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doi:10.3748/wjg.15.3060

World J Gastroenterol 2009 June 28; 15(24): 3060-3064
World Journal of Gastroenterology ISSN 1007-9327

BRIEF ARTICLES

Hepatic injury induced by carbon dioxide pneumoperitoneum in experimental rats

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Supported by The Eleventh-five Medical Science Fund of Chengdu Military Command Area, No. MB07011
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Received: April 2, 2009 Revised: April 29, 2009
Accepted: May 6, 2009
Published online: June 28, 2009

Abstract

AIM: To observe the hepatic injury induced by carbon dioxide pneumoperitoneum in rats and to explore its potential mechanism.

METHODS: Thirty healthy male SD rats were randomly divided into control group (n = 10), 0 h experimental group (n = 10) and 1 h experimental group (n = 10) after sham operation with carbon dioxide pneumoperitoneum. Histological changes in liver tissue were observed with hematoxylin-eosin staining. Liver function was assayed with an automatic biochemical analyzer. Concentration of malonyldialdehyde (MDA) and activity of superoxide dismutase (SOD) were assayed by colorimetry. Activity of adenine nucleotide translocator in liver tissue was detected with the atractyloside-inhibitor stop technique. Expression of hypoxia inducible factor-1 (HIF-1) mRNA in liver tissue was detected with *in situ* hybridization.

RESULTS: Carbon dioxide pneumoperitoneum for 60 min could induce liver injury in rats. Alanine aminotransferase and aspartate aminotransferase were 95.7 ± 7.8 U/L and 86.8 ± 6.9 U/L in 0 h experimental group, and 101.4 ± 9.3 U/L and 106.6 ± 8.7 U/L in 1 h experimental group. However, no significant difference was found in total billirubin, albumin, and pre-albumin in the three groups. In 0 h experimental group, the concentration of MDA was 9.83 ± 2.53 μmol/g in liver homogenate and 7.64 ± 2.19 μmol/g in serum respectively, the activity of SOD was 67.58 ± 9.75 nu/mg in liver and 64.47 ± 10.23 nu/mg in serum respectively. In 1 h experimental group, the concentration of MDA was 16.57 ± 3.45 μmol/g in liver tissue and 12.49 ± 4.21 μmol/g in serum respectively, the activity of SOD was 54.29 ± 7.96 nu/mg in liver tissue and 56.31 ± 9.85 nu/mg in serum, respectively. The activity of ANT in liver tissue was 9.52 ± 1.56 in control group, 6.37 ± 1.33 in 0 h experimental group and 7.28 ± 1.45 (10^{-9} mol/min per gram protein) in 1 h experimental group, respectively. The expression of HIF-1 mRNA in liver tissue was not detected in control group, and its optical density difference value was 6.14 ± 1.03 in 0 h experimental group and 9.51 ± 1.74 in 1 h experimental group, respectively.

CONCLUSION: Carbon dioxide pneumoperitoneum during the sham operation can induce hepatic injury in rats. The probable mechanisms of liver injury include anoxia, ischemia reperfusion and oxidative stress. Liver injury should be avoided during clinical laparoscopic operation with carbon dioxide pneumoperitoneum.

Key words: Carbon dioxide pneumoperitoneum; Hepatic injury; Rat; Anoxia; Laparoscopic operation

Peer reviewer: James Neuberger, Professor, Liver Unit, Queen Elizabeth Hospital, Birmingham B15 2TH, United Kingdom

Xu GS, Liu HN, Li J, Wu XL, Dai XM, Liu YH. Hepatic injury induced by carbon dioxide pneumoperitoneum in experimental rats. *World J Gastroenterol* 2009; 15(24): 3060-3064 Available from: URL: http://www.wjgnet.com/1007-9327/15/3060.asp DOI: http://dx.doi.org/10.3748/wjg.15.3060

INTRODUCTION

Along with the utilization of laparoscope in surgery,

more and more patients can recover with less injuries and complications. However, laparoscopic operation is always limited due to exposure of the organs. Although carbon dioxide pneumoperitoneum is a desirable method to assist in exposing abdominal organs, the high pressure of carbon dioxide in abdominal cavity has some potential side effects, such as impairment of liver, kidney and heart functions[1-3]. Some researches revealed that the continuous high pressure from carbon dioxide during laparoscopic operation can result in ischemia injury in multiple organs, and the longer the operation lasts, the severer the injury is[2]. One of the important reported mechanisms of liver injury is ischemia reperfusion[1,2]. This study was to observe whether sham operation with carbon dioxide pneumoperitoneum causes liver injury and to explore its probable mechanism.

