Subtotal hepatectomy in swine for studying small-for-size syndrome and portal inflow modulation: is it reliable?

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Abstract

Background: Small-for-size syndrome (SFSS) is a feared complication of extended liver resection and partial liver transplantation. Swine models of extended hepatectomy have been developed for studying SFSS and its different treatment options. Although portal inflow modulation (PIM) by splenectomy or splenic artery ligation (SAL) has been proposed in humans to prevent SFSS, such procedures have not yet been evaluated in swine.

Objectives: The present study was designed to evaluate modifications in splanchnic haemodynamics yielded by extended hepatectomy with and without PIM in swine.

Methods: Nineteen animals underwent 70% hepatectomy (H70, n = 7), 90% hepatectomy (H90, n = 7) or sham laparotomy (H0, n = 5). Haemodynamic measurements were performed at baseline, after hepatectomy and after PIM by SAL and splenectomy.

Results: Portal vein flow increased after both H70 (273 ml/min/100 g versus 123 ml/min/100 g; P = 0.016) and H90 (543 ml/min/100 g versus 124 ml/min/100 g; P = 0.031), but the hepatic venous pressure gradient (HVPG) increased only after H90 (10.0 mmHg versus 3.7 mmHg; P = 0.016). Hepatic artery flow did not significantly decrease after either H70 or H90. In all three groups, neither splenectomy nor SAL induced any changes in splanchnic haemodynamics.

Conclusions: Subtotal hepatectomy of 90% in swine is a reliable model for SFSS inducing a significant increase in HVPG. However, in view of the relevant differences between swine and human splanchnic anatomy, this model is inadequate for studying the effects of PIM by SAL and splenectomy.

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Introduction

Small-for-size syndrome (SFSS) is one of the main factors limiting the regeneration of the remnant liver after partial liver transplantation or extended hepatectomy.1,2 It is related to an imbalance between excessive portal venous inflow and insufficient liver volume, which may cause early portal hypertension with subsequent ascites, cholestasis, liver insufficiency and even death in extreme cases.1 In order to decrease portal venous inflow and avoid SFSS, several surgical techniques to achieve portal inflow modulation (PIM) have been reported, including splenic artery ligation (SAL),3–5 splenectomy6,7 and portosystemic shunts.8

Given the similarities between swine and human liver anatomy, porcine models of extended hepatectomy have been widely used to study the mechanisms involved in the process of liver regeneration.9 However, the pathophysiology of SFSS is related to inadequate ratios between whole-body, splanchnic and liver blood flows.1 Thus, a good animal model for
studying SFSS would require ratios similar to those in humans. Further, although experimental models of portocaval or mesocaval shunts in swine have been successfully applied, no experimental studies of PIM by SAL and splenectomy in swine have yet been reported, despite the wide use of these two PIM techniques in the clinical setting.

The aims of the present study were to evaluate the reliability of the porcine model of extended hepatectomy in the study of SFSS by assessing the haemodynamic changes yielded after 70% and 90% hepatectomy, and to establish the effects of PIM by SAL and splenectomy on splanchnic haemodynamic parameters in these two models.

Materials and methods

Animals

Nineteen female domestic white pigs were provided from the Porc Plein Air licensed agricultural settlement (Saint Clair sur Galaure, France). Animals were conditioned for at least 3 days prior to surgery in the study institution’s animal facility (Domaine Rockefeller, Medical University of Lyon-Est, Lyon, France), in which they were fed ad libitum and kept under artificial lighting during daytime.

The study flow chart is reported in Fig. 1. Five animals underwent sham laparotomy; these are designated the H0 group. Seven animals underwent 70% hepatectomy (H70 group) and seven animals underwent 90% hepatectomy (H90 group). In each hepatectomy group, haemodynamic measurements were performed before and after hepatectomy, and thus each animal was considered to provide its own control data.

Anaesthesia

All animals underwent initial sedation with an intramuscular injection of ketamine (Imalgène®, 20 mg/kg; Merial, Lyon, France) and xylazine (Rompun®, 3 mg/kg; Bayer, Diegem, Belgium) before they were transferred to the operating theatre. Anaesthetic induction was attained with an intravenous injection of propofol (Diprivan® 1%, 3 mg/kg; Fresenius Kabi, Lake Zurich, Austria) through a peripheral venous access in the left ear. A 7-mm tracheal tube was inserted and anaesthesia was maintained with a 120–150 mg/h continuous infusion of propofol through a central venous catheter. Analgesia was provided throughout the procedure with sufentanil (Sufenta®, 1 mg/kg/h; Janssen-Cilag, Issy-les-Moulineaux, France) and prophylactic antibiotic treatment (ceftazidime, 1 g) was administered before incision.

