Original Article

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Reversible biliary occlusion in a small animal model: first description of a new technique

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Abstract

Background: Experimental models with reversible biliary occlusion resulted in a high mortality of the animals, up to 20–60% according to the literature. Our aim was to assess a safe and valid technique for reversible biliary occlusion with a low mortality.

Methods: We randomized 30 rats into two groups: with bile duct occlusion (BDO, n = 18) and with sham manipulation of the extrahepatic bile duct (control, n = 12). We used a removable vascular clip for temporary occlusion of the extrahepatic bile duct. The clip was removed on post-operative day (POD) 2. On POD 2, 3, and 5, we measured the hepatocellular injury and metabolic function markers in serum. Activation of mononuclear cells (HIS36) and expression of regeneration markers [cytokeratin 19, hepatic growth factor (HGF)-α, and HGF-β] were determined by immunohistochemistry.

Results: The survival rate was 96.67% (1/30); one animal died. The mortality in the BDO group was 6% (1/18) and that in the control group was 0% (0/12). BDO resulted in a sharp increase of hepatocellular injury and cholestatic parameters on POD 2 with a rapid decline till POD 3. Significantly strongest activation of Kupffer cells and expression of proliferation markers were found until POD 5 after BDO.

Conclusion: The clip technique is a safe, cheap, and valid method for reversible biliary occlusion with an extremely low mortality.

Keywords: CK19; experimental surgery; temporary obstructive cholestasis.

Abbreviations: BDL, bile duct ligation; BDO, bile duct occlusion; BW, body weight; CK19, cytokeratin 19; HGF-α, hepatic growth factor α; HGF-β, hepatic growth factor β; HPF, high-power field; OSKST, one-sample Kolmogorov-Smirnov test; PBS, phosphate-buffered saline; POD, post-operative day.

Introduction

To study the mammalian pathophysiology of obstructive cholestasis, the most favorite model is bile duct ligation (BDL) mimicking a biliary obstruction of varying extent [1–8]. The liver parenchyma develops a histological conversion into a fibrotic or a cirrhotic pattern depending on the duration of biliary obstruction.

Primarily, the focus has been paid to histological alterations like the increase in relative size of the biliary compartment including proliferation of biliary epithelial cells (using CK19) and of interportal fibrous tissue [6, 7]. In these studies, BDL lasted up to 4 weeks [1–6, 8]. Later on, metabolic parameters such as gluconeogenesis, glycogen content, and lipid oxidation were also investigated [1–7, 9]. Several studies have shown that some of these structural and metabolic changes were reversible after relief of biliary obstruction [1–6]. BDL was performed by ligating the common bile duct with several sutures. The relief of the biliary occlusion was facilitated by Roux-en-Y choledochojunostomy, as it is known as a standard procedure for a biliodigestive anastomosis in humans. Because of the impact of the second operation on the reduced hepatocellular regenerative capacity due to cholestasis, the mortality of the animals was mostly as high as 17–40% [2–4, 10]. To avoid mortality, we assessed a new technique for temporary bile duct occlusion (BDO) with inherently less morbidity and mortality.
Materials and methods

Animals

All surgical procedures were performed in inbred male Sprague-Dawley rats (Charles River, Germany) aged 9–10 weeks [body weight (BW) 250–280 g].

Institutional animal care and use committee statement: Rats were fed a standard laboratory diet with water and rat chow ad libitum until harvest. All animals were kept under constant environmental conditions with a 12-h light-dark cycle in a conventional animal facility using environmentally enriched type IV cages in groups of two to three rats. All procedures and housing of the animals were carried out according to the German Animal Welfare Legislation and approved by the local authorities (Hessisches Landesamt für Verbraucherschutz, reg.-nr. 02-025/08). Analgesic treatment was started immediately after wound closure in all animals. Buprenorphine (0.05 mg/kg BW; Temgesic®) was subcutaneously injected; the analgesic therapy was given twice a day during the first 3 days postoperatively. For postoperative monitoring, the rats were weighed daily.

Study design

The 30 animals were randomized into two groups: laparotomy with BDO (n=18) and sham manipulation of the extrahepatic bile duct without occlusion (control, n=12). On postoperative day (POD) 2, the clip was removed in all BDO rats. At three different time points (POD 2, 3, and 5), the animals (BDO with n = 6 per time point, control with n = 4 per time point) were sacrificed and samples (serum, liver tissue) were collected.

