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Effects of carbon dioxide pneumoperitoneum on hepatic function in obstructive jaundice: an experimental study in a rat model

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Abstract

Background and aims The physiology of the patient during laparoscopy differs from that of open surgery. Both pneumoperitoneum and obstructive jaundice impair the hepatic function, but the combined insult has not been previously examined. In this study, we aimed to investigate the effects of carbon dioxide (CO_2) pneumoperitoneum on hepatic function in a rat model of obstructive jaundice.

Methods Forty-four male Sprague–Dawley rats were divided into four groups: group 1 ($n=10$), sham-operated group; group 2 ($n=12$), obstructive jaundice group; group 3 ($n=10$), CO_2 pneumoperitoneum group; and group 4 ($n=12$), obstructive jaundice and CO_2 pneumoperitoneum group. Common bile duct was ligated and divided in the obstructive jaundice groups. After 6 days, a 12-mmHg pneumoperitoneum was induced, maintained for 60 min, and released for 120 min. Blood samples were drawn for the measurement of white blood cell and platelet counts, serum liver enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], total bilirubin). Tissue samples were obtained for analyses of malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase (SOD) levels. We evaluated the degree of liver injury on a grading scale from 0 to 4, histopathologically.

Results Pneumoperitoneum after biliary obstruction resulted in an increase in AST and ALT levels and a decrease in white blood cell and platelet counts. However, changes in liver tissue MDA, GSH, and SOD levels did not correlate with the changes in AST and ALT levels and white blood cell and platelet counts. After sham operation with pneumoperitoneum, the GSH levels in liver homogenate were significantly decreased in the group 3 when compared to the group 2. On the other hand, obstructive jaundice itself caused significant reduction in the SOD activity of liver homogenate in comparison to the group 3. Histopathologically, sinusoidal congestion and vacuolization were more severe in the group 3.

Conclusions Alterations in hepatic function occur in pneumoperitoneum applied jaundiced subjects. However, there were no statistically significant differences between the groups 2 and 4 with regard to white blood cell and platelet counts, serum liver enzymes including AST, ALT, and total bilirubin values, MDA and GSH levels and SOD activity of liver homogenate, and histologic damage. These results indicate that there is no additional risk on liver function associated with pneumoperitoneum performed in obstructive jaundice.

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Introduction

Laparoscopic surgery is now being used in the vast majority of more extensive and time-consuming abdominal surgical operations including antireflux fundoplication, splenectomy, gastric or colorectal resection with its numerous advantages such as decreased postoperative pain, shorter hospital stay, reduction in complications, and lost working days [1]. However, several studies have shown that carbon dioxide (CO_2) pneumoperitoneum (PP) alters adversely systemic and pulmonary hemodynamic parameters, including reduced cardiac output and increased mean arterial pressure, vena caval pressure, and pulmonary arterial wedge pressure [2]. In addition, acid-base changes, bacterial translocation, and splanchnic ischemia have been demonstrated during CO_2 PP [3–5]. On the other hand, the intra-abdominal pressure of 12–14 mmHg of CO_2 traditionally used in laparoscopic surgery is higher than normal pressure values of the portal system (7–10 mmHg). This PP value, therefore, reduces portal flow and causes physiological alterations in liver function.

Biliary obstruction results in development of oxidant injury, hepatic fibrosis, biliary cirrhosis, and portal hypertension [6]. Although the mechanism of acute hepatocellular injury and resultant progressive fibrogenesis in bile flow obstruction is not well elucidated, oxidant stress has been proposed as one process which might be involved in both problems. Cholestasis per se reduces antioxidative capacities in liver mitochondria [7]. Accumulation of hydrophobic bile acids and inflammatory cells in the liver tissue may cause increased production of free radicals in biliary obstruction [8]. Bile acids, especially, enhance reactive oxygen species (ROS) released by polymorphonuclear leukocytes [9]. Moreover, in obstructive jaundice (OJ), the absence of intestinal bile flow, impaired reticuloendothelial system function and immunity, intestinal bacterial overgrowth, and physical disruption of the gut mucosal barrier explain the high incidence of infectious complications [10, 11].

