Video Article

Monitoring of Systemic and Hepatic Hemodynamic Parameters in Mice

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Abstract

The use of mouse models in experimental research is of enormous importance for the study of hepatic physiology and pathophysiological disturbances. However, due to the small size of the mouse, technical details of the intraoperative monitoring procedure suitable for the mouse were rarely described. Previously we have reported a monitoring procedure to obtain hemodynamic parameters for rats. Now, we adapted the procedure to acquire systemic and hepatic hemodynamic parameters in mice, a species ten-fold smaller than rats. This film demonstrates the instrumentation of the animals as well as the data acquisition process needed to assess systemic and hepatic hemodynamics in mice. Vital parameters, including body temperature, respiratory rate and heart rate were recorded throughout the whole procedure. Systemic hemodynamic parameters consist of carotid artery pressure (CAP) and central venous pressure (CVP). Hepatic perfusion parameters include portal vein pressure (PVP), portal flow rate as well as the flow rate of the common hepatic artery (table 1). Instrumentation and data acquisition to record the normal values was completed within 1.5 h. Systemic and hepatic hemodynamic parameters remained within normal ranges during this procedure.

This procedure is challenging but feasible. We have already applied this procedure to assess hepatic hemodynamics in normal mice as well as during 70% partial hepatectomy and in liver lobe clamping experiments. Mean PVP after resection (n= 20), was 11.41±2.94 cmH_2O which was significantly higher ($P<0.05$) than before resection (6.87±2.39 cmH_2O). The results of liver lobe clamping experiment indicated that this monitoring procedure is sensitive and suitable for detecting small changes in portal pressure and portal flow rate. In conclusion, this procedure is reliable in the hands of an experienced micro-surgeon but should be limited to experiments where mice are absolutely needed.

Video Link

The video component of this article can be found at http://www.jove.com/video/51955/

Introduction

The overall goal of this video was to demonstrate a real-time monitoring procedure for acquiring systemic and hepatic hemodynamic parameters. The rationale for developing this procedure is its great value for experimental interventions in mice that require obtaining systemic and hepatic hemodynamic parameters. The procedure can be applied to naïve animals and during or after a given hepato-biliary experimental surgical intervention, such as partial hepatectomy, portal vein ligation and liver transplantation.

Acquisition of hepatic hemodynamic data in rodents requires the proposed invasive procedure. Hepatic perfusion cannot be obtained non-invasively. However, there are alternatives for the acquisition of the systemic blood pressure. Monitoring techniques such as the tail cuff technique[8] have been utilized for acquiring the blood pressure in both rats and mice. The tail cuff technique can be applied in conscious animals. When measuring the blood pressure, the animal needs to be placed and fixed in a specific uncomfortable position. In the manual of the tail-cuff device, the manufacturer states that mice may become nervous and stressed which may diminish the circulation in the tail. Under that circumstance, the peripheral blood pressure acquired in the tail may be much lower than the central blood pressure.

The full monitoring procedure was performed with an integrated multiple-channel monitor using a series of sensors for data acquisition. The blood pressure was obtained by inserting a catheter into the respective vessel after careful microsurgical dissection and exposure under the microscope. The flow rate was measured by placing a transonic flow probe around each vessel.

We already reported a similar intraoperative monitoring procedure for rats resulting in a comprehensive series of physiological hemodynamic data comparable to single data reported from other groups[7]. Therefore we considered this procedure to represent a good basis for adapting it to the mouse, a species 10-fold smaller than the rat. The key difference to the rat procedure is the use of Millar catheters for acquiring blood pressure data instead of a fluid-based catheter system. Flow data were also acquired with transonic flow probes, just much smaller ones than for the corresponding rat vessels.

Due to the small size of the animal, instrumentation of mice is technically challenging, but feasible. Once instrumentation is completed, data acquisition and primary life data analysis is simple, since a predefined setting file can be used. The setting file has to be defined once at the beginning of a series of experiments and can be stored and used for all subsequent experiments.

Up to now we applied this procedure to assess hepatic hemodynamic effects in acute experiments. We measured CAP and PVP before and immediately after 70% partial hepatectomy (PH) and in clamping/de-clamping experiments. We clamped the hepato-duodenal ligament of the right lobe representing 20% of the liver mass followed by brief (5min) clamping of the median and left lateral lobe representing totally 90% of the liver mass. De-clamping started with releasing the clamp from the right lobe followed by freeing the median and left lateral lobe. Maximal clamping time was below 10min.