Surgical procedures

All groups were anaesthetized with an intramuscular injection of ketamine (10 mg/100 g BW) and xylazine (1 mg/100 g BW). The animals were shaved and placed on a small animal operating table. As the operation time for placing and removing the clip was always approximately 10 min, we avoided using a warming plate.

BDO group – laparotomy with BDO: After midline laparotomy, the common bile duct was exposed and occluded 0.5–1 cm above the pancreas using a removable vascular clip. The wounds were closed by running sutures. After 2 days (POD 2), a re-laparotomy was performed and the clip was removed. The wounds were closed.

The vascular titan clip was placed and removed using a special clip-forceps (Aesculap AG, Germany; vascular bulldog clip straight 25 mm). The used forceps ensured correct placing of the clip and simultaneously avoided overtension of the gentle clip and damage of the common bile duct or the surrounding organs. We selected a vascular clip with a low but sufficient closing force of 2.4 N withatraumatic toothing “DeBakey,” as a higher closing force could lead to residual obstruction resulting in remaining dilatation of the extrahepatic bile duct.

Control group – laparotomy with sham manipulation of the bile duct: These animals underwent anesthesia and laparotomy, as described, and exposure of the common bile duct without occlusion (“sham procedure”). The control animals received a second laparotomy at the time point of sacrifice.

Characterization of the animals

Food intake and BW gain were measured daily. No pair-feeding was performed. At the indicated time points of death, liver and spleen weight were measured.

Tissue preparation

At the dedicated time points and under anesthesia, a laparotomy was performed and a blood sample was collected into sterile tubes. The blood was allowed to clot. During all procedures, the samples were kept at 4°C. Immediately after sacrifice of the animals, liver samples were placed in Shandon™ Cryomatrix™ embedding medium (Thermo Fisher Scientific, UK) and quickly cooled in liquid nitrogen for subsequent cryosectioning for immunohistochemistry. For detection of the systemic parameters, blood samples were taken from the retroorbital venous plexus preoperatively and at days 2, 3, 4, and 5. The blood was allowed to clot. During all procedures, the samples were kept at 4°C. All specimens were kept at −80°C until subsequent analyses were performed. Rats do not have a gallbladder.

Laboratory measurements

Measurements in serum: The activities of alkaline phosphatase (U/L), aspartate aminotransferase (U/L), alanine aminotransferase (U/L), γ-glutamyl-transferase (U/L), and the contents of albumin (g/dL), bilirubin (mg/dL), glucose (mmol/L), cholesterol (mg/dL), triglycerides (mg/dL), and whole protein (g/dL) were measured in serum using an automated chemical analyzer (Bayer Advia 1650, Germany).

Staining and immunohistochemistry

For histological evaluation, we used a standardized sampling of three pieces of every liver. Acetone-fixed, 5-μm frozen liver sections were stained with hematoxylin and eosin in order to evaluate alterations of the liver parenchyma and bile duct hyperplasia.

Immunohistological detection of activated Kupffer cells was done by using monoclonal anti-rat-macrophage antibody [HIS36 mouse anti-rat (22231D); Pharmingen Biotechnology, Germany] diluted 1:700 in phosphate-buffered saline (PBS; pH 7.4–7.5, without Ca2+ and Mg2+), applied to the frozen liver sections and incubated at 37°C for 60 min. Staining procedures were performed as already published by our group [11].

Immunohistological detection of biliary neoangiogenesis [cytokeratin 19 (CK19)] was performed by using monoclonal
mouse-anti-rat ready-to-use antibody [CK19, mouse anti-rat (22231D); Pharmingen Biotechnology, Germany] with PBS (pH 7.4–7.5, without 
Ca²⁺ and Mg²⁺) applied to the frozen liver sections and incubated at 
37°C for 60 min. The staining procedure was performed with a com­ 
mercial set provided by Progen Biotechnik GmbH [mouse IgG2b, 
m monoclonal antibody CK19 (Ks 19.2)]. Incubation times were set and 
washing procedures were performed as described in the manufac­
turer’s manual.