Surgery in patients with OJ is associated with increased morbidity and mortality. Laparoscopy can be used in jaundiced patients with biliary tract or pancreatic head carcinoma both for staging and palliative treatment and in patients with common bile duct (CBD) stones for biliary exploration and clearance of their stones. On the other hand, cholestasis can be reproduced in rodents by surgical ligation of the CBD. The model of CBD ligation has been well evaluated and described in rats [12]. Although a detailed description of the morphological and molecular changes following bile duct ligation (BDL) in rats is lacking, this model has been used widely to study cholestatic liver injury [13], fibrogenesis [14], and the impact of OJ on a second hit such as infection or hepatic ischemia [15, 16]. Importantly, the effect of CO_2 PP on injury and repair in the different cellular compartments of the liver in the setting of biliary obstruction has not been characterized. Therefore, in this study, we investigated the impact of CO_2 PP on hepatocellular injury and proliferation, serum markers of cholestasis, and tissue indicators of oxidative stress in CBD-ligated rats

Materials and methods

Animal care and use

Male Sprague–Dawley rats weighing between 250 and 280 g were used in this study. Animals were housed in a light-controlled room with a 12-h light/dark cycle and were allowed free access to food and water. The operative procedure, use of anesthesia, and animal care methods in the experiments were consistent with the guidelines in the National Institute of Health's Guide for the Care and Use of Laboratory Animals (NIH publication number 86-23, revised 1985, Bethesda, Maryland). After obtaining the approval of Institutional Animal Use and Care Committee of Ankara University, Faculty of Veterinary Medicine, the experiment was performed at Experimental Research Center of Selcuk University, School of Medicine.

Operative details and study groups

After an overnight fast, the rats were anesthetized by an intramuscular injection of ketamine 50 mg/kg (Ketalar; Parke Davis, Eczacibasi, Istanbul, Turkey) and xylazine 10 mg/kg (Rompun; Bayer AG, Leverkusen, Germany). Animals were allowed to breathe spontaneously during the surgery. A heating lamp was used to preserve the body temperature at approximately 37°C. A total of 10 mL lactated Ringer's solution was given subcutaneously to prevent dehydration of the rats. After skin shaving and preparation of the abdominal wall with 10% povidone–iodine solution, a midline laparotomy was performed. The animals were allocated into four groups: in group 1 (sham-operated control group, $n=10$), these animals were anesthetized and underwent laparotomy, identical to other groups. The CBD was isolated, and a ligature was passed around the bile duct without ligation, then the abdominal incision was closed. The animals received food and water ad libitum. The animals were followed-up for 6 days to simulate the OJ in other groups. In group 2 (OJ group, $n=$ 12), the CBD was exposed carefully and doubly ligated

with 4-O monofilament glycomer (Biosyn, USSC, USA), then divided to prevent recanalization of the bile duct. In group 3 (CO_2 PP group, $n=10$), identical to sham-operated rats except for the induction of CO_2 PP. After 6 days, these animals were reanesthetized. The abdomen was punctured with a 20-gauge intravenous cannula, and the cannula was connected to an electronic insufflator (Storz & Co., Tutthufen, Germany). A 12 mmHg PP was induced at a flow rate of 0.2 L/min, maintained for 60 min, and released completely for 120 min. In group 4 (OJ and CO_2 PP group, $n=12$), the same procedures as described for groups 2 and 3 were performed.

On postoperative day 6, all animals were anesthetized again, and at the end of 2 h of desufflation period in the CO_2 PP groups 3 and 4, all animals in four groups were sacrificed. Blood samples were drawn by cardiac puncture into two sets of tubes, with and without ethylenediaminete-traacetic acid (EDTA). Then, the abdomen was opened and a small portion of the left lobe (for analysis of tissue malondialdehyde [MDA] level), median lobe (for analysis of tissue glutathione [GSH] level), and right lobe (for analysis of tissue superoxide dismutase [SOD] activity and histologic examination) of the liver were excised and immediately frozen in liquid nitrogen and stored at −80°C until assay.

Laboratory tests

These included white blood cell and platelet counts, serum liver enzymes, liver tissue MDA and GSH levels, and liver tissue SOD activities. All samples were blindly analyzed.

Blood tests

The tubes were centrifuged at 3,000 rpm for 30 min immediately after drawn, and plasma was separated. White blood cell and platelet counts and levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin were measured by automated analyzers using appropriate kits (Coulter AU 400, USA, and Olympus AU 400, Japan).

Analysis of MDA levels

The tissue level of MDA was determined in the liver specimen homogenized in a ratio of 1:10 (w:v) in 1.15% cold KCl solution. Zero point five milliliter homogenate was mixed with 3 mL of 1% phosphoric acid and 1 mL of 0.6% thiobarbituric acid solution. The mixture was heated in a water bath for 45 min. After cooling to room temperature, 4 mL of n-butanol was added, and the mixture was shaken vigorously. After centrifugation at 3,000 rpm for 5 min, the n-butanol layer's absorption was measured at

535 and 520 nm. The difference between the two absorptions was expressed as the MDA level in nanomoles per milligram of liver tissue [17].

