model of ABDL (Fig. 3). There were no death cases in this study. After seven days, the skin color of the auricle was characterized by yellowing in rats who underwent ABDL (Figs. 4a and 4b). Interestingly, the color of the skin returned to a normal red color and the yellow color disappeared seven days after reversal of ABDL (R-ABDL) (Fig. 4c). The changes in auricle skin color indicated that the biliary obstruction was effectively established by means of ABDL, and that the obstruction was successfully relieved by R-ABDL. Then, the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), and albumin (ALB) levels were determined to assess the impacts of bile duct obstruction and biliary reversal on liver function. Compared with the sham operation (SH) group, the serum levels of ALT (Fig. 4d), AST (Fig. 4e), and TBIL (Fig. 4f) were significantly increased after seven days in the BDL and ABDL groups, while the serum ALB level (Fig. 4g) decreased. There was no significant difference in the serum level of ALT, AST,

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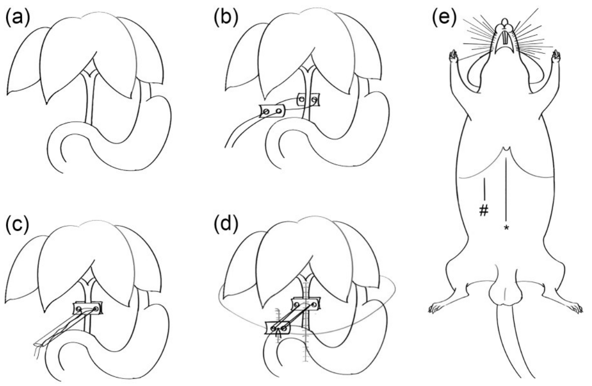

Fig. 1 Diagrammatic representation of the ameliorated bile duct ligation (ABDL) model. (a) Exposure of the bile duct; (b) Placement of spacers with sutures anterior and posterior to the bile duct; (c) Insertion of a silicone tube; (d) Knotting of sutures over the silicone tube to the third spacer embedded in the subcutaneous layer; (e) * and # represent the median and right abdominal incisions, respectively.

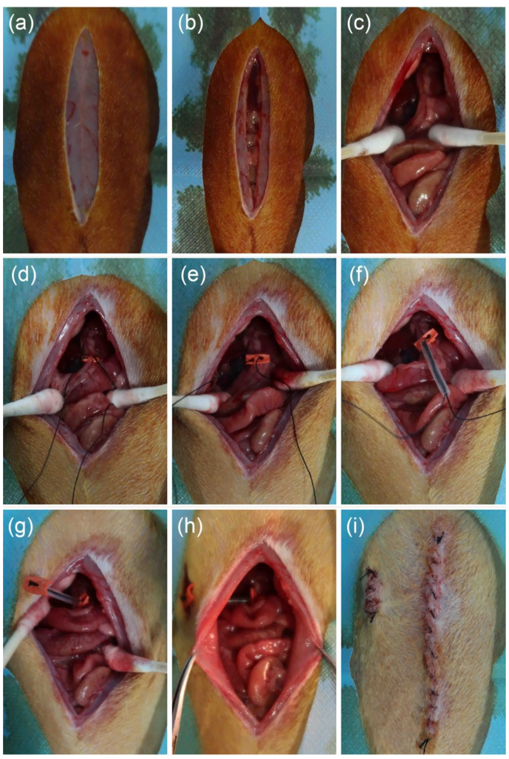

Fig. 2 Representative images of the operational procedure used to cause biliary obstruction by ameliorated bile duct ligation (ABDL). (a) Median abdominal incision; (b) Opening of the abdominal cavity; (c) Exposure of bile duct; (d) Placement of the spacer posterior to the bile duct; (e) Placement of the spacer anterior to the bile duct; (f) Insertion of a silicone tube; (g) Making a knot in the spacer; (h) Embedding the third spacer subcutaneously on the right of the median abdominal incision; (i) Closing the abdomen in layers.