The immunohistological detection of hepatic growth factors 
(HGF-α and -β) followed the above-described procedure with dilution 
of the primary antibodies at 1:50 in PBS [anti-HGF-α and anti-HGF-β 
(N9) sc-1356], application to the frozen liver sections, and incubation 
at 37°C for 60 min. The next steps were performed with a commer­ 
cial set of secondary and tertiary antibodies provided by Santa Cruz 
Pharmaceuticals (Goat ABC Staining System Kit; Santa Cruz Biotech­
nology, sc-2023). Incubation times were set and washing procedures 
were performed as described in the manufacturer’s manual.

Negative and positive controls were made using five slides for 
each staining (primary antibodies) and with additional control slides 
from random organs.

Quantification of immunohistochemistry

Liver cryostat sections were analyzed microscopically using a mor­
phometric analysis system (CBA 8000-Manager; Leica, Germany). We 
manually counted CK19-positive cells in 10 high-power fields (HPFs, 
20× magnification) and calculated the ratio of CK19-positive cells per 
total cholangiocytes. The expression was graduated into four grades 
(Table 1). Kupffer cells, CK19, and HGF-α+β were evaluated according 
to this standardized protocol [11].

Statistics

Data are presented as mean ± standard deviation (SD). We used the 
parametric descriptors due to the type of data distribution and per­
formed statistical tests in coordination with a statistician.

The data were analyzed for the type of distribution by using the 
one-sample Kolmogorov-Smirnov test (OSKST). Normal distribution 
was accepted if p > 0.05 (two-tailed significance, corrected for ties). For 
all parameters, the OSKST showed no normal distribution. Therefore, 
statistical evaluation for significance was performed using the 
Kruskal-Wallis test. Significance was accepted in all cases at p < 0.05 
two-tailed significance, corrected for ties. All statistical pro­
cedures were performed using SPSS® 22.0 for Windows (SPSS Science

| Grade | Description |
|-------|-------------|
| 0     | No positively stained cells are visible |
| 1     | 1% > x ≤ 25% positively stained cells |
| 2     | 25% > x ≤ 50% positively stained cells |
| 3     | 50% > x ≤ 75% positively stained cells |
| 4     | 75% > x ≤ 100% positively stained cells |

Results

Safe and easy administration of the vascular clip for temporary biliary occlusion

In all BDO animals, the clip was easy and safe to place at the extrahepatic bile duct above the pancreas. As we placed the clip under the control of a microscope and 
by using the clip-forceps, we avoided any impairment of the branches of the hepatic artery, portal vein, and pan­
creas. Furthermore, the microscope and forceps assured the 
placement of the clip below the bile duct branches of 
the lower liver lobes (right lobes and caudate lobes), pre­venting a non-intended incomplete BDO (Figure 1A–D). In all BDO animals, we found a macroscopically visible 
dilatation of the extrahepatic bile duct above the clip 
(Figure 1A–D) until POD 2. We have not seen any dis­
location of the clip or damage of the neighboring liver lobes. On POD 2, the clip was easy to remove using the 
clip-forceps without any additional surgical manipula­
tion (e.g. adhesiolysis, thermal or mechanical coagulation) besides the re-laparotomy. Interestingly, shortly after 
(≤5 min) the retrieval of the clip, the massive dilatation of the main bile duct was reduced to a slightly dilated 
common bile duct. No persistent dilatation of extrahepatic bile duct has been noted on POD 3 and POD 5, respectively. In all BDO animals, we found a slightly green staining of the 
liver indicating intrahepatic cholestasis after 48 h of 
BDO (POD 2). Until 72 h (POD 5) after the removal of the 
clip, the tissue staining disappeared in all BDO animals. Few drops of clear ascites were found only in two BDO 
animals (2/18): in n = 1 on POD 3 and n = 1 on POD 5.

The control animals showed neither ascites nor a 
modified coloring of the liver.

Situs pictures

Survival and recovery of the animals

The survival rate of all animals was 96.67%; only one 
animal (1/30, 3.33%) died. Death occurred in a BDO animal on 
POD 3 (24 h after clip removal). The mortality rate in the 
BDO group was 6% (1/18) and that in the control group was 
0% (0/12). Autopsy could exclude surgical complications 
like bleeding, ischemia, organ damages, or peritonitis.
Therefore, it seems that an anesthesia-related problem led to the death of the animal. All surviving animals showed an uncomplicated recovery during the observation period. No significant differences between the groups were found regarding BW, food intake, and liver and spleen weights (data not shown).