Quantification of reduced GSH levels

The reduced-form GSH contents of liver tissue samples were measured by the method of Ellman [18]. Tissue samples were homogenized in 1 mL of 0.5 M metaphosphoric acid. This homogenate was centrifuged at 3,500 rpm for 10 min, and the supernatant was assayed. A standard solution was prepared in 3 mL amounts by mixing 0.3 mL of water, 0.3 mL of tamponate, and 2.4 mL of dithioni-trobenzoic acid. The sample mixtures were each prepared by mixing 0.1 mL of water, 0.3 mL of tamponate, 2.4 mL of dithionitrobenzoic acid, and 0.2 mL of supernatant. The absorption of the two mixtures was measured at 410 nm, and the change in absorption was read as optical density. The protein concentration of the same samples was measured by the method of Lowry [19]. The amount of GSH in the tissues was then expressed as micromoles per milligram of protein.

Determination of SOD activity

Liver samples were homogenized in 0.1 M Tris/HCl buffer, pH 7.2, containing 154 mM NaCl. The homogenate was centrifuged at 13,000 rpm for 15 min. The protein concentration of the supernatant was measured by the method of Lowry [19]. SOD activities of the samples were determined by inhibition of nitroblue tetrazolium reduction, and xanthine–xanthine oxidase was used as a superoxide generator in the same supernatants [20]. The results were expressed as unit per milligram of protein. One unit of SOD is defined as the amount that inhibits the rate of nitroblue tetrazolium reduction by 50%.

Histopathologic evaluation

A part of each liver was fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin (HE). Blind analysis was performed on all samples by a pathologist based on the criteria used by Suzuki et al. [21]. Histologic changes were scored from 0 to 4 based on sinusoidal congestion and vacuolization, necrosis of paren-chymal cells, and proliferation of bile ducts as shown in Table 1.

Statistical analysis

The results of liver injury scoring were expressed as median (range), whereas, other results were expressed as mean±SEM (the standard error of the mean). The

Table 1 Histologic criteria for assessment of liver damage

Numerical assessment	Sinusoidal congestion/vacuolization	Necrosis of parenchymal cells	Proliferation of bile ducts
0	–	–	None
1	None	None	Minimal
2	Minimal	Single-cell necrosis	Mild
3	Moderate	<30%	Moderate
4	Severe	>30%	Severe

differences among the groups were evaluated by using one-way analysis of variance followed by post-hoc test (Tukey's HSD test). The differences among the groups in terms of liver injury scores were evaluated using Kruskal–Wallis rank test, and multiple comparisons between the groups were performed with Mann–Whitney U test. The results were considered to be of statistical significance when $p < 0.05$. The data were analyzed by statistical software (SPSS for Windows 18.0; SPSS, Chicago, Illinois, USA).

Results

All animals, but four, tolerated the surgical procedures. Four rats in the OJ groups died during the experiment; one at postoperative day 2 and one at postoperative day 5 (group 2) and two during PP (group 4).

Blood test results

White blood cell and platelet counts and serum liver enzymes including AST, ALT, and total bilirubin values are shown in Table 2.

The levels of AST, ALT, and total bilirubin in the OJ groups (groups 2 and 4) were significantly higher than the values of the sham-operated group 1 and the PP-only group 3 ($p = 0.001$ and $p = 0.01$, respectively; Fig. 1). However, there were no significant differences between

the groups 2 (OJ+/PP-) and 4 (OJ+/PP+) in terms of the levels of AST, ALT, and total bilirubin ($p > 0.05$). PP itself (group 3) elevated the levels of AST and ALT significantly compared to the sham-operated group 1 ($p = 0.001$ for AST level and $p = 0.01$ for ALT level) but statistically lower than the groups 2 (OJ+/PP-) and 4 (OJ+/PP+; $p = 0.01$). On the other hand, group 4 (OJ+/PP+) had higher AST and ALT levels than the groups 2 (OJ+/PP-) and 3 (OJ-/PP+).

The white blood cell counts in the groups 2 (OJ+/PP-), 3 (OJ-/PP+), and 4 (OJ+/PP+) were significantly lower than the values of the sham-operated group 1 ($p = 0.007$, $p = 0.003$, and $p = 0.004$, respectively; Fig. 2). However, there were no significant differences between the groups 2 (OJ+/PP-) and 4 (OJ+/PP+) in terms of the white blood cell counts ($p > 0.05$). PP itself (group 3) decreased the white blood cell counts compared to the sham-operated group 1 ($p = 0.003$), but this reduction was not statistically significant in comparison to the OJ groups (groups 2 and 4; $p > 0.05$). On the other hand, group 4 (OJ+/PP+) had lower white blood cell counts than the groups 2 (OJ+/PP-) and 3 (OJ-/PP+).