Surgical procedures

After midline laparotomy and exposure of the upper abdominal cavity, the hepatoduodenal ligament was dissected. The gastroduodenal artery and gastrohepatic artery (a gastric arterial branch supplying the proximal portion of the stomach) were divided, and the inferior vena cava (IVC), portal vein and hepatic artery were dissected and individualized (Fig. 2) to facilitate the placement of captors. Haemodynamic data recorded at this step represented the reference data for each group.

Animals in the H70 group underwent extended hepatectomy including the removal of the left lateral lobe and both left and right medial lobes, as described by Court et al. The arterial branches and biliary ducts of these three lobes were divided; the left portal branch was divided (Fig. 3), and the right portal branch was dissected within the liver parenchyma at approximately 1 cm. The portal branch supplying the right medial lobe was selectively divided. Parenchymal transection was started in an upward direction following the right portal fissure at 2 cm. Glisson’s capsule was dissected laterally 1 cm below the IVC, allowing a vascular clamp to be placed across the parenchymal bridge between the right lateral and right medial lobes. The excised liver comprising three lobes was removed, leaving a small portion of parenchyma beneath the IVC (Fig. 4). An oversewing stitch was performed back and forth around the vascular clamp and, finally, the clamp was...
removed. After the procedure, the remnant liver comprised the right lateral lobe and segment I.

Animals in the H90 group underwent the additional removal of the right lateral lobe after the completion of a 70% hepatectomy as described. Parenchymal transection was started upward 1 cm from the fissure between segment I and the posterior segment to avoid the injury of collateral branches of the right hepatic vein (Fig. 5). The transection was then performed from bottom to top at 1 cm below the IVC. A vascular clamp was placed across the remaining parenchymal bridge and the right lateral lobe was excised. After the procedure, the remnant liver comprised only segment I.

Haemostasis was achieved by bipolar cauterization and several stitches using vascular 4-0 Prolene® threads.

At the end of each procedure, the animal was killed. The remnant liver and surgical specimens were weighed in order to calculate the remnant liver : body and remnant liver : whole liver ratios.

**Surgical portal inflow modulation**

Surgical PIM was simulated by two modalities: SAL, which was simulated by clamping of the splenic artery, and splenectomy, which was simulated by clamping of the three vascular pedicles of the spleen (Fig. 6). Each modality of PIM was performed at least 20 min after hepatectomy, for a period of 5 min, and was followed by a 10-min interval before the other modality was performed.

**Haemodynamic measurements and calculations in animals**

All measurements were recorded after haemodynamic stabilization, with a 20-min delay after hepatectomy. Systemic blood pressure and heart rates were recorded through an arterial carotid catheter. Intrahepatic IVC pressure and portal venous pressure (PVP) were recorded with two intravascular Millar® (Millar Instruments, Houston, TX, USA) pressure catheters (Fig. 7). Portal vein flow (PVF) and hepatic artery flow (HAF) were recorded with, respectively, 10-mm and 4-mm time-transit flowmeter probes (Transonic Systems, Inc., Ithaca, NY, USA) (Fig. 4). In some animals, splenic vein flow was recorded with the 4-mm probe. All data were recorded simultaneously with PowerLab® Version 7.2.4 (AD Instruments Pty Ltd, Bella Vista, NSW, Australia). Both HAF and PVF were expressed in millilitres per minute.

The hepatic venous pressure gradient (HVPG) was calculated with the following formula: HVPG = PVP − IVC.

Values of HVPG of <5 mmHg were considered normal and
values of >10 mmHg were considered pathological. The PVF : liver weight and HAF : liver weight ratios were calculated with the following formulae:

\[
PVF\text{-to-liver weight (ml/min/100 g)} = \frac{PVF}{\text{weight of the remnant liver}} \times 100
\]

\[
HAF\text{-to-liver weight (ml/min/100 g)} = \frac{HAF}{\text{weight of the remnant liver}} \times 100
\]

Portal vein resistance (PVR) was estimated using the hydraulic analogy of Ohm’s law: resistance = difference of pressure between two points/flow (PVR = HVPG/PVF : liver weight).