**Serum parameters**

BDO resulted in a significantly strong increase in all systemic parameters compared to control on POD 2 (Table 2). With restored bile flow, nearly all parameters declined to normal values on POD 3 (Table 3). Only the activities of aspartate aminotransferase and alkaline phosphatase remained significantly elevated until POD 5 (Table 2). Glucose was significantly depressed after BDO until POD 3 and returned to normal levels until POD 5 (Table 3). Cholesterol was increased on POD 2 after BDO, followed by a rapid decline to normal values (Table 3). Whole protein content and triglycerides were not significantly altered after BDO or in control.

**Immunohistochemistry**

We found a three-fold increase of the expression of HGF-α and HGF-β after BDO till POD 5 in comparison to control (Table 4). HGF-α was predominantly expressed in the area between the central veins and the portal fields, whereas HGF-β was expressed in the periportal areas and near the central veins (Figures 2A–D and 3A–D).

Following BDO, CK19 showed a constant and nearly two-fold increase till POD 5 (Table 4). A strong expression was localized around the portal fields and a weaker expression toward the central veins (Figure 4A–D).

Activation of Kupffer cells (HIS36) showed a significant increase on POD 3 after BDO, followed by a stepwise decrease till POD 5 in comparison to control. The strongest increase in the numbers of resident macrophages was
found at the portal fields, whereas a weaker activation was found toward the central veins (Figure 5A–D).

**Discussion**

Within the last decades, numerous studies explored the impact of temporary biliary occlusion on liver function and liver histology [1–3, 5, 6]. Only few authors reported about their survival data; the mortality of the animals was mostly as high as 17–40% [2–4, 10]. Most of them described the highest mortality within 1–5 days after the biliary drainage operation [3, 5]. The authors identified the combination of additional surgical trauma, the pro-inflammatory response, and the increased hepatocellular metabolic and proliferative demand due to the second operation with a reduced hepatic regenerative capacity due to the biliary occlusion to be the critical factors for the high mortality in this experimental setting [2–5].

To avoid these unfavorable results, we searched for an alternative technique for temporary BDO with inherently less morbidity and mortality. We used a titan vascular clip to occlude the common bile duct (Figure 1A–D). The clip was easy to place and to remove without any additional surgical trauma within the second operation. In our study, only 1 of the 30 animals died (survival: 96.67% with 29/30; mortality: BDO 6% with 1/18 vs. control 0% with 0/12). Autopsy could exclude surgical complications. The application of the clip devise was always done under direct control of a microscope, assuring the ascertained arterial and portal-venous blood supply. Therefore, the new technique for temporary BDO using a vascular clip is a safe, fast, and inexpensive method without significant mortality. Similar observations concerning safety and usage of a