Although the platelet counts in the groups 2 (OJ+/PP-), 3 (OJ-/PP+), and 4 (OJ+/PP+) were lower than the values of the sham-operated group 1, only the difference between the sham-operated group 1 and the group 4 (OJ+/PP+) was statistically significant ($p = 0.008$; Fig. 3). In addition, there were no significant differences between the groups 2 (OJ+/PP-) and 4 (OJ+/PP+) in terms of the platelet counts ($p > 0.05$). PP itself (group 3) reduced the platelet counts, but this decrease was not statistically significant when compared

Table 2 White blood cell (WBC) and platelet counts, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin values

Groups	WBC (×10³/μL)	Platelet (×10³/μL)	AST (U/L)	ALT (U/L)	Total bilirubin (mg/dL)
1 (OJ-/PP-)	17,430.0±1,826.2	776.3±36.7	136.8±7.4	44.2±2.7	0.1±0.02
2 (OJ+/PP-)	10,400.0±1,826.2	647.4±157.9	751.5±168.1	180.6±24.3	18.5±1.1
3 (OJ-/PP+)	9,770.0±1,257.0	510.9±141.9	347.4±48.0	67.6±8.0	0.1±0.05
4 (OJ+/PP+)	8,880.0±1,796.0	400.4±139.0	906.7±133.6	204.4±12.9	15.0±1.4

Results were given as mean±SEM (one-way analysis of variance (ANOVA) and Tukey's HSD test) for WBC and platelet counts and AST, ALT, and total bilirubin values

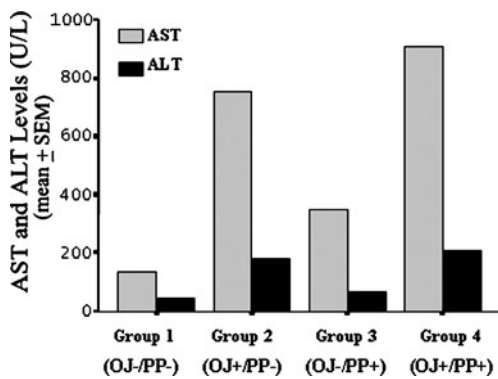*OJ* obstructive jaundice, *PP* pneumoperitonem

Fig. 1 The levels of AST and ALT in the groups (mean±SEM). *OJ* obstructive jaundice, *PP* pneumoperitoneum. There were no significant differences between the groups 2 and 4 ($p > 0.05$). PP itself (group 3) elevated the levels of AST and ALT significantly compared to the group 1 ($p = 0.001$ for AST level and $p = 0.01$ for ALT level) but statistically lower than the groups 2 and 4 ($p = 0.01$; one-way analysis of variance (ANOVA) and Tukey's HSD test)

to the OJ groups (groups 2 and 4; $p > 0.05$). On the other hand, group 4 (OJ+/PP+) had lower platelet counts than the groups 2 (OJ+/PP-) and 3 (OJ-/PP+).

Briefly, group 4 (OJ+/PP+) had lower white blood cell and platelet counts and higher levels of AST, ALT, and total bilirubin in comparison to the group 2 (OJ+/PP-), although these were not statistically significant ($p > 0.05$).

MDA and GSH levels and SOD activity of liver homogenate

The levels of MDA and GSH and SOD activity are shown in Table 3. The MDA and GSH levels and the SOD activities were not significantly different between the
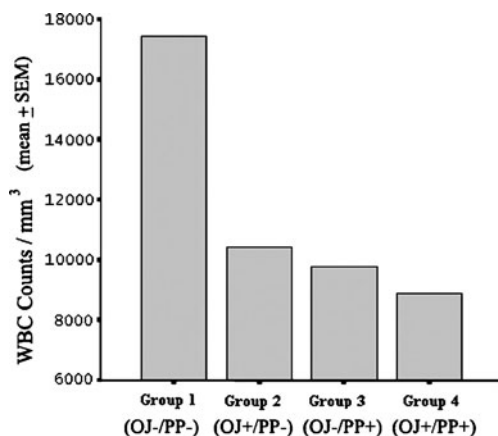