MATERIALS AND METHODS

Animals

Thirty healthy male SD rats were randomly divided into control group (n = 10), 0 h experimental group (n = 10), and 1 h experimental group (n = 10) after sham operation with carbon dioxide pneumoperitoneum. All experimental rats received sham operation for 1 h. Rats in the two experimental groups accepted carbon dioxide pneumoperitoneum during operation. The pressure of carbon dioxide was 15 mmHg. Liver tissue and serum were collected for further test.

Reagents

Oligo-nucletide probe of hypoxia inducible factor 1 (HIF-1) mRNA was produced by Shanghai Shenneng Biotechnology Company (China). ^{3}H-ADP and atractyloside (ATR) were obtained from Sigma Company (USA).

Methods

All rats were anaesthetized with pentobarbital sodium muscular injection. Rats in the two experimental groups received carbon dioxide pneumoperitoneum for 1 h during sham operation. Rats in the control group only underwent sham operation for 1 h. Blood samples and liver tissues were taken immediately from rats in 0 h experimental group and control group, and from rats in 1 h experimental group after sham operation, respectively. Liver function was detected with an automatic biochemistry analyzer. Histological changes in liver tissue were observed with hematoxylin-eosin (HE) staining under optical microscope. Concentration of malonyldialdehyde (MDA) in liver homogenate and serum was measured by thio-barbituric acid colorimetry using a spectrophotometer at the wave length of 532 nm and expressed as μmol/g. Activity of superoxide dismutase (SOD) was detected by xanthine oxidase colorimetry and expressed as nu/mg. Mitochondria in liver tissue were isolated by centrifugation. Activity of ANT in liver tissue was detected with the ATR-inhibitor stop technique. Mitochondria were initiated by adding ^{3}H-ADP and terminated after 12 s by adding ADR. Radioactivity in each group was measured and activity of ANT was expressed as 10^{-9} mol/min per gram protein. Expression of HIF-1 mRNA in liver tissue was detected with *in situ* hybridization (ISH). The results of ISH were quantified with an electronic computer and shown as absorbance (A) value.

Statistical analysis

Experimental data were expressed as mean ± SD. All data were analyzed by t test using SPSS 10.0 statistical software.

RESULTS

Liver function

Liver function in the two experimental groups was disturbed obviously compared with the control group (Table 1). After sham operation with carbon dioxide pneumoperitoneum, the level of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was 86.8 ± 6.9 U/L and 95.7 ± 7.8 U/L respectively in 0 h experimental group A, which was higher than that in control group (P < 0.05). The level of AST and ALT was 106.6 ± 8.7 U/L and 101.4 ± 9.3 U/L respectively in 1 h experimental group, which was higher than that in control group (P < 0.05). No significant difference was observed in the levels of total bilirubin (TB), albumin (A) and pre-albumin (Pre-A) between the two experimental groups.

MDA concentration and SOD activity in liver homogenate and serum

In control group, the concentration of MDA in liver homogenate and serum was 4.69 ± 1.31 μmol/g and 3.98 ± 1.05 μmol/g, respectively. After sham operation with carbon dioxide pneumoperitoneum, the concentration of MDA in liver homogenate and serum was significantly elevated in the two experimental groups (Table 2), indicating that liver is more susceptible to hypoxia injury. The activity of SOD in control group was 80.56 ± 12.43 nu/mg in liver homogenate and 75.66 ± 11.35 nu/mg in serum, respectively. The activity of SOD in liver homogenate and serum was significantly decreased in the two experimental groups (P < 0.05), demonstrating that more oxygen radicals are produced in the two experimental groups and SOD is consumed after elimination of oxygen radicals.

