Effects of Prolonged Pneumoperitoneum on Hepatic Perfusion During Laparoscopy

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Objectives: To assess the influence of prolonged pneumoperitoneum (PP) on liver function and perfusion in a clinically relevant porcine model of laparoscopic abdominal insufflation.

Background: PP during laparoscopic surgery produces increased intra-abdominal pressure, which potentially influences hepatic function and microcirculatory perfusion.

Methods: Six pigs (49.6 ± 5.8 kg) underwent laparoscopic intra-abdominal insufflation with 14 mm Hg CO2 gas for 6 hours, followed by a recovery period of 6 hours. Two animals were subjected to 25 mm Hg CO2 gas. Hemodynamic parameters were monitored, and damage parameters in the blood were measured to assess liver injury. Liver total blood flow and function were determined by the indocyanine green (ICG) clearance test. Intraoperative hepatic hemodynamics were measured by simultaneous reflectance spectrophotometry (venous oxygen saturation StO2 and relative tissue hemoglobin concentration rHb) and laser Doppler flowmetry (blood flow and flow velocity). Postmortem liver samples were collected for histological evaluation.

Results: A decrease in microvascular perfusion was observed during PP. After 6 hours of PP, ICG clearance increased (P < 0.001), indicating a compensatory improvement of overall liver blood flow resulting in concomitantly improved microcirculatory perfusion (P = 0.024). Minimal parenchymal damage (aspartate aminotransferase) of the liver was seen after 6 hours of PP (P = 0.006), which seemed related to PP pressure. Minor histological damage was observed.

Conclusions: The liver sustains no additional damage due to prolonged PP during laparoscopic surgery. Our findings suggest that prolonged PP does not hamper liver function or cause liver damage after extended laparoscopic procedures.

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Adverse effects of laparoscopic surgery are well recognized and include reduced postoperative pain, shorter length of hospital stay, and superior cosmetic results.1,2 Within the field of laparoscopy, the use of laparoscopy has increased substantially in recent years.3 Disadvantages associated with laparoscopic surgery include prolonged operation times and elevations in intra-abdominal pressure. Laparoscopic operations require insufflation of the abdominal cavity (pneumoperitoneum, PP) with carbon dioxide (CO2) gas to achieve adequate surgical exposure for instrumentation and surgical maneuvers. However, PP obviously produces elevated intra-abdominal pressure with continuous compression of intra-abdominal organs, which potentially influences hepatic microcirculatory perfusion. Intrapleural pressures during PP are higher (14 mm Hg) than the normal pressure in the hepatic portal system (7–10 mm Hg) and may, therefore, cause changes in liver portal blood flow and, consequently, hepatic function. Indeed, elevations in aspartate aminotransferases and alanine aminotransferases levels have been reported after laparoscopic operations; however, these elevations were transient and returned to normal within the first 3 postoperative days.4–6 Several studies described the effects of PP on intra-abdominal blood flow and reported diminished blood flow in the portal vein and the hepatic artery.1,7–10 Because these studies focused on the effects of blood flow after 1 hour of PP, 2 interesting questions emerged: first, to what extent does diminished blood flow influence liver function? And, second, what are the consequences after prolonged PP? The indocyanine green (ICG) clearance test is the most frequently used test for determining liver total blood flow and function11,12 and has previously been shown to be significantly reduced after 1 hour of PP, suggesting a decrease of liver function. However, it has been shown that this short-lived decline in liver function is associated with only a transient elevation of liver enzymes (aspartate aminotransferases and alanine aminotransferases). With laparoscopic liver resection, however, the duration of PP increases with longer periods of surgery. In addition to its effects on blood flow and liver function, the impact of continuous insufflation on hepatic microcirculation, as the principal site of metabolic exchange between the blood and tissue parenchymal cells, is also essential for assessing the influence of prolonged PP. These issues become more relevant with laparoscopic liver resection in which the liver may be subjected to additional injury when applying vascular inflow occlusion (Pringle maneuver).

The Oxygen to See is an instrument designed to perform simultaneous laser Doppler flowmetry and tissue spectrophotometry, using a single compact probe to detect microperfusion parameters.13 This device is capable of intraoperatively providing the characteristics of hepatic microcirculation.14,15 The Oxygen to See system has been extensively used for the determination of microcirculatory parameters in a wide range of surgical procedures in maxillofacial,16 cardiothoracic,17,18 plastic,19,20 and neurosurgical21,22 specialties.