Fig. 2 White blood cell counts of the groups (mean±SEM). *OJ* obstructive jaundice, *PP* pneumoperitoneum. The white blood cell counts in the groups 2, 3, and 4 were significantly lower than the values of the group 1 ($p = 0.007$, $p = 0.003$, and $p = 0.004$, respectively). However, there were no significant differences between the groups 2 and 4 ($p > 0.05$; one-way analysis of variance (ANOVA) and Tukey's HSD test)
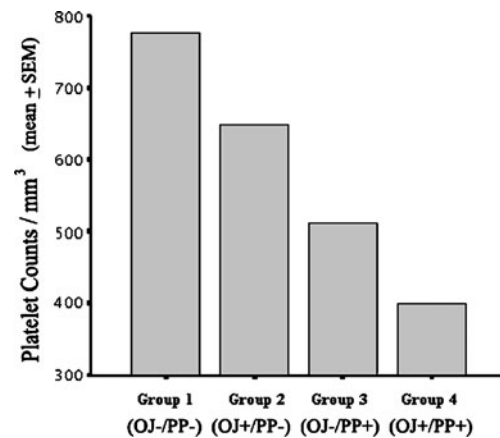

Fig. 3 Platelet counts of the groups (mean±SEM). *OJ* obstructive jaundice, *PP* pneumoperitoneum. Although the platelet counts in the groups 2, 3, and 4 were lower than the values of the group 1, only the difference between the groups 1 and 4 was statistically significant ($p = 0.008$). However, there were no significant differences between the groups 2 and 4 ($p > 0.05$; one-way analysis of variance (ANOVA) and Tukey's HSD test)

groups 2 (OJ+/PP-) and 4 (OJ+/PP+; $p > 0.05$). However, there were significant differences between the groups 2 (OJ+/PP-) and 3 (OJ-/PP+) in terms of the GSH levels ($p = 0.02$) and SOD activities ($p = 0.04$). After sham operation with CO_2 PP, the GSH levels in liver homogenate were significantly decreased in the group 3 when compared to the sham-operated group 1 and the group 2 (OJ+/PP-; $p = 0.001$ and $p = 0.02$, respectively; Fig. 4). On the other hand, OJ itself (group 2) caused significant reduction in the SOD activity of liver homogenate in comparison to the PP-only group 3 ($p = 0.04$). CO_2 PP-induced liver tissue injury might lead to a perturbation in antioxidant defense mechanism of liver, suggesting that more oxygen free radicals were produced in the CO_2 PP-only group 3, and GSH was rapidly consumed after elimination of free oxygen radicals. Similarly, OJ-induced liver tissue insult might cause an impairment in antioxidant capacity of liver, meaning that more ROS was generated in the OJ-only group 2, and SOD was quickly consumed before GSH after removal of free oxygen radicals.

Histopathological values

The degree of histologic damage seen in liver tissue is shown in Fig. 5. No morphological damage was observed in any of the rats in the sham-operated group 1. OJ and PP each caused sinusoidal congestion and vacuolization, but these were more severe in the PP-only group 3 than in the OJ-only group 2 and the group 4 (OJ+/PP+; $p = 0.001$ and $p = 0.04$, respectively). However, there were no significant differences between the groups 2 (OJ+/PP-) and 4 (OJ+/PP+) in terms of sinusoidal congestion and vacuolization ($p > 0.05$). Up

Table 3 Malondialdehyde (MDA) and glutathione (GSH) levels and superoxide dismutase (SOD) activity

Groups	MDA (nmol/mg tissue)	GSH (μmol/mg protein)	SOD (U/mg protein)
1 (OJ-/PP-)	43.9±3.6	1.6±0.07	21.2±1.7
2 (OJ+/PP-)	50.9±5.5	1.4±0.1	17.0±1.3
3 (OJ-/PP+)	44.2±3.2	1.1±0.04	22.0±2.0
4 (OJ+/PP+)	47.0±4.5	1.3±0.1	19.4±1.7

Results were given as mean±SEM (One-way analysis of variance (ANOVA) and Tukey's HSD test) for MDA and GSH levels, and SOD activity

OJ obstructive jaundice, *PP* pneumoperitonem

to 30% necrosis was seen in three rats in group 2 (OJ+/PP-), in two rats in group 3 (OJ-/PP+), and in two rats in group 4 (OJ+/PP+). In addition, apoptosis was seen in two rats in group 4 (OJ+/PP+). In terms of necrosis, however, there were no significant differences between any of the groups. Bile duct proliferation was observed only in the OJ groups (groups 2 and 4). Addition of the PP to the OJ did not affect the mean bile duct proliferation value (*p*>0.05), but severe ductal proliferation was seen only in the group 4 (OJ+/PP+).