Histological changes in liver tissue

Liver samples were embedded in paraffin, stained with HE and then observed under an optical microscope. The livers of control group showed a normal lobular architecture with central veins and radiating hepatic cords, indicating that sham operation with routine anesthesia does not cause histopathological damage to liver. Mild hepatic fatty degeneration and necrosis were found in livers of the two experimental groups

Table 1 Changes in liver function (mean ± SD)

Group	TB (μmol/L)	ALT (U/L)	AST (U/L)	A (g/L)	Pre-A (mg/L)
Control	24.5 ± 5.1	48.5 ± 8.2	45.6 ± 7.7	38.5 ± 4.2	203.5 ± 76.4
0 h experimental group	23.1 ± 3.7	95.7 ± 7.8[a]	86.8 ± 6.3[a]	37.4 ± 3.1	195.8 ± 41.5
1 h experimental group	25.8 ± 3.5	101.4 ± 9.3[a]	106.6 ± 8.7[a]	40.6 ± 3.9	182.9 ± 58.4

[a]$P < 0.05$ *vs* control group.

Table 2 MDA concentration and SOD activity in liver homogenates and serum (mean ± SD)

Group	MDA (liver) (μmol/g)	SOD (liver) (nu/mg)	MDA (serum) (μmol/g)	SOD (serum) (nu/mg)
Control group	4.69 ± 1.31	80.56 ± 10.43	3.98 ± 1.05	75.66 ± 9.35
0 h experimental group	9.83 ± 2.23[a]	67.58 ± 9.75[a]	7.64 ± 2.39[a]	64.47 ± 10.23[a]
1 h experimental group	16.57 ± 3.45[a]	54.29 ± 7.96[a]	12.49 ± 4.21[a]	56.31 ± 9.87[a]

[a]$P < 0.05$ *vs* control group.

Table 3 Activity of ANT in mitochondria of liver (mean ± SD)

Group	ANT (10^{-9} mol/min per gram protein)
Control group	9.52 ± 1.76
0 h experimental group	6.37 ± 1.23[a]
1 h experimental group	7.21 ± 1.05[a]

[a]$P < 0.05$ *vs* control group.

(Figure 1A). The hepatic injury was more severe in 1 h experimental group B than in 0 h experimental group, suggesting that carbon dioxide pneumoperitoneum can cause hepatic injury (Figure 1B).

Activity of ANT in mitochondria of liver

In control group, the activity of ANT was 9.52 ± 1.56 (10^{-9} mol ADP/min per gram protein). In 0 h experimental group A, it was only 6.37 ± 1.33 ($P < 0.05$ compared with control group), indicating that energy metabolism in mitochondria of liver is damaged by carbon dioxide pneumoperitoneum. One hour after carbon dioxide pneumoperitoneum, the activity of ANT was slightly increased (7.21 ± 1.05) compared with control group ($P < 0.05$). However, it was lower in 1 h experimental group than in control group, indicating that energy metabolism in mitochondria of liver is recuperated to some extent after sham operation with carbon dioxide pneumoperitoneum (Table 3).

Expression of HIF-1 mRNA in liver tissue

No expression of HIF-1 mRNA was found in liver tissue from control group, indicating that sham operation without carbon dioxide pneumoperitoneum does not cause hypoxia stimulation in liver (Figure 2A). The expression of HIF-1 mRNA was significantly increased in the two experimental groups. The *A* value for HIF-1 mRNA was 6.14 ± 1.03 in 0 h experimental group and 9.51 ± 1.74 in 1 h experimental group ($P < 0.05$). Brown positive particles of HIF-1 mRNA, mainly located in cytoplasm of liver cells, were more in stromal cells than in hepatocytes (Figure 2B). Whether the stromal cells are hepatic stellate cells or endotheliocytes remains unknown. The expression of HIF-1 mRNA was increased more significantly in 1 h experimental group compared with 0 h experimental group, indicating that there exists persistent hypoxia stimulation in liver after carbon dioxide pneumoperitoneum (Figure 2C).

