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Full Length Research Paper

Portal vein arterialization used in partial hepatectomy maintains liver regeneration.

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Portal vein arterialization (PVA) has been used in some hepatobiliary surgeries and the liver transplants. There is considerable debate regarding the effect of PVA on the liver regeneration. This study was to investigate the effect of PVA on the liver regeneration. Sprague-Dawley rats were randomly divided into PVA group (rats received 68% hepatectomy, right nephrectomy and PVA) and control group (rats received 68% hepatectomy and right nephrectomy) , and the serum and liver tissues were collected at designed time points. Serum ALT level was measured with an automatic biochemical analyzer. The proliferation of hepatocytes was detected by immunohistochemistry for Ki-67 and PCNA. TUNEL staining was done to detect the apoptosis of hepatocytes and the liver regeneration rate (LRR) was calculated. At 1, 4 and 7 days after surgery, the ALT level in the PVA group was similar to that in the control group (*P*>0.05), while it on day 2 was higher than that in the control group (*P*<0.05). At 2 days after surgery, the number of Ki-67 and PCNA positive cells in the PVA group was higher than that in the control group (*P*<0.05). No significant difference in the proliferation was found between two groups at other time points. The number of apoptotic cells and LRR were also similar between two groups at different time points. PVA can initiate and maintain the proliferation of hepatocytes at the early stage following partial hepatectomy in rats.

Key words: Portal vein arterialization; liver regeneration; liver resection.

INTRODUCTION

Portal vein arterialization (PVA) was previously applied in patients with portal hypertension to maintain adequate blood supply to the liver. Due to the improvement of image-guided interventional technology and the development of liver transplantat technology, PVA is seldom used to in patients with portal hypertension (Maggi et al., 2010). Nowadays, studies showed the successful application of PVA in the treatment of extrahepatic bile duct cancer, acute liver failure(ALF) and orthotopic liver transplantation with portal vein thrombosis (Tsivian et al., 2007; Zhang and Meng, 2011). Considering the limited cases, unanatomical and unphysiological procedures, the clinical application of PVA is still controversial.

Increasing studies focused on the blood flow control during the surgery and the effects of PVA on the liver regeneration. Rat underwent 68% hepatectomy (PH) has been used in the liver regeneration model(Martins et al.,2008). In this model, the blood flow in the PVA is controlled with a 0.5 mm-diameter stent, and then the portal venous flow is at the physiological level. The average flow of an arterialized portal vessel is between 8±3 and 9±3 ml/min, similar to the recorded portal

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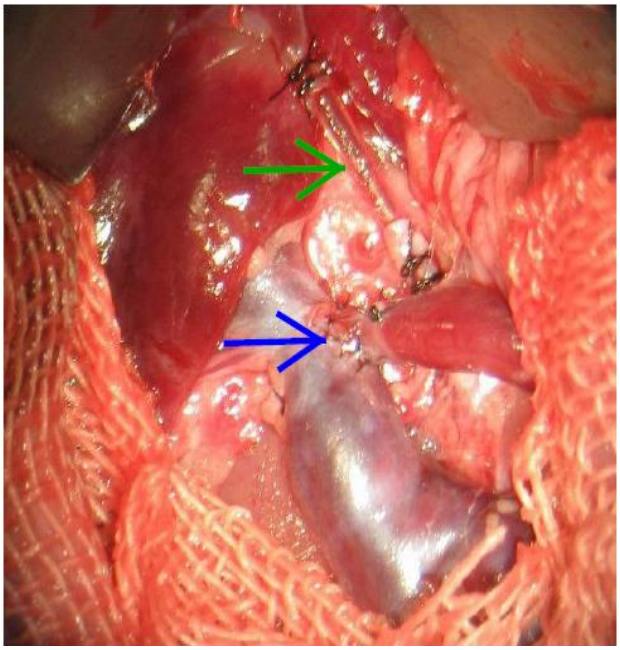

Figure 1. The proximal end of portal vein was anastomosed end-to-end to right kidney artery by a 0.5 mm-diameter polyethylene catheter (green arrow).The distal end of portal vein was anastomosed end-to-side to Venae Cavae (blue arrow).Using an operating microscope with 4 magnification.

venous inflow after a 68% PH (minimum: 7±2 ml/min; maximum: 8±2 ml/min). The normal liver is perfused by a dual blood supply via the hepatic artery and portal vein; about 80% of the total blood flow is poorly oxygenated venous blood delivered through the portal system. While PVA is performed, the portal venous blood will be completely or partially replaced with arterial blood. Thus, it is necessary to revaluate the effects of PVA on the liver regeneration. Recent studies suggested that PVA could maintain the liver regeneration(Fan et al.,2002; Chen et al.,2008); however, a series of studies carried out by Schleimer K et al revealed that PVA could increase hepatocyte apoptosis and inhibit the liver weight gain and thus could not achieve long-term effectiveness(Schleimer et al., 2008). Therefore, further studies are required to investigate the effect of PVA on the liver regeneration.

This study was to explore the effect of PVA on the liver regeneration in an animal model in which rats underwent 68% hepatectomy and PVA, which may provide evidence for the medical application of PVA.