### Statistical analysis

All calculations and statistical tests were performed using IBM spss Statistics for Macintosh Version 20.0 (IBM Corp., Armonk, NY, USA). Data analyses were performed with the two-sided non-parametric Wilcoxon test for matched continuous variables and the Mann–Whitney test for unmatched continuous variables. Results are expressed as median values with the first to third interquartile range. The measurement units used were millimetres of mercury (mmHg) for pressures, and millilitres per minute per 100 g liver tissue for flows. Differences between groups were considered statistically significant if they achieved a P-value of <0.05.

### Results

No deaths occurred during these experiments. All systemic and splanchnic haemodynamic measurements were obtained.

#### Volumetry

Animal and whole-liver weights were similar in the H70 and H90 groups (Table 1). The median percentage of resected liver was 71% in the H70 group and 89% in the H90 group. The liver : body weight ratio was 2.85% in the H0 group, 0.82% in the H70 group and 0.33% in the H90 group.

#### Systemic and splanchnic haemodynamics

In the H70 group, mean arterial pressure (83 mmHg versus 85 mmHg; \( P = 0.22 \)) and pulse rate (120 bpm versus 131 bpm; \( P = 0.67 \)) did not differ significantly in values.
obtained before and after 70% hepatectomy (Table 2). In the H90 group, mean arterial pressure decreased after hepatectomy (85 mmHg versus 61 mmHg; \( P = 0.016 \)), whereas pulse rate remained unchanged (121 bpm versus 137 bpm; \( P = 0.47 \)).

Portal vein flow decreased by 25% after 70% hepatectomy \( (P = 0.16) \) and by 46% after 90% hepatectomy \( (P = 0.016) \). When normalized to liver weight, PVF increased significantly after both 70% and 90% hepatectomy, by 122% \( (P = 0.016) \) and 338% \( (P = 0.031) \), respectively.

Hepatic artery flow decreased by 75% after 70% hepatectomy \( (P = 0.031) \) and by 89% after 90% hepatectomy \( (P = 0.016) \), but once HAF had normalized to liver weight, the difference observed was not statistically significant.

The HVPG increased significantly only after 90% hepatectomy \( (3.7 \text{ mmHg to } 10.0 \text{ mmHg}; \ P = 0.016) \). Whereas PVR values were similar before and after 90% hepatectomy, PVR decreased by more than half after 70% hepatectomy in comparison with the control group (Table 2).

**Comparison of SVF : PVF ratio between swine and humans**

In comparison with that in humans, the SVF : PVF ratio in swine was three times lower (Table 4).

**Discussion**

The present study demonstrates that subtotal hepatectomy of 90% but not of 70% represents a reliable model for SFSS, but with anatomical limitations. Indeed, subtotal hepatectomy of 90% was shown to reproduce the haemodynamic setting of SFSS by increasing both PVF and HVPG significantly, whereas the 70% hepatectomy model induced an increase in PVF only, with no increase in HVPG. These findings are consistent with those of the study by Court et al.,\(^{13}\) which was the first to compare the two models of 90% and 70% hepatectomy; it concluded that 90% hepatectomy provided a reproducible model of postoperative liver insufficiency, but that after 70% resection, the liver was still able to regenerate properly, without liver insufficiency. Subtotal hepatectomy of 70% may induce postoperative liver insufficiency when combined with prolonged remnant ischaemia,\(^{14}\) but the liver failure observed in the latter is not related to SFSS but to direct hepatic ischaemia.
However, the increase in PVP yielded in the earlier study did not correlate with the present findings. The increase in both PVP and PVF values after 75% and 83% hepatectomies was non-linear: PVR dramatically decreased between animals and humans. These findings are consistent with those of former studies reporting a lack of correlation between HVPG and PVR. In this study, both models of hepatectomy induced substantial decreases in HAF, which were apparently proportional to the volume of liver resected (75% in H70, 89% in H90). However, when normalized to liver weight, the decrease in HAF was not significant and therefore no conclusion on HABR activation could be drawn. Further studies in larger groups of animals will be necessary to study the mechanisms involved in HAF variations in the setting of post-hepatectomy SFSS.

In the clinical setting, the recognized cut-off for the liver : body weight ratio (LBWR), below which the liver remnant is considered to be insufficient, is 0.6% after extended hepatectomy and 0.8% after partial liver transplantation.2 Interestingly, in the present study, the median LBWRs observed in the H70 group (0.82%) and H90 group (0.33%) were, respectively, above and below the extended hepatectomy LBWR cut-off value of 0.6%, which suggests that the same cut-off values may be relevant in swine. However, this conclusion needs to be confirmed by further longterm studies reporting postoperative results.