This study was undertaken to investigate the influence of prolonged PP on liver function and hepatic microcirculatory parameters in a clinically relevant porcine model of extended abdominal insufflation.

METHODS

The study protocol was approved by the institutional Animal Experimentation Committee of the Academic Medical Center of the University of Amsterdam. Care and handling of the animals were in accordance with the European guidelines for the Institutional and Animal Care and Use committees.

Animals

Twelve female Landrace pigs (van Beek, The Netherlands) with a mean body weight of 47.0 ± 1.7 kg were used in this study. All animals were allowed to feed and drink ad libitum and remained
quarantined 1 week before the start of the investigation to permit adaptation to environmental conditions. All experiments were initiated in the morning after an overnight fast. An intramuscular injection of premedication consisting of ketamine (Nimetek; Eurovet, Bladel, The Netherlands; 15 mg/kg of body weight), midazolam (Dormicum; Actavis, Hafnarfjordur, Iceland; 1.5 mg/kg of body weight), and atropine sulfate (Pharmachemie, Haarlem, The Netherlands; 0.01 mg/kg of body weight) was administered before induction of anesthesia. The pigs were resting in a dorsally recumbent position on the operating table, and core body temperature was maintained at 37°C for the duration of the experimental procedures. All animals were orotracheally intubated. Before surgery, the animals received bolus doses of ketamine, midazolam, sufentanil (Hamlæn Pharmaceuticals, Hameln, Germany), and pancuronium bromide (Pavulon; Organon, Oss, The Netherlands). Anesthesia was continued with sufentanil (5–8 μg/kg/h of body weight), ketamine (10–14 mg/kg/h of body weight), midazolam (1–1.5 mg/kg/h of body weight), and pancuronium bromide (0.10–0.15 mg/kg/h of body weight) intravenously. Anesthesia was maintained with oxygen and/or air FiO2 45% O2 (1.5–3 L/min). All animals received 0.9% NaCl (Baxter, Utrecht, The Netherlands; 8–10 mL/kg/h) and 6% cloehaes (Tetraspan, Braun, Melsungen, Germany; 2–3 mL/kg/h) for electrolyte and metabolic homeostasis. Glucose 20% (Baxter Benelux, Brussels, Belgium) was given intravenously to maintain glucose levels between 5 and 10 mmol/L. Catheters were placed in the brachial artery, popliteal artery, and jugular vein, respectively, to continuously monitor heart rate, mean arterial blood pressure, and central venous pressure and for collection of blood samples. Open introduction was performed for bladder catheterization and placement of a 10-mm trocar in the abdomen for laparoscopy, followed by closure of the abdominal wall.

Experimental Design

Six animals underwent laparoscopic intra-abdominal insufflation for 6 hours via a 10-mm trocar (Olympus, Zoeterwoude, The Netherlands) with CO2 gas (14 mm Hg), followed by a recovery period of 6 hours. Two animals were subjected to 25 mm Hg intra-abdominal insufflation during 6 hours and were observed over a simulated recovery phase of 2 hours. Four pigs served as controls and did not undergo intra-abdominal insufflation. Hemodynamic parameters, such as arterial oxygen saturation (S\text{p}O\text{2}), heart rate, mean arterial blood pressure, central venous pressure, rectal temperature, and respiratory minute volume were continuously recorded. Arterial blood gas samples derived from the brachial artery were collected every hour and were analyzed using an automated analyzer (Blood Gas, Oximeter, and Electrolyte Systems, Acid Base Laboratory, Radiometer Medical, Copenhagen, Denmark), and measurements were obtained for pH, oxygen saturation, carbon dioxide, sodium, potassium, and hemoglobin. Glucose levels were also determined every hour.

The experiments started after a stabilization period of 60 minutes (0 mm Hg), in which baseline values were recorded. After 6 hours of insufflation (14 or 25 mm Hg), blood samples were collected for the determination of liver injury parameters and measurements of liver function using the ICG clearance test. Post-PP recovery measurements were performed at 2 and 6 hours. Aspartate aminotransferases, alanine aminotransferases, alkaline phosphatase, gamma-glutamyltransferase (γ GT), bilirubin, lactate, and lactate dehydrogenase levels were evaluated by routine clinical chemistry for the assessment of liver function. The left peripheral ear vein was cannulated for the administration of ICG solution for assessment of liver function. Hepatic hemodynamics were examined continuously for the entire duration of the protocol by simultaneous reflectance spectroscopy (StO2 and rHb) and laser Doppler flowmetry (blood flow and flow velocity), using Oxygen to See (LEA Medizintechnik GmbH, Giessen, Germany). At the end of each experiment, all animals were killed by infusion of KCl (60–90 mmol) under general anesthesia. For histological examination, postmortem biopsies of both the left and right liver lobes were obtained. An overview of the experimental design and measurement time points is presented in Figure 1.