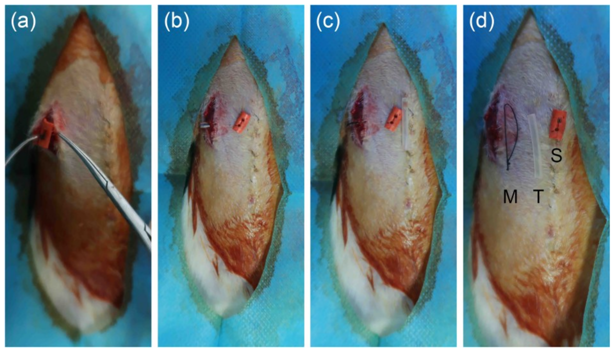

Fig. 3 Representative images of the reversal of ameliorated bile duct ligation (R-ABDL) procedure. (a) Making a small incision and exposure of the spacer; (b) Cutting the thread node; (c) Removal of the silicone tube; (d) Removal of the suture. M, T, and S indicate the Mersilk suture, tube, and spacer, respectively.

TBIL, or ALB between the groups at seven days after BDL and ABDL. However, the serum ALT, AST, and TBIL levels were significantly decreased, and the serum ALB level was significantly increased in rats seven days after they underwent R-ABDL as compared with the values at seven days following ABDL. Moreover, the serum level of ALT, AST, TBIL, or ALB showed no significant difference between the group that underwent R-ABDL after seven days and the SH group. These results indicated that ABDL is an effective and reliable means to induce biliary obstruction similar to BDL, and that the reversal of biliary obstruction after ABDL can be easily achieved. Furthermore, the deranged liver function occurring seven days after biliary obstruction could return to normal seven days after the relief of biliary obstruction.

Hematoxylin and eosin (H&E) staining, Masson's trichrome staining, and Sirius red staining of the tissues were performed to study the histopathological changes and to quantify the extent of liver fibrosis caused by biliary obstruction and reversal. The livers in the SH group displayed normal histological structure and only a small amount of collagen was observed in the portal area (Figs. 5a and 5b). However, as compared with the SH group, seven days after BDL and ABDL, the portal area appeared expanded due to pseudo bile-duct proliferation and infiltration by inflammatory cells. There was no obvious hepatic necrosis in either the BDL or ABDL group seven days after the procedure. Interestingly, cholestasis was markedly alleviated seven days after R-ABDL as compared with the BDL and ABDL groups at seven days; however, it did not