Protocol

Housing and all procedures carried out were in accordance with German Animal Welfare Legislation.

1. Sensors calibration. (Follow manufactures instructions for sensors calibration).

1.1) Millar catheter calibration. Pre-soak the tip of the catheter in sterile water or saline for 30 minutes prior to balance (zeroing) and calibration.

1. Connect the millar sensor to the millar1 channel of bridge amplifier and insert the millar sensor tip into water column.
2. Set the water column value to 0 cmH_2O. In data analysis software window, choose bridge amplify and zero it. The baseline value 0 cmH_2O can be set.
3. Set the water column value to 20 cmH_2O. Run data analysis software window progress, and stop. Choose “units” in the window of bridge amplify, set the baseline of 0 and 20 cmH_2O accordingly. Adjust the “unit” to cmH_2O.
4. Calibrate the millar2 for measuring CAP in the same way (set two base line 90 and 110 cmH_2O).

1.2) Blood flow probe calibration

1. Put the probe into deionised water. Connect the probe with transonic flow probe system.
2. In data analysis software window, choose Input amplify to zero the flow probe. Adjust the units.
3. Press the button to “test channel” to collect the signal: if the signal has 3-4 bars, it means the signal is good. In case a good signal is acquired, the procedure can be continued.
4. Press the button to “zero channel” and scale channel to see whether the value has been calibrated or not.
5. Press the button to “measure channel” for later measuring.

2. Prepare the mouse for the surgical procedure

1. Place the mouse in an induction chamber and anesthetize the mouse with 2% isoflurane and 0.3ml/min oxygen. The operation can be performed if the toe-pinch withdrawal reflex of the mouse is absent.
2. Shave the fur of surgical regions, which include the left neck and abdomen.
3. Place the mouse on the operation table and fix it using tapes. Use vet ointment on eyes to prevent dryness during the operation period.
4. Place a gauze cushion under the neck for optimal exposure of the operation field of neck.
5. Disinfect the operation field and place sterilized gauzes to cover the mouse only leaving the surgical field open.

3. Vital parameters measurement

1. Insert the ECG needles subcutaneously into the paws of mouse.
2. Place the respiratory sensor under the back of the mouse.
3. Place the temperature probe into the rectum of the mouse.
4. Record temperature, ECG and respiratory rate of the mouse in data analysis software.

4. Neck operation for systemic cardiovascular monitoring

4.1) Vessel dissection

1. Identify the middle line of the neck, middle point of clavicle, the angle of mandible.
2. Make a 2cm longitudinal incision from the angle of mandible to the middle point of clavicle which is 0.5cm to the left side of the middle line.
3. Dissect the submandibular gland, turn it over and cover it with saline soaked gauze.
4. Identify the jugular vein, dissect it and place three 6-0 silk sutures under the vein for later ligation and fixation.
5. Identify the sternocleidomastoid muscle, separate it from the superior belly of omohyoid and posterior belly of digastric muscle, and pull it with a retractor for easy exposure of the carotid artery.
6. Dissect the carotid artery and place three 6-0 silk sutures under the artery for later ligation and fixation.

4.2) Carotid artery blood flow measurement

1. Place the transonic probe around the carotid artery, keep it stable, and optimize the contact using ultrasound gel or saline.
2. Record blood flow velocity of the carotid artery as indicated on the small screen of the transonic device using data analysis software

3. Remove the probe after completing the measurement

4.3) Carotid artery pressure measurement (CAP)

1. Ligate the distal end of the carotid artery and clamp its proximal end.
2. Place 2 fixing sutures around the carotid artery. Use 10-0 prolene for the stay suture.
3. Make a small incision on the anterior wall of the vessel.
4. Insert the millar catheter and fix it with pre-placed sutures.
5. Record the CAP in data analysis software.

4.4) Jugular vein blood flow measurement

1. Lift the jugular vein and place the transonic flow probe to measure the flow rate.
2. Record the flow rate in data analysis software.

4.5) Central venous pressure measurement (CVP)

1. Clamp the proximal end of the jugular vein and ligate the distal end.
2. Cut a small incision using microscissors on the anterior wall of the vessel.
3. Insert the fluid-filled catheter and fix it with the pre-placed suture lines.
4. Record the CVP in data analysis software.