Assessment of Hepatic Function and Perfusion

Hepatic Function

Hepatic function was determined using laboratory measurements and the ICG clearance test. The levels of aspartate aminotransferases, alanine aminotransferases, alkaline phosphatase, and γ GT were determined as liver damage parameters. ICG clearance was determined by the LiMON method. A 0.5 mg/kg of ICG solution (ICG-PULSION; Medical Systems AG, Munich, Germany) dissolved in 5 mL of sterile distilled water was injected in the left peripheral ear vein in all animals and for all measurements of liver perfusion. The LiMON device (LiMON, Pulsion Medical Systems AG, Munich, Germany) measures ICG elimination by pulse spectrophotometry. Details of this technique have been described elsewhere. Briefly, accurate and continuous recording of ICG blood levels was possible using a dichromatic densitometer, placed on the tail of the pig. Liver blood flow and function were determined by calculating the ICG clearance from the ICG retention rate 15 minutes after administration. As ICG clearance depends on liver perfusion, it is also a parameter of total liver blood flow.

Hepatic Microperfusion Assessments

Hepatic microperfusion parameters were assessed using Oxygen to See. The Oxygen to See device (Type LW 1/1/1/1, LEA Medizintechnik GmbH, Giessen, Germany) combines laser Doppler flowmetry and tissue spectrophotometry in 1 flat probe (LF1.027, LEA Medizintechnik GmbH, Giessen, Germany) and detects a wide range of microperfusion parameters. The optical methods for measuring these parameters have been previously described in

FIGURE 1. Timeline of experimental design (in 14 mm Hg PP group). All animals were subjected to the same procedures.

- Continuous assessments: hemodynamics;
- Every hour: blood gas analysis, glucose levels;
- Baseline measurements: continuous Oxygen to See, and after 1 hour, blood samples (liver function), and ICG clearance;
- Repeated measurements after 6, 8, and 12 hours:
  - Continuous Oxygen to See,
  - Blood samples for liver function, and
  - ICG clearance;
- 12 hours: liver biopsy for histological examination.
Central venous pressure increased significantly during insufflation and returned to baseline during desufflation in the 14 and 25 mm Hg groups \(^* P < 0.01\) for 3 and 6 hours of PP vs baseline in both groups; \(P < 0.001\) for 0 and 2 hours of recovery vs 6 hours of PP in both groups and 6 hours recovery vs 6 hours of PP in the 14 mm Hg group.

**Blood Gas Analysis**

The levels of pco\(_2\) increased significantly during PP (baseline 36.7 ± 0.7 mm Hg vs 46.1 ± 1.1 mm Hg after 6 hours of PP, \(P = 0.019\)), which is related to the CO\(_2\) gas used for PP. This resulted in respiratory acidosis and, consequently, a decrease in pH and pO\(_2\) values. pH decreased significantly from 7.49 ± 0.01 at baseline to 7.41 ± 0.01 after 6 hours of PP (\(P < 0.05\)). Baseline mean pO\(_2\) was 247.5 ± 5.9 mm Hg and after 6 hours of PP decreased to 202.7 ± 4.9 mm Hg (\(P < 0.05\)). Similar findings were observed for PP at 25 mm Hg but with greater differences. A pco\(_2\) baseline value of 48.4 ± 22.1 mm Hg was observed, which increased to 66.1 ± 35.6 mm Hg after 6 hours of PP (NS); pH at baseline was 7.47 ± 0.08 and decreased to 7.35 ± 0.12 after 6 hours of PP (NS), whereas pO\(_2\) decreased from baseline 192.8 ± 27.1 mm Hg to 180.9 ± 34.5 mm Hg after PP (NS). After desufflation, a significant decrease of pco\(_2\) was observed in the 14 mm Hg PP group at 0, 2, and 6 hours of recovery as compared with 6 hours of PP. As a consequence, pO\(_2\) and pH increased significantly after desufflation in the same group. Figure 3 shows the overall results of blood gas analysis.