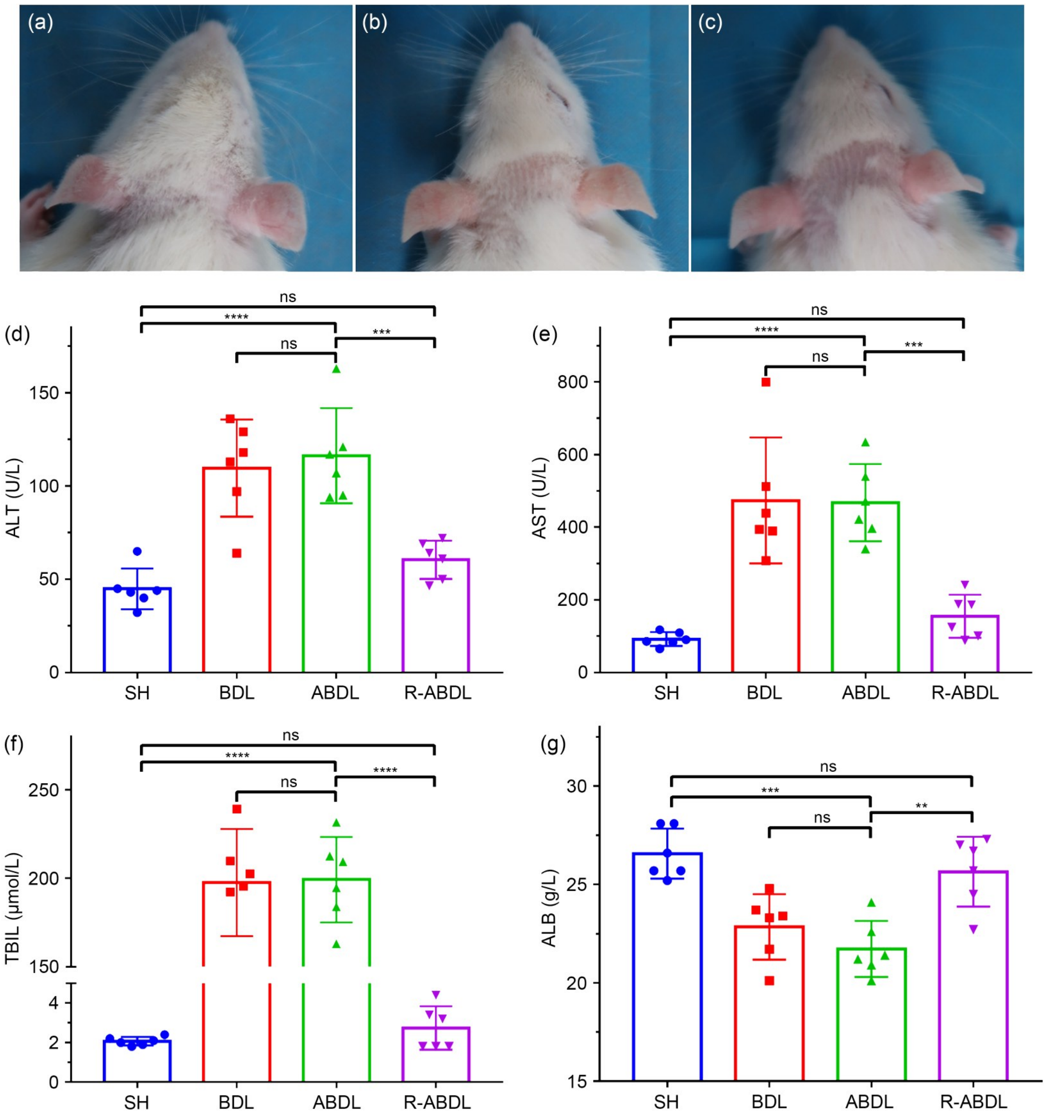

Fig. 4 Skin color of the auricle of rats after various procedures and the levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), and albumin (ALB) in the various groups seven days after the procedure. Skin color of the auricle in the sham operation (SH) group (a), after seven days in the ameliorated bile duct ligation (ABDL) group (b), and after seven days in the reversal of ABDL (R-ABDL) group (c) is shown. The rat in the ABDL (b) and R-ABDL (c) groups represents the same rat that underwent ABDL and then R-ABDL. Serum levels of ALT (d), AST (e), TBIL (f), and ALB (g) were shown. All values are presented as mean±standard deviation (SD), *n*=6. ** *P*<0.01, *** *P*<0.001, **** *P*<0.0001; ns: not significant.

completely return to normal. Both Masson's trichrome staining and Sirius red staining (Figs. 5b and 5c) showed an abundant deposition of collagen seven days after BDL and ABDL, and this was significantly higher than that in the SH group. Moreover, a significant reduction in the area of collagen deposition was observed seven days after R-ABDL, but this was still higher than the collagen deposition area in the SH group. The area of fibrotic lesions and the percentage of collagen in the BDL and ABDL groups at seven days were significantly higher than those in the SH group. Importantly, a significant reduction in the areas of fibrotic lesions and collagen deposition was observed seven days after R-ABDL. The above results indicated that obstructive jaundice could be effectively induced by ABDL in a similar manner to BDL, and it could be reversed by R-ABDL. Liver fibrosis and the accumulation of collagen at seven days after obstruction were both significantly decreased, but did completely diminish seven days after R-ABDL. Cytokeratin