### Table 2: Hepatocellular injury and cholestasis parameters.

|                | Control (n=12) | BDO (n=18) | p-Value (BDO vs. control) |
|----------------|---------------|------------|---------------------------|
| **Preoperative** |               |            |                           |
| Aspartate aminotransferase (U/L) | 72 ± 15.4 | 64 ± 40.2 | *                     |
| Alanine aminotransferase (U/L) | 35 ± 3.7 | 35 ± 14.6 | *                     |
| Alkaline phosphatase (U/L) | 278 ± 70.5 | 238 ± 10.4 | *                     |
| Bilirubin (mg/dL) | 0.1 ± 0.1 | 0.15 ± 0.05 | *                     |
| γ-Glutamyl-transferase (U/L) | 3 ± 0.4 | 3 ± 0.6 | *                     |
| **POD 2** |               |            |                           |
| Aspartate aminotransferase (U/L) | 48 ± 28.1 | 544 ± 262 | 0.001                   |
| Alanine aminotransferase (U/L) | 41 ± 5 | 478 ± 235 | 0.001                   |
| Alkaline phosphatase (U/L) | 227 ± 34 | 579 ± 244 | 0.002                   |
| Bilirubin (mg/dL) | 0.05 ± 0.04 | 0.75 ± 0.1 | 0.001                   |
| γ-Glutamyl-transferase (U/L) | 3 ± 0.6 | 9 ± 3 | 0.002                   |
| **POD 3** |               |            |                           |
| Aspartate aminotransferase (U/L) | 90 ± 2 | 154 ± 84 | 0.003                   |
| Alanine aminotransferase (U/L) | 51 ± 5 | 91 ± 21 | 0.056                   |
| Alkaline phosphatase (U/L) | 191 ± 21 | 298 ± 44 | *                     |
| Bilirubin (mg/dL) | n.d. | 0.3 ± 0.2 | n.d.                   |
| γ-Glutamyl-transferase (U/L) | 2.5 ± 0.5 | 2.9 ± 1.1 | *                     |
| **POD 4** |               |            |                           |
| Aspartate aminotransferase (U/L) | 55 ± 1 | 57 ± 3 | *                     |
| Alanine aminotransferase (U/L) | 39 ± 0 | 41 ± 8 | *                     |
| Alkaline phosphatase (U/L) | n.d. | 260 ± 4 | n.d.                   |
| Bilirubin (mg/dL) | 0.1 ± 0.01 | 0.2 ± 0.01 | *                     |
| γ-Glutamyl-transferase (U/L) | 2.5 ± 0.5 | 2.8 ± 1.3 | *                     |
| **POD 5** |               |            |                           |
| Aspartate aminotransferase (U/L) | 34 ± 5 | 55 ± 8 | 0.004                   |
| Alanine aminotransferase (U/L) | 29 ± 4 | 33 ± 5 | *                     |
| Alkaline phosphatase (U/L) | 204 ± 2 | 282 ± 67 | *                     |
| Bilirubin (mg/dL) | 0.1 ± 0.01 | 0.2 ± 0.01 | *                     |
| γ-Glutamyl-transferase (U/L) | 2.5 ± 0.5 | 3.3 ± 0.5 | *                     |

*p > 0.05 vs. control; n.d., not determined. The rats studied had either BDO for 2 days or a sham laparotomy (control). On POD 2, the bile flow was restored by removal of the clip devise. Data are given as mean ± SD.
Table 3: Liver metabolic parameters.

|                  | Control (n=12) | BDO (n=18) | p-Value (BDO vs. control) |
|------------------|----------------|------------|---------------------------|
| **Preoperative** |                |            |                           |
| Proteins (g/dL)  | 5.8 ± 0.3      | 5.8 ± 0.3  |                          |
| Albinin (g/dL)   | 4.1 ± 0.01     | 3.9 ± 0.2  |                          |
| Glucose (mmol/L) | 7.3 ± 5.4      | 7.7 ± 5.7  |                          |
| Triglycerides (mg/dL) | 121.4 ± 9.3 | 120 ± 5.1  |                          |
| Cholesterol (mg/dL) | 79.2 ± 19.8  | 79.8 ± 27.8|                          |
| **POD 2**        |                |            |                           |
| Proteins (g/dL)  | 5.6 ± 0.4      | 5.3 ± 0.3  |                          |
| Albinin (g/dL)   | 2.7 ± 0.4      | 2.5 ± 0.4  |                          |
| Glucose (mmol/L) | 10.9 ± 5.2     | 9.1 ± 2.6  | 0.05                      |
| Triglycerides (mg/dL) | 117 ± 9.3     | 113 ± 9.7  |                          |
| Cholesterol (mg/dL) | 75.2 ± 18.2  | 148.5 ± 68.9| 0.001                    |
| **POD 3**        |                |            |                           |
| Proteins (g/dL)  | 5.1 ± 0.3      | 4.5 ± 0.2  |                          |
| Albinin (g/dL)   | 2.5 ± 0.3      | 2.0 ± 0.1  |                          |
| Glucose (mmol/L) | 18.4 ± 0.6     | 8.7 ± 1.2  | 0.006                     |
| Triglycerides (mg/dL) | 119 ± 6.5     | 124 ± 9.7  |                          |
| Cholesterol (mg/dL) | 85.0 ± 32     | 86.6 ± 17  |                          |
| **POD 4**        |                |            |                           |
| Proteins (g/dL)  | 5.3 ± 0.4      | 5.6 ± 0.1  |                          |
| Albinin (g/dL)   | 2.8 ± 0.5      | 2.3 ± 0.01 |                          |
| Glucose (mmol/L) | 10.8 ± 0.1     | 12.6 ± 0.8 | 0.035                     |
| Triglycerides (mg/dL) | 119.9 ± 10.1  | 117 ± 7.2  | 0.01                      |
| Cholesterol (mg/dL) | 71.0 ± 12.73  | 84.0 ± 7   |                          |
| **POD 5**        |                |            |                           |
| Proteins (g/dL)  | 5.0 ± 0.15     | 4.9 ± 0.39 |                          |
| Albinin (g/dL)   | 2.2 ± 0.1      | 1.9 ± 0.15 |                          |
| Glucose (mmol/L) | 22.3 ± 0.1     | 17 ± 3.5   |                          |
| Triglycerides (mg/dL) | 121 ± 7.3     | 120 ± 5.3  |                          |
| Cholesterol (mg/dL) | 67.0 ± 19.8   | 78.4 ± 8.3 |                          |