Experimental porcine models of extended hepatectomy have been widely used in the study of SFSS and surgical modalities of PIM,10,11,21 and have used various techniques to achieve portal diversion into the systemic circulation. These modalities are more complex and demanding than SAL and splenectomy, which are the two most commonly used surgical modalities of PIM.4,6,12,22 Surprisingly, despite the wide experience in

### Table 3 Haemodynamics after surgical modulation of the liver inflow

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|---------------------------------|---------------------------------|-----------------|-----------------|-----------------|
| **Splenectomy**                  | **Splenectomy**                  | **P-value**     | **P-value**     | **P-value**     |
| **Before**                       | **After**                       |                 |                 |                 |
| **H0 group**                     | **H70 group**                   |                 |                 |                 |
| Animals, n                       | 5                               | 5               |                 |                 |
| HVPG, mmHg, median (IQR)         | 2.1 (1.7–2.2)                   | 2.1 (1.9–2.3)   | 0.16            | 1.2 (1.1–1.5)   | 1.0 (0.8–1.6)   | 0.76 |
| PVF/liver weight, ml/min/100 g, median (IQR) | 104 (104–139)                   | 118 (112–127)   | 0.56            | 105 (86–130)    | 114 (108–130)   | 0.81 |
| HAF/liver weight, ml/min/100 g, median (IQR) | 21.7 (14.9–31.3)               | 26.0 (13.6–31.3)| 0.29            | 25.6 (7.3–31.3) | 27.8 (10.9–28.1)| 0.65 |
| **H90 group**                    |                                 |                 |                 |                 |
| Animals, n                       | 4                               | 4               |                 |                 |
| HVPG, mmHg, median (IQR)         | 5.0 (3.6–6.5)                   | 5.8 (3.9–6.3)   | 0.97            | 4.1 (2.2–5.9)   | 2.5 (1.6–4.2)   | 0.33 |
| PVF/liver weight, ml/min/100 g, median (IQR) | 325 (239–408)                   | 259 (225–304)   | 0.41            | 233 (201–279)   | 233 (168–274)   | 0.98 |
| HAF/liver weight, ml/min/100 g, median (IQR) | 17.5 (13.6–21.3)               | 23.0 (20.8–24.7)| 0.49            | 24.7 (18.1–31.3)| 23.8 (22.8–24.9)| 0.99 |

### Table 4 Comparison of splenic vein flow : portal vein flow ratio between animals and humans

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|---------------------------------|---------------------------------|-----------------|-----------------|
| **Animals (n = 8)**             | **Humans (n = 6)**              | **P-value**     | **P-value**     |
| SVF : PVF, %, median (IQR)      | 9 (8–12)                        | 31 (26–34)      | <0.001          |

IQR, interquartile range (first–third quartiles); PVF, portal vein flow.
porcine experimental liver surgery reported, no previous study had focused on the impacts of SAL and splenectomy on splanchnic haemodynamics in swine. This is, to the present authors’ knowledge, the first study to assess the effects of these two surgical modalities of PIM after extended hepatectomy, and shows that neither SAL nor splenectomy induced any significant change in portal or arterial haemodynamics at baseline and after 70% or 90% hepatectomy in non-diseased liver. This demonstrates that the porcine model of extended hepatectomy may not be reliable for studying the impacts of surgical modalities of PIM involving splenic venous flow.

The vascular anatomies of the porcine and human livers are considered very similar, and the reported lack of correlation in their haemodynamics may seem paradoxical. To better understand this phenomenon, the present study included a secondary comparison of the SVF:PVF ratios in swine and humans. This demonstrated a dramatic difference between humans and swine (31% versus 9%; P < 0.001). The difference may be explained by differences between swine and humans in the length of the large bowel, and the very small splenic volume in swine compared with humans.

The present study is subject to some limitations. It was limited to the recordings of intraoperative data, although postoperative outcomes in terms of liver regeneration, ascites, liver insufficiency and histopathological data would have been valuable. Further studies on postoperative outcome are required to confirm the present findings. Moreover, the PIM procedures performed were simulated procedures as the corresponding vascular pedicles were clamped in place of true SAL or splenectomy. This protocol allowed the number of animals used in this study to be decreased.

In conclusion, subtotal hepatectomy of 90% in swine is a reliable experimental model for the study of post-hepatectomy SFSS, which induces significant acute portal hypertension, whereas the remnant liver volume after 70% hepatectomy remains oversized and does not reproduce an SFSS setting. However, because of differences in the visceral anatomies of swine and humans, this model is not suitable for the study of PIM by SAL and splenectomy.

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Conflicts of interest
None declared.

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