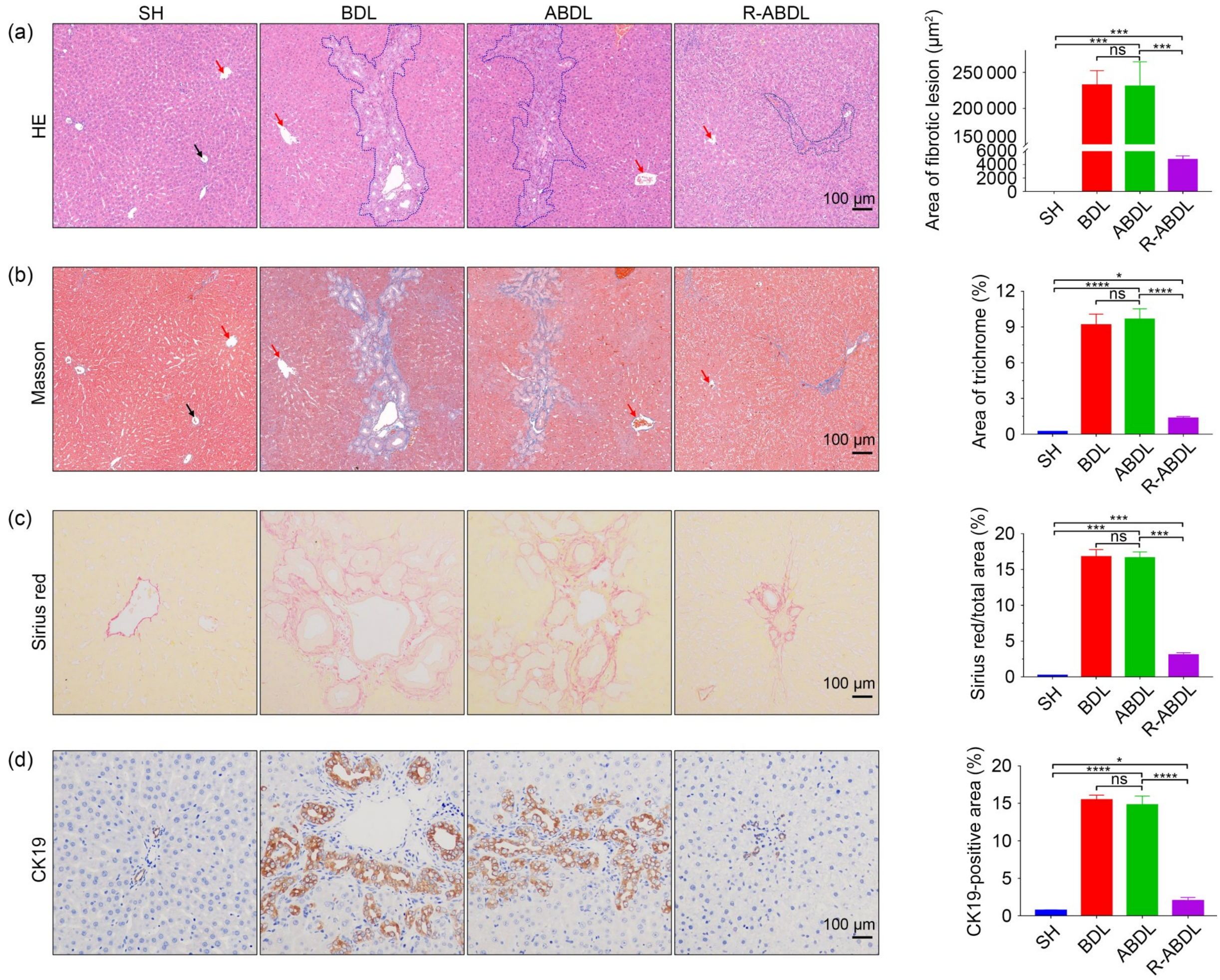

Fig. 5 Hematoxylin and eosin (H&E) staining (a), Masson's trichrome staining (b), Sirius red staining (c), and cytokeratin 19 (CK19) immunostaining (d) in the various groups. The red and black arrows represent central vein and the portal area, respectively. The blue dashed lines represent the approximate borders for fibrotic tissue. All values are presented as mean±standard deviation (SD), *n*=5. * *P*<0.05, * *P*<0.001, **** *P*<0.0001; ns: not significant. SH: sham operation; BDL: bile duct ligation; ABDL: ameliorated BDL; R-ABDL: reversal of ABDL.**

19 (CK19) protein was considered as a biliary marker and associated with the progression of liver fibrosis. Immunohistochemical staining was further performed to investigate the intrahepatic biliary mass. As shown in Fig. 5d, CK19 was mainly expressed in bile duct endothelial cells for the SH group. In comparison, high expression of CK19 was observed in the BDL and ABDL groups, indicating an abundance of reactive bile ductules in the portal regions. Furthermore, there was no difference for the CK19-positive area between the BDL and ABDL groups. The positive area of CK19 was significantly lower at seven days in the R-ABDL group compared with those in the BDL and ABDL groups, but slightly higher than that in the SH group, indicating that liver fibrosis was significantly alleviated after biliary reversal.