*p > 0.05 vs. control. The rats studied had either BDO for 2 days or sham laparotomy (control). On POD 2, the bile flow was restored by removal of the clip devise. Data are given as mean ± SD.

Table 4: Results of immunohistochemistry.

|                  | Control (n=12) | p-Value (BDO vs. control) | BDO (n=18) | p-Value (BDO: POD 2 vs. POD 3) |
|------------------|----------------|---------------------------|------------|--------------------------------|
| **POD 2**        |                |                           |            |                                |
| HIS36            | 1.0 ± 0.07     | 0.0001                    | 2.28 ± 0.39| 0.001                          |
| CK19             | 1.05 ± 0.25    | 0.156                     | 1.44 ± 0.58| 0.015                          |
| HGF-α            | 0.83 ± 0.39    | 0.002                     | 1.61 ± 0.44| 0.015                          |
| HGF-β            |                |                           |            |                                |
| **POD 3**        |                |                           |            |                                |
| HIS36            | 0.9 ± 0.07     | 0.0001                    | 3.17 ± 0.69| 0.001                          |
| CK19             | 1.0 ± 0.2      | 0.0001                    | 2.14 ± 0.37| 0.004                          |
| HGF-α            | 1.38 ± 1.19    | 0.003                     | 2.5 ± 0.81 | 0.047                          |
| HGF-β            | 0.8 ± 0.3      | 0.0001                    | 2.56 ± 0.54| 0.003                          |
| **POD 5**        |                |                           |            |                                |
| HIS36            | 1.1 ± 0.09     | 0.0001                    | 2.16 ± 0.7 | 0.669                          |
| CK19             | 1.1 ± 0.2      | 0.0001                    | 2.53 ± 0.38| 0.001                          |
| HGF-α            | 1.40 ± 1.2     | 0.0001                    | 3.06 ± 0.15| 0.0001                         |
| HGF-β            | 0.75 ± 0.45    | 0.0001                    | 3.33 ± 0.53| 0.0001                         |

The rats studied had either BDO for 2 days or sham laparotomy (control). On POD 2, the bile flow was restored by removal of the clip devise. Data are given as mean ± SD.
vascular clip device were published by Jorge with experimental intermittent hepatic pedicle clamping and temporary choledochal clamping (~10 min) in rats [7, 12, 13]. The second endpoint in our study was the suitability of the clip to induce comparable systemic and histological alterations, as already described with the “ligation
Figure 4: Immunohistochemical assessment of the liver after BDO for 2 days or control. Staining for CK19 (red: CK19; blue: nuclei, 200× magnification). (A) Control, POD 2: control animal without BDO. (B) BDO, POD 2: after 2 days of BDO. (C) BDO, POD 3: 24 h after relief of BDO. (D) BDO, POD 5: 72 h after relief of BDO.

Figure 5: Histologic assessment of the liver after BDO for 2 days or control. Staining for HIS36 (brown: HIS36; blue: nuclei, 200× magnification). (A) Control, POD 2: control animal without BDO. (B) BDO, POD 2: after 2 days of BDO. (C) BDO, POD 3: 24 h after relief of BDO. (D) BDO, POD 5: 72 h after relief of BDO.
Conflicts of interest: The authors have declared that no conflicts of interest exist. Informed consent: Informed consent is not applicable. Ethical approval: The research related to the animal use complies with all the relevant national regulations and institutional policies. All procedures and housing of the animals were carried out according to the German Animal Welfare Legislation and approved by the local authorities (Hessisches Landesamt für Verbraucherschutz, reg.-nr. 02-025/08).