DISCUSSION

Laparoscopic operation, performed frequently in recent years, has many advantages over conventional surgery, such as less injuries and complications. Thus patients who accept laparoscopic operation can recover with a shorter healing time and less operative scars. However, exposure of organs is always not enough. Carbon dioxide pneumoperitoneum is a desirable method to assist in exposing abdominal organs. Some researches have shown that it has some potential side effects, such as impairment of liver, kidney, and heart functions[1-4]. It has been reported that the continuous high pressure from carbon dioxide during laparoscopic operation can result in ischemia injury of multiple organs, and the longer the operation lasts, the severer the injury is[5,6].

In this study, 1 h after sham operation with carbon dioxide pneumoperitoneum, the serum ALT and AST levels were increased while the levels of TB, A and Pre-A were not significantly changed. Since the half life of albumin is 14 d, the reduced albumin level can demonstrate the chronically impaired synthetic function of liver. However, the half life of prealbumin is only 2 d, and accordingly, a low level of prealbumin in serum indicates acute impairment of liver synthetic function. This study showed that liver function injury was not severe enough to cause hypoproteinemia. However, more susceptible markers of the liver function, ALT and AST, demonstrated mild impairment of liver function. It has been shown that necrosis of even a few hepatocytes results in a high level of transaminase[7-10]. In HE stained liver samples, fatty degeneration was found in some hepatocytes, indicating that ischemia or anoxia occurs during sham operation with carbon

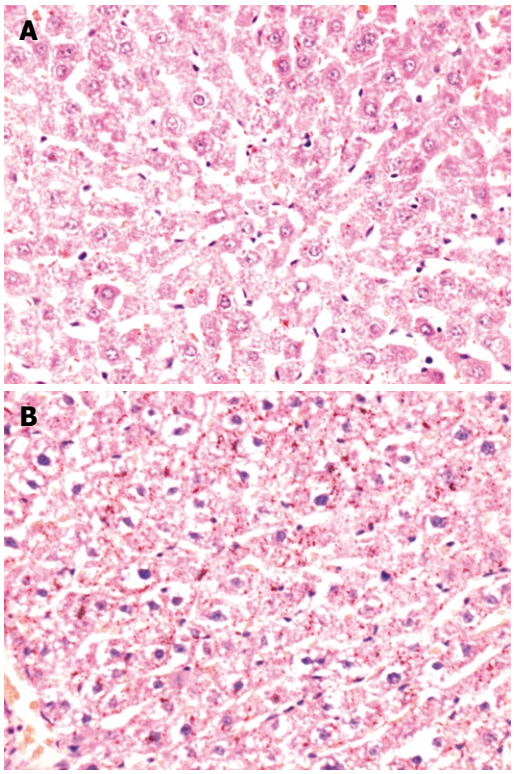

Figure 1 Liver injury in 0 h experimental group (A) and 1 h experimental group (B).