Previous research demonstrated that cellular senescence plays a vital role in BDL-hepatic fibrosis. The senescence-related proteins p16 and p21 were analyzed by western blot (Fig. 6). The protein expression of p16 and p21 was significantly higher in the BDL and ABDL groups than in the SH group. Moreover, compared with the former two groups, a significant reduction of p16 and p21 was observed in the R-ABDL group, indicating that the extent of liver fibrosis was markedly alleviated after R-ABDL.

The survival rate of rats was further analyzed to investigate whether biliary reversal could prevent death caused by obstructive jaundice. The various groups were further evaluated in this regard. The BDL group had no intervention performed, whereas the reversal of biliary obstruction was performed in the ABDL

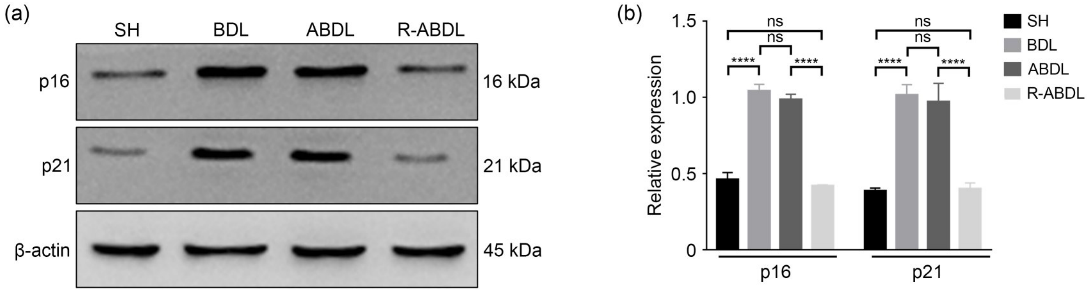

Fig. 6 Expression of senescence markers reduced by R-ABDL in rats with biliary obstruction. (a) The expression of p16 and p21 proteins was measured by western blot in each group; (b) The semi-quantitative data were shown. The protein expression was normalized to the β-actin. All data were presented as mean±standard deviation (SD), *n*=3. **** *P*<0.0001; ns: not significant. SH: sham operation; BDL: bile duct ligation; ABDL: ameliorated BDL; R-ABDL: reversal of ABDL.

group at seven days. As shown in Fig. S1, the survival of rats in the BDL group was less than 50 d. Five rats in the R-ABDL group survived longer than 100 d without any complications, whereas one rat in this group died after three days due to biliary fistula. Thus, rats treated by R-ABDL had significantly longer survival than rats who underwent BDL alone (*P*<0.001), demonstrating that the reversal of biliary obstruction was successfully achieved after R-ABDL.

The present study established a novel rat model of reversible obstructive jaundice that could be easily performed, avoid a second laparotomy, and result in a low mortality rate. As is well known, a second laparotomy makes the reversal of bile duct obstruction difficult in the rat because of tissue adhesion and hemorrhage. Only one rat died after bile duct reversal using our method, which is an accepted threshold value compared with previous approaches. Moreover, the direct injury to the bile duct caused by suture ligation was effectively avoided in our model.

The effectiveness of our experimental model was verified by evaluating the hepatic function and histopathology of the liver. The serum ALT, AST, and TBIL levels measured at seven days after obstructive jaundice returned to normal seven days after relieving the obstruction. Although the liver fibrosis and proliferation of the biliary ductules after seven days of obstruction relief were significantly alleviated, they did not return to complete normalcy, which is consistent with previous results (Aronson et al., 1993; Hiratani et al., 2019). Therefore, the recovery of liver function precedes histological changes. To summarize, an ameliorated rat model of reversible obstructive jaundice was successfully achieved in our study.