## Author Contributions

Beate Richter: conceptualization; data curation; formal analysis; investigation; methodology; project administration; software; validation; writing – original draft; writing – review and editing. Semik Khodaverdi: investigation. Wolf Otto Bechstein: financial support. Carsten Gutt: financial support. Lukas Krähenbühl: financial support. Thomas Schmandra: supervision; writing – review and editing.

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Reviewer Assessment

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Reversible biliary occlusion in a small animal model: first description of a new technique

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Reviewers’ Comments to Original Submission

Reviewer 1: anonymous
Jun 24, 2018

| Reviewer Recommendation Term: | Accept |
|-------------------------------|--------|
| Overall Reviewer Manuscript Rating: | 90 |

| Custom Review Questions | Response |
|-------------------------|----------|
| Is the subject area appropriate for you? | 5 - High/Yes |
| Does the title clearly reflect the paper’s content? | 5 - High/Yes |
| Does the abstract clearly reflect the paper’s content? | 5 - High/Yes |
| Do the keywords clearly reflect the paper’s content? | 4 |
| Does the introduction present the problem clearly? | 4 |
| Are the results/conclusions justified? | 5 - High/Yes |
| How comprehensive and up-to-date is the subject matter presented? | 4 |
| How adequate is the data presentation? | 5 - High/Yes |
| Are units and terminology used correctly? | 5 - High/Yes |
| Is the number of cases adequate? | 4 |
| Are the experimental methods/c clinical studies adequate? | 4 |
| Is the length appropriate in relation to the content? | 4 |
| Does the reader get new insights from the article? | 5 - High/Yes |
| Please rate the practical significance. | 4 |
| Please rate the accuracy of methods. | 4 |
| Please rate the statistical evaluation and quality control. | 4 |
| Please rate the appropriateness of the figures and tables. | 4 |
| Please rate the appropriateness of the references. | 4 |
| Please evaluate the writing style and use of language. | 3 |
| Please judge the overall scientific quality of the manuscript. | 5 - High/Yes |
| Are you willing to review the revision of this manuscript? | Yes |

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Comments to Authors:
This is a really nice study with a significant result. It shows clearly that the described clip technique is a safe and easy method for reversible biliary occlusion with a very low mortality. The clip is easy to remove in a second small surgery. In other studies, the animals needed a biliodigestive anastomosis to reverse the biliary occlusion with higher mortality perhaps also because of the bigger surgical trauma. The laboratory measurements show a fast recovery after removing the clip. The paper is well structured, giving a good overview about the published literature. The methods and results are clearly described by the author.

Reviewer 2: anonymous
Jul 05, 2018

Reviewer Recommendation Term: Accept
Overall Reviewer Manuscript Rating: 76

Custom Review Questions Response
Is the subject area appropriate for you? 4
Does the title clearly reflect the paper’s content? 5 - High/Yes
Does the abstract clearly reflect the paper’s content? 5 - High/Yes
Do the keywords clearly reflect the paper’s content? 4
Does the introduction present the problem clearly? 4
Are the results/conclusions justified? 4
How comprehensive and up-to-date is the subject matter presented? 5 - High/Yes
How adequate is the data presentation? 5 - High/Yes
Are units and terminology used correctly? 5 - High/Yes
Is the number of cases adequate? 4
Are the experimental methods/clinical studies adequate? 5 - High/Yes
Is the length appropriate in relation to the content? 5 - High/Yes
Does the reader get new insights from the article? 4
Please rate the practical significance. 3
Please rate the accuracy of methods. 4
Please rate the statistical evaluation and quality control. 5 - High/Yes
Please rate the appropriateness of the figures and tables. 4
Please rate the appropriateness of the references. 4
Please evaluate the writing style and use of language. 5 - High/Yes
Please judge the overall scientific quality of the manuscript. 4
Are you willing to review the revision of this manuscript? Yes

Comments to Authors:
The author presents in this experimental paper with accurate methods a modified technique of reversible biliary occlusion resulted in a reduces mortality compared to former studies. As described in the conclusions further investigations with long-term-outcomes are needed to prove superiority of these methods.