Discussion

Laparoscopic surgery has become the standard procedure of cholecystectomy and is being increasingly used for more complex procedures. However, the use of laparoscopic surgery for jaundiced patients has not yet been established. The adverse effects of CO_2 PP on cardiac, pulmonary and renal function have been described in both experimental and clinical studies [22, 23]. Nevertheless, the effects of

CO_2 PP on hepatic function have not been studied in jaundiced patients. In the current study, a standardized rat model of BDL has been used to investigate the effects of CO_2 PP on hepatic function in OJ. No statistically significant differences were found between the groups 2 (OJ+/PP-) and 4 (OJ+/PP+) with regard to white blood cell and platelet counts, serum liver enzymes including AST, ALT, and total bilirubin values, MDA and GSH levels and SOD activity of liver homogenate, and histologic damage.

The insufflation of CO_2 into the peritoneal cavity to create the PP deteriorates hepatic cellular integrity with a subsequent increase in liver enzymes and alteration of the hepatic microcirculation [24–26]. Hepatic function during and after laparoscopic surgery has been examined in a number of clinical studies, and AST and ALT serum activities were found to be increased as a result of intra-abdominal pressure increase [24, 25, 27, 28]. Nickkholgh et al. clearly demonstrated the ischemia/reperfusion (I/R) injury after PP in an in vivo microscopy study of the liver in a rat model [26]. After deflation of the PP, they observed
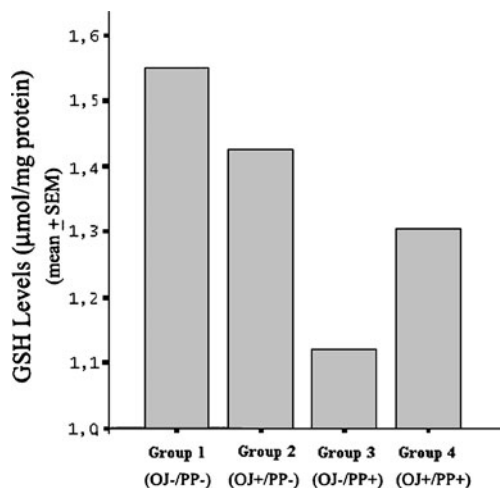

Fig. 4 GSH levels of the groups (mean±SEM). *OJ* obstructive jaundice, *PP* pneumoperitoneum. There were no significant differences between the groups 2 and 4 (*p*>0.05). Only the differences between the groups 1 and 3 and between the groups 2 and 3 were statistically significant (*p*=0.001 and *p*=0.02, respectively; one-way analysis of variance (ANOVA) and Tukey's HSD test)
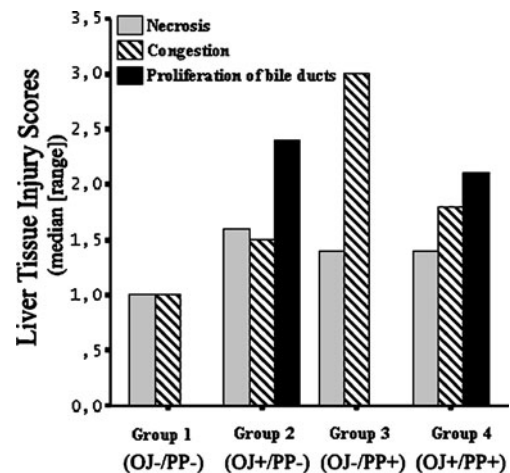

Fig. 5 The degree of histologic damage seen in liver tissue (median [range]). *OJ* obstructive jaundice, *PP* pneumoperitoneum. There were no significant differences between the groups 2 and 4 (*p*>0.05). Although sinusoidal congestion and vacuolization were developed in the groups 2, 3, and 4, these were more severe in the group 3 than in the groups 2 and 4 (*p*=0.001 and *p*=0.04, respectively; Kruskal–Wallis test and Mann–Whitney *U* test)

a release of transaminases as well as an increasing number of leukocytes and platelets permanently adherent to the endothelium in all subacinar zones together with activated Kupffer cells indicating microcirculation abnormalities. It has been shown that necrosis of even a few hepatocytes results in a high level of transaminase [29]. Therefore, the elevation of AST and ALT after CO_2 PP may be attributed to hepatocellular damage due to intra-abdominal hypertension-induced ischemic injury to the hepatocytes [24, 25, 27]. From the present study, it is clear that elevation of hepatic transaminases can occur after CO_2 PP or biliary obstruction. However, these risings of liver enzymes were not significant after laparoscopic insufflation in the jaundice group.