Various methods have been attempted to establish the reversal of bile duct obstruction (Du et al., 2016). For example, external drainage was used to establish reversible obstructive jaundice; however, tube-related complications such as blockage, cannulation failure, and tube dislodgement have restricted its application. In addition, the risk of biliary infection using this technique is increased due to exposure of the biliary cannula.

The internal drainage approach has also been used to establish reversible obstructive jaundice. This was considered superior to external drainage due to its physiological similarity (Ji et al., 2017). Internal drainage can be accomplished by anastomotic and non-anastomotic methods. The first anastomotic procedure, the Roux-en-Y choledochojejunostomy that paralleled the clinical situation to some extent, was successfully established and widely used for relieving biliary obstruction (Lyu et al., 2021). Although the death rate was reduced with the improvement of operation technology, its requirements of skilled technique and lengthy surgery time have limited its application. The second anastomotic procedure to establish reversible obstructive jaundice was choledochocholedochostomy. This procedure had the disadvantage of requiring a second laparotomy, and the duodenal side of the bile duct was difficult to be exposed during the second operation (Huang et al., 2017). The third anastomotic procedure, as a classic technique to achieve reversible obstructive jaundice, was choledochoduodenostomy. In this technique, the reversal of obstruction after BDL was obtained by inserting and connecting the duodenum and the dilated bile duct with a short polyvinylchloride tube. This model mimicked choledochoduodenostomy and endoscopic biliary stent insertion performed in humans. However, this model had a high mortality and poor stability due to the high incidence of tube-related complications. In addition, it was not applicable in the long term due to frequent blockage of the tube caused by a solid diet. Overall, traditional anastomotic methods to establish reversible obstructive jaundice had several drawbacks that restricted their wide application.

Hence, to reduce mortality, methods other than those using anastomotic techniques were explored to establish a model of reversible obstructive jaundice. For example, a titanium clip was employed to clip the bile duct and induce obstructive jaundice, and the obstruction was relieved by the removal of the titanium clip without a second laparotomy in mice and rats. The simplicity, feasibility, and low mortality of this approach could be used to establish an animal model of reversible obstructive jaundice. However, this procedure was limited by the difficulty in removing the titanium clip, and the bile duct was often damaged due to the traction exerted during removal (Hiratani et al., 2019). Oruç et al. (2009) tied a knot over the plastic venous cannula, which was inserted over the bile duct to produce obstructive jaundice. Cutting this knot caused the relief of obstructive jaundice. This procedure avoided direct injury caused by suture ligation, and the technique was easy to perform. However, this procedure required a second laparotomy and hence limited its application. Kahramansoy et al. (2012) used rapidly absorbable sutures to ligate the bile duct to establish reversible obstructive jaundice. The biliary obstruction was reversed when these materials were degraded. This procedure required no second laparotomy either, and was easy to perform. The main disadvantage of this method was that tight ligation by absorbable sutures could cause direct injury to the bile duct. Moreover, the method was inconsistent due to individual differences in the absorption of absorbable sutures (Huang et al., 2017). Although all these methods could establish a model of reversible obstructive jaundice (Huang et al., 2017), they have not gained popularity due to the related technical difficulty and lack of consistency. Hence, anastomotic methods remain more common to establish reversible obstructive jaundice models.

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

Acknowledgments

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Author contributions

Yongkang ZOU designed the research, established the animal models, analyzed the date, and wrote the original the manuscript. Pengpeng YUE performed the experiment and analyzed the experimental data. Hankun CAO and Liqin WU analyzed the experimental data. Li XU was responsible for collecting the relevant literature. Zhongzhong LIU guided the experiment. Shuangquan WU guided the experiment and revised this manuscript. Qifa YE contributed to funding acquisition and project administration, designed the study, and revised this manuscript. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Yongkang ZOU, Pengpeng YUE, Hankun CAO, Liqin WU, Li XU, Zhongzhong LIU, Shuangquan WU, and Qifa YE declare that they have no conlfict of interest.

All animal procedures were performed in accordance with the Experimental Animal Management Ordinance of Wuhan University. This research was approved by the Institutional Animal Care and Use Committee of Wuhan University Center for Animal Experiments (No. WP20210013).