**Biochemical Parameters**

The mean baseline aspartate aminotransferases level was 36.5 ± 3.8 U/L in the 14 mm Hg PP group (Fig. 4). A marginal, but statistically significant, increase in aspartate aminotransferases was found after 6 hours of PP (57.0 ± 16.6 U/L, \(P = 0.006\)). The elevated aspartate aminotransferases values persisted during the recovery period, whereas alanine aminotransferases levels did not show any statistically significant differences (results not shown). In the 25 mm Hg PP group, the liver damage parameter aspartate aminotransferases also increased (baseline: 38.8 ± 11.7 U/L; 6 hours of PP: 69.0 ± 46.7, \(P = 0.001\)). Aspartate aminotransferases levels were significantly higher in the 25 mm Hg group than in the 14 mm Hg group (\(P < 0.001\)).

**Liver Perfusion and Microcirculation**

ICG clearance increased significantly after 6 hours of PP and continued to increase during desufflation (\(* P < 0.001\) for 6 hours of PP and 2 hours of recovery vs baseline; Fig. 5), suggesting an improved total liver blood flow over time. The profile of ICG clearance in the 25 mm Hg group was comparable with ICG clearance at 14 hours of PP and returned to baseline after desufflation in the 14 and 25 mm Hg groups (\(P < 0.01\) for 3 and 6 hours of PP vs baseline in both groups; \(P < 0.001\) for 0 and 2 hours of recovery vs 6 hours of PP in both groups and 6 hours recovery vs 6 hours of PP in the 14 mm Hg group).
FIGURE 3. A, The levels of pCO₂ increased significantly during 6 hours of PP (∗P < 0.05 for 3 and 6 hours of PP vs baseline) in the 14 mm Hg PP group. After desufflation, a significant decrease of pCO₂ was observed at 0, 2, and 6 hours of recovery as compared with 6 hours of PP (∗P < 0.05). B, As a consequence, pO₂ decreased significantly during insufflation (∗P < 0.05 for 3 and 6 hours of PP vs baseline) in the 14 mm Hg PP group, after which an increase was observed after desufflation (∗P < 0.05 for 2 and 6 hours of recovery vs 6 hours of PP), as well as for pH. C, ∗P < 0.05 for 3 and 6 hours of PP vs baseline and 0 and 2 hours of recovery vs 6 hours of PP.

FIGURE 4. Changes in aspartate aminotransferases as a function of intra-abdominal pressure (PP) and time. Aspartate aminotransferases increased after 6 hours of PP (∗P = 0.006) in the 14 mm Hg PP group. The elevated aspartate aminotransferases levels persisted during the recovery period (∗P = 0.008). In the 25 mm Hg PP group, aspartate aminotransferases also increased after 6 hours of PP and was significantly higher than that in the 14 mm Hg PP group (∗P = 0.001).

FIGURE 5. ICG clearance increased significantly during insufflation in the 14 mm Hg PP group, which continued during desufflation (∗P < 0.001 for 6 hours of PP and 2 hours of recovery vs baseline; †P = 0.017 for 6 hours of recovery vs baseline). In the 25 mm Hg PP group, ICG clearance significantly increased at 2 hours of recovery compared with baseline (∗P = 0.006). No significant differences in ICG clearance were observed in the 14 and 25 mm Hg PP groups.

Blood flow velocity also slightly decreased during 6 hours of PP (NS, Fig. 6B). Oxygen saturation was elevated after an insufflation period of 6 hours, which subsequently normalized in the recovery period (14 mm Hg PP group). There were no significant differences between the 14 and 25 mm Hg PP groups.

Histology

No significant differences were seen for any of the histological parameters evaluated between the right and left liver lobes of the same pig or between the 14 and 25 mm Hg PP groups at all time points. No steatosis or centrolobular ischemic changes were found in any animal; portal and lobular inflammation was mild in all pigs. Sinusoidal dilatation in both groups ranged from 0 to 2 (median grade 2), focal lytic necrosis from 0 to 1 (median 0), portal edema from 1 to 3 (median grade 1), and confluent necrosis from 0 to 1 (median grade 0) (NS, within or between groups).
Effects of Prolonged Pneumoperitoneum on Hepatic Perfusion

We used the Oxygen to See probe to assess hepatic microcirculatory blood flow after prolonged PP. To our knowledge, no studies have correlated the effects of PP on hepatic function as measured by ICG clearance and on liver microcirculation. A significant negative correlation was seen for ICG clearance and microvascular blood flow in both groups, with restoration of ICG clearance after desufflation (P = 0.024).