5. Abdominal operation for acquisition of hepatic hemodynamics

5.1) Vessel identification

1. Make a transverse incision on the abdomen.
2. Eventerate the intestines to the left side and cover with wet gauze.
3. Identify the inferior vena cava, the portal vein, the common hepatic artery and the proper hepatic artery.
4. Drop some warm saline in the abdomen and on the surface of the intestines every five minutes during the whole monitoring procedure.

5.2) Measurement of portal blood flow

1. Dissect the portal vein.
2. Place 6-0 silk under the portal vein to facilitate lifting of the vessel when placing the flow probe.
3. Place the transonic flow probe around the portal vein and measure its blood flow rate.
4. Record the blood flow rate of the portal vein.

5.3) Measurement of Common hepatic artery flow

1. Dissect the common hepatic artery cautiously.
2. Place one 6-0 silk suture around the vessel to facilitate lifting of the vessel.
3. Place the flow probe around the artery.
4. Measure its blood flow and acquire the data.

5.4) Measurement of portal vein pressure (PVP)

1. Choose one branch of the mesenteric vein with few side branches, which drains straight into the portal vein.
2. Ligate the distal end of the selected mesenteric vein. Make sure that ligation is close to the intestinal tube. Ligate its small branches
3. Place 2 fixing sutures using 6-0 prolene around the vein. The key point of this procedure is to avoid touching the mesenteric artery when ligating the vein.
4. Clamp the proximal end of the portal vein.
5. Place 2 stay sutures using 10-0 prolene. Some bleeding will occur since the stay suture should penetrate the vascular wall of the fine mesenteric vein.
6. Make a small incision on the vein using a microscissor obliquely at a 45 degrees angle.
7. Insert the Millar catheter through the mesenteric vein into the portal vein and fix it
8. Record the portal vein pressure. At the end of the procedure, sacrifice the mice by exsanguination under anesthesia.

Representative Results

Vital parameters of the mice such as respiratory rate and heart rate are obviously much higher than in rat. Mean systemic blood pressure and jugular vein pressure are similar to rat values and even similar to the human data.

Hepatic hemodynamic data are obviously different. We obtained normal values from 8 mice. Portal blood flow in normal mice ranged between 1.6 to 2.3ml/min. Flow in the common hepatic artery ranged from 0.10 to 0.35 ml/min. Portal vein pressure in normal animals was in the wide range from 4.4 to 11.2 cmH_2O with a mean value of 8.09 ± 2.47 cmH_2O (Table 1). This wide range may lead to small but not significant differences when comparing the mean value of small groups of normal animals.

Since we observed considerable inter-individual differences especially in the portal pressure, we tested whether small intra-individual differences could be detected with this technique. We evaluated this procedure in two different experimental settings: partial hepatectomy and liver lobe clamping/de-clamping. Portal pressure before and immediately after 70% partial hepatectomy (n=20) in the same animal (Figure1) increased

by 2-fold from 6.87±2.39 and 11.41±2.94cmH_2O (P =<0.001). These results were in a similar range as observed in other species [5,12] and also in humans [7].

Normal portal vein pressure and portal pressure before resection were acquired from two different groups (one from normal monitoring parameters group, the other from 70%PH group). Due to the wide range of portal pressure in normal mice (4 to 11 cmH_2O), the mean values of small groups of animals may be slightly different, as observed in our experiment (8.09 ± 2.47 cmH_2O versus 6.87±2.39 cm H_2O). However, when analyzing the data, we found that there was no statistically significant difference between these two groups (P = 0.237).

The clamping/de-clamping experiment was designed to demonstrate that the procedure is sensitive enough to pick up even smaller changes in the portal pressure. Clamping of the right lobe representing 20% of the liver mass resulted in an increase of about 17%. Further clamping of the median and left lateral lobe caused an increase of at least 2-3 folds compared to the starting portal pressure. Portal pressure returned gradually to the starting pressure, when releasing the clamp from the right lobe resulting in clamping of 70% of the liver. The pressure returned to the starting level when removing the clamp from the left portal vein supplying the median and left lateral lobe (Figure2 and Table2). The MAP of the sham operation group remained stable within 1h after opening the abdomen. The MAP of mice in the control group, obtained at the comparable time point as in the clamping experiment, had no significant difference compared with the MAP of the experimental group. The results of both experiments revealed that even small intra-individual changes of less than 20% could be detected with this procedure.