There is limited information on liver histologic changes during PP. The consequence of the histologic changes in humans appears to be clinically nonsignificant because the hemodynamic changes are completely reversible [30, 31]. An electron microscopy study by Izumi et al. found hepatic vascular endothelium changes after CO_2 PP in a murine model [32]. Schachtrupp et al. found low-grade paracentral hypoxic necrosis and moderate sinusoidal leukocyte infiltration after 24 h of CO_2 PP in a porcine model [33]. A study by Alexakis et al. showed a time course increase in portal inflammation, intralobular inflammation, edema, sinusoidal dilation, sinusoidal hyperemia, centrilobular dilation, centrilobular hyperemia, pericentrilobular ischemia, and focal lytic necrosis scores in a porcine model [34]. On the other hand, as early as 2 to 3 h after BDL, patches of hepatic necrosis are evident [35]. Kupffer cells adjacent to the necrotic areas are prominent and packed with phagocytic vacuoles and slight congestion of the sinusoids is apparent. Twenty-four hours after ligation, the proximal bile duct remnant is slightly dilated. After 2 to 3 days, dilatation and proliferation of the small intralobular and interlobular bile ducts occur [36]. Proliferation of fibroblasts is seen 7 days after BDL [37]. Later, infiltration by leukocytes and fibrosis occur with continued proliferation of ducts. The current study found liver histomorphology impairment after both CO_2 PP and BDL. In HE stained liver samples, OJ and PP each caused sinusoidal congestion and vacuolization in hepatocytes, but this was more severe in the PP-only group 3 than in the OJ-only group 2 and PP applied jaundiced subjects (group 4), indicating that ischemia or anoxia occurs during CO_2 PP [34]. These changes indicate hepatic tissue damage. However, there were no significant differences between the OJ-only group 2 and PP applied jaundiced subjects (group 4) in terms of sinusoidal congestion and vacuolization. Up to 30% necrosis was seen in three rats in group 2, in two rats in group 3, and in two rats in group 4. In addition, apoptosis was seen in two rats in group 4. In terms of necrosis, however, there were no significant differences between any

of the groups. Bile duct proliferation was observed only in the OJ groups (groups 2 and 4). Addition of the PP to the OJ did not affect the mean bile duct proliferation value, but severe ductal proliferation was seen only in PP applied jaundiced subjects (group 4).

The role of elevated intra-abdominal pressure generated by the PP in hepatic function and cell-conveyed immune response is well established. Several studies have showed that there is a direct correlation between portal blood flow changes and immediate effects on liver clearance function and phagocytic activity [38, 39]. Local defense and immune mechanisms might be suppressed by the altered pH during CO_2 insufflation. Macrophages, for example, exhibit less phagocytic activity under acidic conditions [40]. In a study reported by Gutt et al., phagocytic activity in the liver was analyzed during laparoscopy and laparotomy in a rat model. They found a decreased antigen elimination during CO_2 PP [41]. On the other hand, biliary obstruction in the rats results in a significant depression of the reticuloendothelial system phagocytic function, which may cause impaired systemic bacterial clearance and is associated with decreased survival following Escherichia coli endotoxemia [42]. In our study, we observed that leukocyte counts were decreased in both OJ-only group 2 and PP-only group 3. However, the white blood cell count in PP applied jaundiced subjects (group 4) was not significantly decreased in comparison to the OJ-only group 2. The decrease in white blood cell counts observed in our study might be explained by the same mechanisms. Although we did not measure portal blood flow and Kupffer cell activity, our observation may correlate well with the functional disturbances of the liver observed in our rat model.

MDA is an end product of peroxidative decomposition of polyenic fatty acids in the lipid peroxidation process. The concentration of MDA in liver homogenate is a direct marker for the level of oxygen radicals. There is some evidence that free radicals and lipid peroxides are generated and contribute to the pathogenesis of cholestatic liver injury [8, 43]. Liu et al. demonstrated excessive production of superoxide radicals and hydroxyl radicals in blood and liver in rats with OJ induced by ligation of the CBD [44]. On the other hand, abdominal deflation at the end of a laparoscopic procedure results in the generation of oxygen-derived free radicals, possibly as a result of an I/R phenomenon induced by the inflation and deflation of the PP. ROS provoke severe changes at cellular level leading to cell death, especially the Kupffer and the endothelial cells of the hepatic sinusoids, because of their extreme reactivity [45]. They attack to essential cell constituents, such as proteins, lipids and nucleic acids, and cause the modification of proteins leading to the formation of protein carbonyl groups [46]. Eleftheriadis et al. previously demonstrated that elevated intra-abdominal pressure with a 15-mm Hg CO_2