In conclusion, a decrease in liver microvascular perfusion was observed during 6 hours of PP in both groups, with restoration of microcirculatory flow after desufflation (P < 0.01). A negative correlation was also observed between ICG clearance and blood flow velocity 6 hours after PP (r = −1.000, P < 0.01). No other significant differences were found.

It is important to consider that clearance of ICG from the blood depends on total blood flow of the liver. Therefore, the ICG clearance rate also reflects total liver circulatory dynamics. Several authors report a decrease in liver blood flow, as intra-abdominal pressure increased in pigs with different cutoff values for intra-abdominal pressure. This finding is in line with the results of this study in which microvascular perfusion decreased during 6 hours of PP as the intra-abdominal pressure increased, with a concomitant increase in microcirculatory parameters after desufflation, and increased ICG clearance.

Some recent experimental studies have demonstrated an impairment of liver function after PP. Hepatic injury was shown after 60 to 90 minutes of PP in rat models. Also liver regeneration rate was impaired, and oxidative stress and hepatocellular damage increased in rats undergoing PP before hepatectomy. These findings are not in accordance with our study. However, these differences may be explained by the choice of animal model, of which the porcine model obviously is more compatible with the clinical situation. Using a swine model too, Nsadi et al reported no hepatocellular injury or microcirculatory changes in pigs undergoing PP, which is in line with our results. Yet, with the application of portal triad clamping, the authors found increased hepatocellular damage parameters and an increased necrotic index. These results suggest caution when combining PP with portal triad clamping.

In literature, only 1 clinical study demonstrated the feasibility of Oxygen to See for intraoperative evaluation of hepatic microcirculation. Studies focusing on hepatic perfusion during prolonged PP and its effect on liver function are lacking. With this in mind, it is important to consider some weaknesses of the Oxygen to See device. First, disturbances through motion can falsify values for relative microcirculatory blood flow and velocity. This problem can be resolved by immobilizing the Oxygen to See probe on the liver surface, as was taken care of in our study. Second, there are no absolute values for single parameters and therefore the measured values cannot be interpreted without baseline values. In all animals of this study, baseline measurements were obtained to assess changes in hepatic microcirculation while each animal served as its own control. Third, if the probe is not properly affixed, stray light can influence oxygen saturation and hemoglobin concentration values. In this study, the probe was isolated from any external light sources because the abdomen was closed after the placement of the trocar for laparoscopy. Several other methods for evaluation of liver perfusion have been used, such as a transonic hepatic blood flow measurement, intravital fluorescence microscopy, or transesophageal Doppler ultrasonography. However, because most of these techniques are invasive, they are less useful for evaluating liver perfusion in a (pre)clinical setting.

Not only the level of intra-abdominal pressure during laparoscopic surgery is responsible for changes in liver function and perfusion but also the duration of PP influences (micro)circulatory hemodynamics. Hypoxia due to elevated levels of carbon dioxide potentially triggers vasoregulatory mechanisms and alters blood pressure, and thus, deprives the liver of the much needed oxygen to support the rich metabolism of the liver. One study evaluated the effects of 15 mm Hg of intra-abdominal pressure over a period of 24 hours and reported a reduction in function and morphological changes in the liver, lungs, kidneys, and bowel. Serum alanine aminotransferases and alkaline phosphatase levels were significantly elevated, and low-grade liver necrosis was observed. No literature currently exists establishing the effects of prolonged PP on hepatic perfusion and liver damage. In this study, we chose a clinically relevant PP time of 6 hours representing the duration of several complex abdominal laparoscopic procedures used today, such as major liver resections of 3 Couinaud segments or more.

In conclusion, a decrease in liver microvascular perfusion was detected during 6 hours of PP. After desufflation, hepatic microcirculatory blood flow was restored with a concomitant increase of ICG clearance, indicating a compensatory improvement of overall liver blood flow. The liver thereby sustained limited parenchymal damage during PP, which was related to PP pressure. Our findings suggest...
that prolonged PP does not hamper liver function after extended laparoscopic procedures.

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REFERENCES