Typical complications like severe bleeding and congestion may occur during the procedure. Since severe blood loss would cause significant decrease of MAP as well as PVP, the results of mice with this complication should be eliminated. To avoid venous congestion and thrombosis when performing liver lobe clamping experiment, we suggest injecting a small dose heparin (500U/kg) intra-operatively before clamping. Common hepatic artery can undergo a transient vessel spasm upon handling such as lifting the vessel and placing the probe. This might cause a transient brief ischemia of the liver. In general, the spasm may resolve spontaneously within minutes. A short vascular spasm in the CHA is not posing a serious problem to the life of the animal, but might interfere with experimental results, when focusing on hepatic ischemia reperfusion injury.

In conclusion, this procedure is challenging but feasible. It requires some training even for experienced micro-surgeons. Due to the high inter-individual variability comparison of pressure data obtained in different animals before and after an intervention may not lead to conclusive results. Therefore we recommend this procedure to study short term regulation of hepatic hemodynamics in acute experiments by acquiring the data before and after the intervention within the same animal.

Parameters		Own data obtained	Parameters reported
Vital parameters	Heart rate (n=8)	418±55 BPM	389(353-566) BPM [1]
	respiratory rate (n=2)	162±11 BPM	254 ± 28 BPM [2]
	Temperature (n=8)	33.56±0.54°C	36.1–36.6°C[11]
Systemic blood pressure (CAP) (n=8)		130.54 ± 20.47cmH_2O (=96.02 ± 15.06 mmHg)	94 ± 15 mmHg [9]
Central venous pressure (CVP) (n=2)		8.90 ± 3.25 cmH_2O	5.9 ± 2.0 cmH_2O [11]
Hepatic hemodynamics	Portal vein flow (n=6)	2.03 ± 0.24 ml/min	3.0 (2.5-3.1) ml/min [1]
			3.3 ml/min [1]
	Common hepatic artery flow (n=6)	0.20 ± 0.09 ml/min	Not found
	PVP (n=8)	8.09 ± 2.47 cmH_2O (=5.95 ± 1.82mmHg)	5.3±1.4 cm saline [3]
			8.7 ± 2.1 mmHg [4]
			4mmHg [6]

Table 1. Normal systemic and hepatic hemodynamic parameters of mice acquired using this monitoring procedure. CAP: carotid artery pressure; MAP: mean arterial pressure; CVP: central venous pressure; PVP: portal vein pressure.