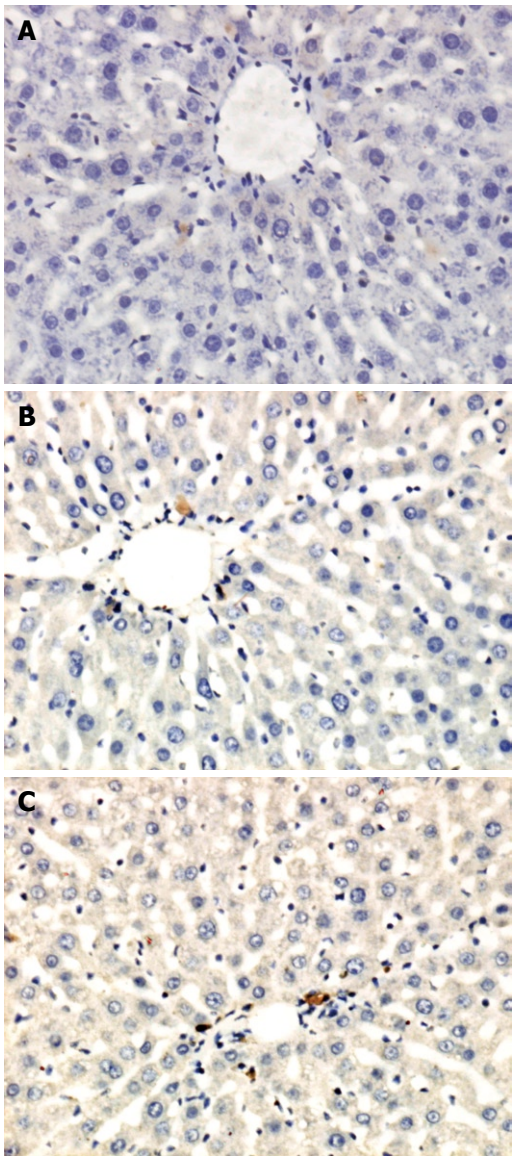

Figure 2 Expression of HIF-1 mRNA in liver tissue from control group (A), 0 h experimental group (B), and 1 h experimental group (C).

dioxide pneumoperitoneum[11]. The elevated expression level of HIF-1 mRNA in liver homogenate further indicates that anoxia injury is induced by carbon dioxide pneumoperitoneum. MDA is the end product of lipid peroxidation. The concentration of MDA in liver homogenate or serum is a direct marker for the level of oxygen radicals. SOD is one of the important scavenger enzymes of oxygen radicals. The activity of SOD would decrease after oxygen radicals are cleaned. In this study, the concentration of MDA was elevated and the activity of SOD was reduced in liver homogenate and serum, indicating that the number of oxygen radicals is increased after carbon dioxide pneumoperitoneum. Since liver is a motochondria-abundant organ, it is more susceptible to hypoxia than other organs. In this study, the activity of ANT, a marker of energy metabolism in mitochondria[12], was reduced after carbon dioxide pneumoperitoneum. The activity of ANT was mildly elevated 1 h after carbon dioxide pneumoperitoneum compared with the control group. It has been shown that blood-supply is obviously decreased in portal vein during carbon dioxide pneumoperitoneum[13]. In addition, hypercapnemia is related to ischemia injury of abdominal organs, while high pressure during operation and immediate relief of carbon dioxide after operation can induce ischemia reperfusion injury of multiple organs and apoptosis of hepatocytes after carbon dioxide pneumoperitoneum[14,15]. In this study, hepatic injury in rats and the possible mechanism of carbon dioxide pneumoperitoneum were elicited. Since pathophysiological changes in rats are not always identical as those in human beings, injury of carbon dioxide pneumoperitoneum should be observed closely in clinical practice. Although impairment of liver function usually does not cause severe complications, it should be alleviated or avoided especially in patients with preceding liver diseases during sustaining laparoscopic operation[16-20]. It has been shown that liver injury is pressure-dependant[21]. The pressure of carbon dioxide used in pneumoperitoneum is 10-12 mmHg, which is higher than that (7-10 mmHg) in the port system[22]. The higher pressure from carbon dioxide influences the systemic and portal blood flow dynamics[23-26], and causes apoptosis of hepatocytes[27,28]. A shorter time or a lower carbon dioxide pressure in pneumoperitoneum might help to alleviate liver injury. Stepwise increasing carbon dioxide insufflation might also be an ischemic preconditioning method to reduce liver injury[29]. Further study is needed on the precise mechanism of carbon dioxide pneumoperitoneum and more effective methods should be found to avoid liver injury[30].

COMMENTS

Background

Along with the utilization of laparoscope and carbon dioxide pneumoperitoneum in surgery, potential side effects of carbon dioxide pneumoperitoneum have been noticed in recent years.

Research frontiers

Some researches have shown that carbon dioxide pneumoperitoneum can induce impairment of liver, kidney, and heart functions. Apoptosis of hepatocytes was observed in this study.

Innovations and breakthroughs

This study showed mild injury of liver function in rats. At the same time, the expression level of hypoxia inducible factor-1 mRNA in liver tissue was increased as an evidence of anoxia in liver.

Peer review

This describes liver injury caused by carbon dioxide pneumoperitoneum in experimental rats. Although the injury does cause significant complications in rats, close attention should be paid to hepatic injury or other injury in clinical practice. The results of this study are of practical values.

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S- Editor Li LF **L- Editor** Wang XL **E- Editor** Zheng XM