PP for 60 min in rats led to intestinal, spleen, liver, and lung oxygen free radical production [47]. In that study, significant quantities of MDA were released from the intestine, spleen, liver, and lung 30 min after intra-abdominal pressure abolition. We observed an increase in MDA concentrations in the liver tissue of bile duct-ligated rats. The increase in hepatic MDA levels with PP, however, was not significant in the group with OJ. This nonsignificant increase may be due to the short period of obstruction. In the early phase of OJ, antioxidants and free-radical scavengers may compensate for the injury. With prolonged obstruction, the peroxidation level may become high, resulting in liver injury, possibly due to decreased amounts of antioxidants.

GSH, a key antioxidant, is an essential component of the cellular defense mechanism against oxidative stress that induced by ROS. The primary site for de novo GSH synthesis is the liver, which supplies approximately 90% of the circulating plasma GSH [48]. Reduced GSH, which constitutes the main component of endogenous nonprotein sulfhydryl pool, is known to be a major low molecular weight scavenger of free radicals in the cytoplasm [49]. Because of their exposed sulfhydryl groups, nonprotein sulfhydryls bind a variety of electrophilic radicals and metabolites that may be damaging to the cells [50]. Oxidation of hepatic GSH occurs during biliary obstruction. It is possible that free radical generation in the liver is related to an increased oxidative stress in cholestatic rats and could spill over to other organ systems [51]. Huang et al. studied the mitochondrial functions in bile duct-ligated rats and suggested that biochemical and molecular changes are related to oxidative stress in the liver [52]. GSH is protective against postischemic injury by reducing lipid peroxidation. When animals with different preischemic GSH contents subjected to a 90-min of ischemia, followed by a 3-h of reperfusion, it was found that the early postischemic cell death was more extensive in rats with low initial GSH content than in rats with high GSH content [53]. Bickel et al. reported that, in a clinical setting, induction of positive pressure PP during elective laparoscopic cholecystectomy created I/R changes and revealed a decrease in total glutathione level after 30 min of PP termination [54]. In our study, by comparing hepatic GSH content after PP with changes after OJ, it was seen that the effect of PP alone was more severe than its effect after OJ. The decrease in GSH level suggests the same role in protecting the liver during PP. The decrease of GSH content after a long-term insult like OJ might be compensated for and the additional insult of PP might then cause only a nonsignificant reduction in GSH content.

The production of oxygen-derived free radicals by postischemic tissues may contribute to cell damage during I/R injury. The source of free oxygen radicals has been shown to be from xanthine oxidase (XO), an important enzyme in purine metabolism. XO has a role in free radical metabolism and catalyzes the conversion reaction of hypoxanthine to xanthine. In the presence of adequate purine substrate and oxygen, XO generates reactive oxygen metabolites such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH). The use of scavengers of superoxide and hydroxyl radicals, such as SOD, has been suggested to alleviate the problem of oxidative injury in various tissues. Recent studies suggest that disturbance of the oxidant–antioxidant balance might be responsible for cholestatic liver injury and that SOD enzymatic activity is hepatoprotective and play important roles in preventing oxidative stress [55]. The dismutation of free oxygen radicals with SOD leads to the creation of H_2O_2. H_2O_2 plays an important role as a free radical with different intra-abdominal pressures during CO_2 PP [56]. The activity of SOD would decrease after oxygen radicals are cleaned in liver tissues, indicating that the number of oxygen radicals is increased after CO_2 PP [57]. In our study, compared with the jaundice-only group 2, the PP applied group with jaundice (group 4) had not significant changes in the SOD activity. The decreased activity of SOD may be a sign that it had been used to scavenge free radicals in injured liver tissues. But the change in SOD activity was not correlated with the changes in GSH level. Further studies are needed to elucidate these findings.

Conclusion

In summary, the study was designed to be as clinically relevant as possible. To our knowledge no published reports have addressed the effect of PP after BDL. Alterations in hepatic function occur in PP applied jaundiced subjects. However, there were no statistically significant differences between the groups 2 and 4 with regard to white blood cell and platelet counts, serum liver enzymes including AST, ALT, and total bilirubin values, MDA and GSH levels and SOD activity of liver homogenate, and histologic damage. Our results indicate that there is no additional risk on liver function associated with PP performed in OJ. Despite experimental studies have proven to be useful, clinical studies are needed to test these findings in the clinical setting.

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