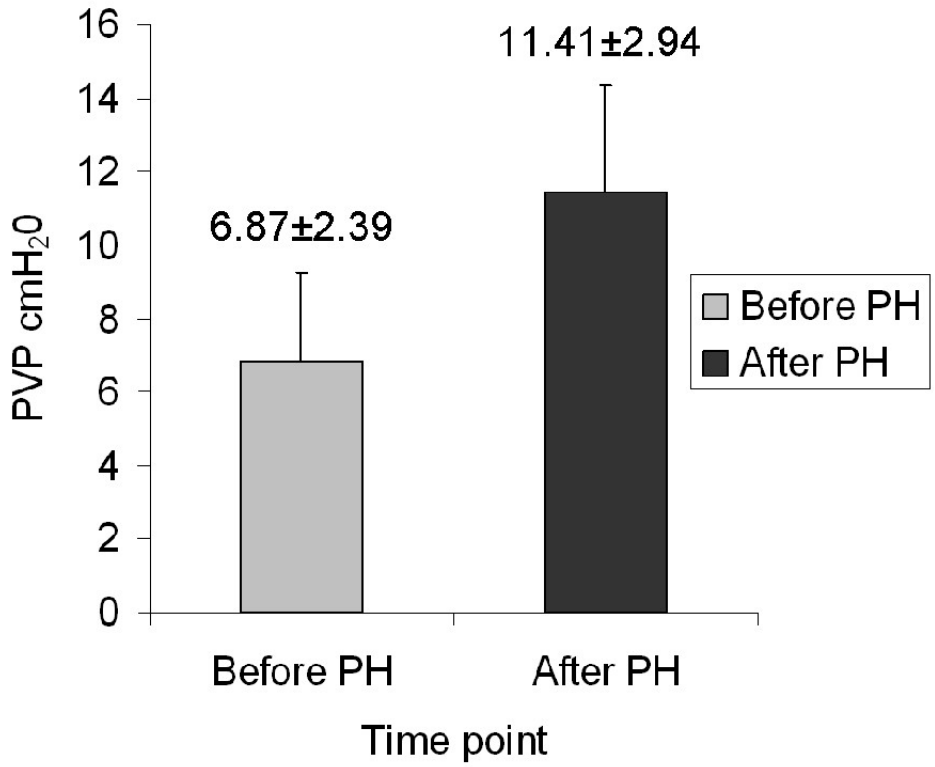

Figure 1. PVP before and after 70%PH. Portal vein pressure before and after 70%partial hepatectomy (n=20) were 6.87±2.39 and 11.41±2.94cmH_2O. Please click here to view a larger version of this figure.

Parameters (cmH2O)	After laparotomy	After clamping 20% of liver (RL)	After clamping 90% of liver (RL+ML+LLL)	After clamping 70% of liver (ML+LLL)	At the end (release all clamps)
PVP - Clamping exp group (n=10)	9.59±4.00	10.45±3.89	25.78±8.99	16.91±9.86	11.14±4.48
Mean CAP - Clamping exp group (n=10)	121.50±18.67	95.89±32.76	74.41±35.35	93.88±42.96	89.44±44.20
Mean CAP - Sham group (n=3)	123.33±12.42	121.0±5.57	124.00±8.66	127.33±7.23	123.00±8.89

Table 2. Hemodynamic response after clamping and declamping of different liver lobes (n=10). RL: right lobe, ML: median lobe (including right median lobe and left median lobe), LLL: left lateral lobe.