References

Aronson DC, Chamuleau RAFM, Frederiks WM, et al., 1993. Reversibility of cholestatic changes following experimental common bile duct obstruction: fact or fantasy? *J Hepatol*, 18(1):85-95.
https://doi.org/10.1016/s0168-8278(05)80014-5

Blacker S, Lahiri RP, Phillips M, et al., 2021. Which patients benefit from preoperative biliary drainage in resectable pancreatic cancer? *Expert Rev Gastroenterol Hepatol*, 15(8):855-863.
https://doi.org/10.1080/17474124.2021.1915127

Du YH, Chen H, Xuan ZF, et al., 2016. Bile deficiency induces changes in intestinal glucose absorption in mice. *Surgery*, 160(6):1496-1507.
https://doi.org/10.1016/j.surg.2016.05.040

Frissen M, Liao LJ, Schneider KM, et al., 2021. Bidirectional role of NLRP3 during acute and chronic cholestatic liver injury. *Hepatology*, 73(5):1836-1854.
https://doi.org/10. 1002/hep.31494

Hiratani S, Mori R, Ota Y, et al., 2019. A simple and easily reproducible model of reversible obstructive jaundice in rats. *In Vivo*, 33(3):699-706.
https://doi.org/10.21873/invivo.11528

Huang LT, Hsieh CS, Chou MH, et al., 2004. Obstructive jaundice in rats: cause of spatial memory deficits with

recovery after biliary decompression. *World J Surg*, 28(3): 283-287.
https://doi.org/10.1007/s00268-003-7209-z

Huang X, Li CH, Zhang AQ, et al., 2017. A simple rat model of *in situ* reversible obstructive jaundice *in situ* reversible obstructive jaundice model. *Ann Surg Treat Res*, 92(6):389-395.
https://doi.org/10.4174/astr.2017.92.6.389

Ji CG, Xie XL, Yin J, et al., 2017. Bile acid receptor TGR5 overexpression is associated with decreased intestinal mucosal injury and epithelial cell proliferation in obstructive jaundice. *Transl Res*, 182:88-102.
https://doi.org/10.1016/j.trsl.2016.12.001

Kahramansoy N, Erkol H, Yilmaz EE, et al., 2012. A new model of reversible obstructive jaundice using rapidly absorbable suture materials. *Clin Invest Med*, 35(6):E351-E357.
https://doi.org/10.25011/cim.v35i6.19206

Li W, Chung SCS, 2001. An improved rat model of obstructive jaundice and its reversal by internal and external drainage. *J Surg Res*, 101(1):4-15.
https://doi.org/10.1006/jsre.2001.6240

Liu JY, Qu JL, Chen HY, et al., 2021. The pathogenesis of renal injury in obstructive jaundice: a review of underlying mechanisms, inducible agents and therapeutic strategies. *Pharmacol Res*, 163:105311.
https://doi.org/10.1016/j.phrs.2020.105311

Lyu SC, Wang J, Xu WL, et al., 2021. Therapeutic effect of combining anisodamine with neostigmine on local scar formation following Roux-en-Y choledochojejunostomy in a novel rat model. *Front Pharmacol*, 12:700050.
https://doi.org/10.3389/fphar.2021.700050

Oruç MT, Özmen MM, Han U, 2009. A new technique for inducing and releasing obstructive jaundice in rats. *Eur Surg Res*, 43(4):354-359.
https://doi.org/10.1159/000247061

Pavlidis ET, Pavlidis TE, 2018. Pathophysiological consequences of obstructive jaundice and perioperative management. *Hepatobiliary Pancreat Dis Int*, 17(1):17-21.
https://doi.org/10.1016/j.hbpd.2018.01.008

Wu LL, Li W, Wang ZK, et al., 2013. Bile acid-induced expression of farnesoid X receptor as the basis for superiority of internal biliary drainage in experimental biliary obstruction. *Scand J Gastroenterol*, 48(4):496-503.
https://doi.org/10.3109/00365521.2012.763173

Supplementary information

Fig. S1; Materials and methods