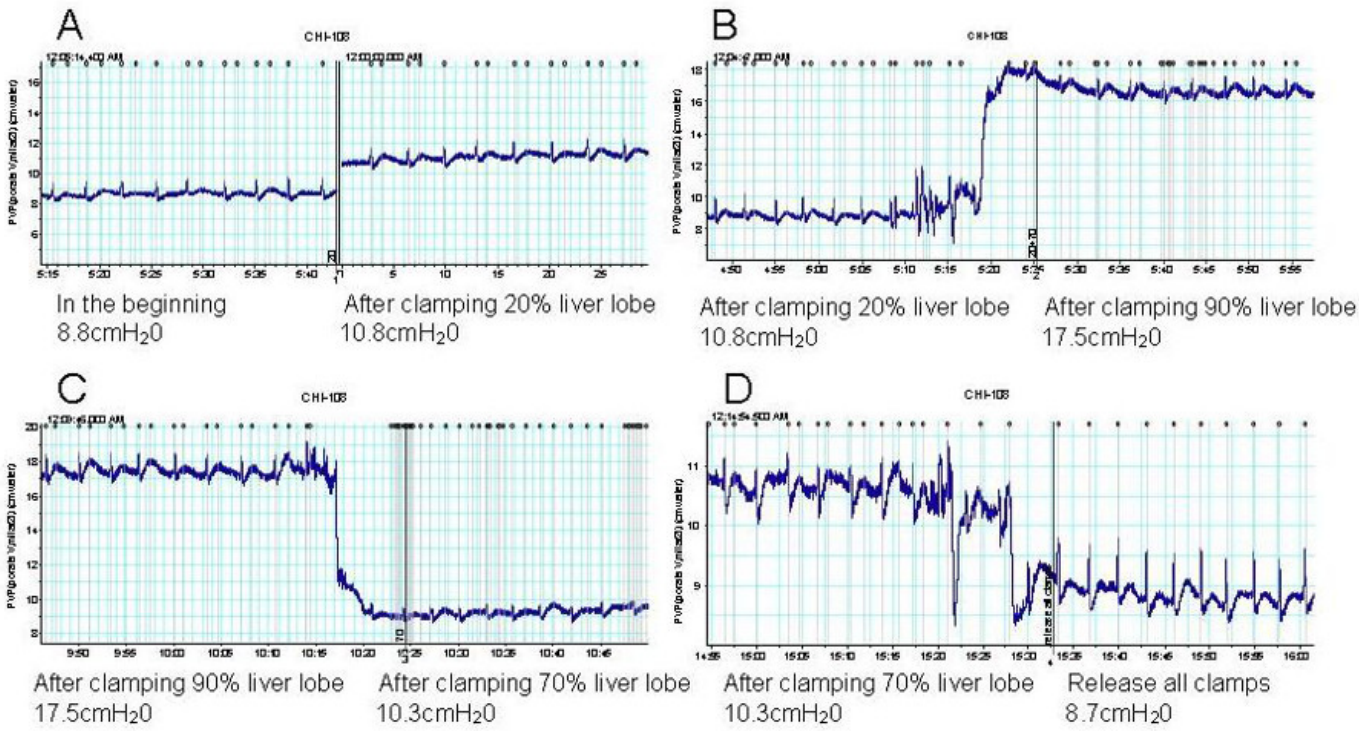

Figure 2. Hemodynamic response after clamping and declamping of different liver lobes (animal ID: CHI-108). A. PVP right after inserting the millar catheter was 8.8cmH_2O. PVP after clamping 20% liver lobe was 10.8cmH_2O. B.PVP rose to 17.5cmH_2O after clamping 90% liver lobe. C. PVP decreased to 10.3cmH_2O when releasing the clamp of the right lobe. D.PVP went back to 8.7cmH_2O as in the beginning after releasing all the clamps. Please click here to view a larger version of this figure.

Discussion

Monitoring of hepatic hemodynamics is an important research tool in hepatology and hepatobiliary surgery. Acquisition of hepatic hemodynamic data helps to characterize the effect of hepatobiliary procedures on the circulatory system. Acquisition of hepatic hemodynamic data is also needed to study the effect of drugs affecting portal pressure and portal flow, e.g. as needed in studies evaluating vasoactive drugs.

Despite the small size, the vital parameters, systemic and hepatic hemodynamics can be monitored in mice. The critical steps within the protocol were as follows: First, it is important to place the animal on a warming pad during the whole procedure to avoid hypothermia which may lead to circulatory dysfunction. Second, it is crucial to be very cautious when dissecting the vessels of a mouse, since the vessel wall of mouse is very fragile and thin. It seems best to fix the vessel wall by grabbing some fat tissue on the surface instead of grabbing the vessel wall itself. Third, it is vital to avoid accidental ligation of mesenteric artery for not impairing the arterial supply when placing the fixing sutures around the mesenteric vein.

However, the invasive mentoring echnique has some limitations. The **first** one is its invasiveness by itself. This monitoring technique is an invasive method, which requires a surgical intervention. Therefore the monitoring procedure itself may cause side effects for the animals. Hence, we only used this procedure to acquire hemodynamic parameters in normal animals and in acute experiments, but not in survival experiments. In a next step, we want to evaluate this technique in survival experiments. The **second** limitation of this procedure is that it requires substantial microsurgical experience. Hemodynamic monitoring in mice should only be performed by specifically trained microsurgeons. The most difficult part of this monitoring procedure is the insertion of the Millar catheter in the small mesenteric vein, since this vein is extremely fragile. In our hands, about 10 training operations were needed for an experienced microsurgeon prior to technically mastering this procedure. Experience was defined of having successfully performed more than 50 vascular anastomosis (carotid artery, jugular vein) in rats or mice. The **third** limitation is that the portal pressure obtained with this method may be below the physiological range for a normal animal. Mesenteric vein ligation and catheter insertion may reduce the total volume of blood draining into the portal vein by an estimated 10%. However, this physiological range cannot be acquired using the currently available devices. Similarly, the effect of anesthesia itself on PVP cannot be excluded[13]. However, since all animals are subjected to the same intervention, the potential error would be a systematic error. Therefore, data interpretation should be done with caution focusing on relative changes within an animal and not necessarily on absolute differences between animals.

However, there are little alternatives to the invasive hemodynamic monitoring in rodents. Non-invasive monitoring is limited to the acquisition of the systemic blood pressure. Portal pressure or portal flow cannot be determined non-invasively in mice. Telemetric monitoring is also limited to the acquisition of the systemic blood pressure. No reports were found regarding the telemetric acquisition of other hemodynamic parameters.

This full intraoperative monitoring procedure is needed to understand hepatic physiological processes such as regulation of liver perfusion, liver regeneration and hepatobiliary surgery comprehensively. The ability to monitor and collect data of laboratory animals intraoperatively in real-time thus represents a significant advance in the study of liver disease and portal hypertension.

Disclosures

The authors declare that they have no competing financial interests.

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