MATERIALS AND METHODS

Male Sprague-Dawley (SD) rats weighing 280 to 350 g were purchased from the Beijing VITAL RIVER Laboratory Animal Technology Co., Ltd. Animals were allowed to acclimatize for 1 week and given *ad libitum* access to food and water. The animals received no nourishment other than water *ad libitum* for a period of 12 to 24 h preoperatively. The present study was approved by the Animal Ethics Committee of Inner Mongolia Medical College and animal care was in accordance with the National Institutes of Health guidelines. A total of 48 rats were randomly divided into two groups (n=24 per group): control group (rats received 68% PH and right nephrectomy) and PVA group (rats received 68% PH, right nephrectomy and PVA). Six rats of each group were sacrificed respectively on postoperative days 1, 2, 4, 7. According to detection index , the serum and liver tissues were collected at designed time points.

Animal anesthesia and surgical procedures

All animals were anesthetized by intraperitoneal injection of ketamine at 100 mg/kg. Operations were carried out under a surgical microscope (4-25×; YZ20T4; Beijing Suohong Electronics Co., Ltd). Right nephrectomy and 68% PH were performed in all animals according to previously reported (Higgins and Anderson,1931). In the control group, the portal vein was blocked for 10 min following 68% PH. In the PVA group, the proximal end of portal vein and the right renal artery were anastomosed with a polyethylene tube (0.5 mm in diameter), and the blood flow was observed at high magnification; the end-to-side anastomosis of the distal end of portal vein to the inferior vena cava was performed (Figure 1). Hepatic artery of all animals was kept intact.

Serum alanine aminotransferase test

Serum alanine aminotransferase (ALT) was tested using an

Table 1. Postoperative ALT activity.

Group	n	1 day	2 days	4 days	7 days
PVA group	24	367.67±126.12	198.17±26.65*	115.00±49.51	57.83±20.56
Control group	24	411.00±72.26	145.67±43.56	118.33±53.51	61.67±18.82

Abbreviation: ALT, Alanine Transarninase; PVA, Portal Vein Arterialization compared with control group, *P ＜0.05.

automatic biochemical analyzer (Roche Modular DPP System, Germany).

Measurement of liver weight and liver regeneration rate

Before surgery, all animals were weighed. During the operation, part of liver was removed and immediately weighed. The weight of the remaining liver was calculated by the following formula: whole liver weight - weight of the resected liver. The whole liver weight was about 3.1 ± 0.34% of body weight which was determined in previous experiments in 50 animals. Six rats in each group were killed at postoperative days 1, 2 and 7. The remaining liver was collected and weighed. LRR（abbreviation of Liver Regeneration Rate）is one of important index to valuate liver regeneration, and is more objective than liver weight alone (Vanheule et al., 2011, Yao et al., 2009). According to previously described (Fan et al., 2002; Child et al., 1953), the liver regeneration rate (LRR) at different time points was determined.

$$\frac{\text{(Liver weight at autopsy - estimated residual liver weight at the time of surgery)}}{\text{Weight of resected liver}} \times 100\%$$

Detection of hepatocyte proliferation and apoptosis

Immunohistochemistry SABC (immunohistochemical strept-avidin-biotin complex) method was employed to detect the expressions of Ki67 and PCNA (Zyada, 2009). Cells positive for Ki67 and proliferating cell nuclear antigen (PCNA) have brownish yellow or yellow granules in the nucleus. The number of positive cells was counted as follow: five fields were randomly selected in each section and representative image was captured at high magnification (×100). The number of positive cells at high magnification was calculated in each field and the data were averaged.

Terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL method, kit purchased from Bio Co., Ltd KGI, Nanjing, China) was used to detect the apoptosis of hepatocytes. In brief, paraffin sections were prepared and depariffinized; *in situ* cell apoptosis detection was performed according to manufacturer's instructions. PBS was used instead of TdT as negative control(Loo, 2002; Costa et al.,2010). The brownish yellow nucleus was defined as positive. One section was obtained from each sample in each group. Five fields were selected at high magnification (×400) and the total number of cells and the number of TUNEL positive cells counted followed by averaging.

Statistical analysis

Data were expressed as mean ± SEM and analyzed using SPSS version 13.0. Comparisons between two groups were conducted with Mann-Whitney-U-test and T-test. A value of P <0.05 was considered statistically significant.

RESULTS

All animals in the control group survived; however, in the PVA group, four animals died postoperatively due to a postoperative hemorrhage or a narcosis incident. The postoperative survival rate in the PVA group was 83%. Therefore, surgery was performed in other rats and another 4 rats post-operatively survived were recruited into the PVA group.

ALT detection

The degree of liver injury is negatively correlated to the state of liver regeneration. Detection of aminotransferase level after hepatectomy can indirectly reflect liver regeneration. A 1 day after surgery, the ALT level in both groups reached a peak level, and then gradually decreased. On post-operative day 2, the ALT level in the control group was markedly lower than that in the PVA group (P<0.05), whereas no significant difference was found between two groups at 4 and 7 days after surgery (P>0.05) (Table 1).

LRR

Throughout study, LRR increased gradually, but there was no significant difference between two groups at different time points (Table 2).

Hepatocyte proliferation

At 2 days after surgery, the number of Ki-67 positive cells increased in both groups, but the increase in the PVA group was more obvious (P<0.05). At 4 and 7 days after surgery, the Ki67 positive cells decreased in both groups and no significant difference was found between two groups at different time points (P > 0.05) (Figure 2A, 2B, Figure 3A).

Table 2. Liver regeneration rate at different time points.

Group	1 day	2 days	7 days
Control group	24.50±9.29	52.41±6.4	94.55±6.92
PVA group	28.62±8.67	49.39±4.8	92.95±6.68

Liver regeneration rate between two groups had no significant difference at different tested time points.

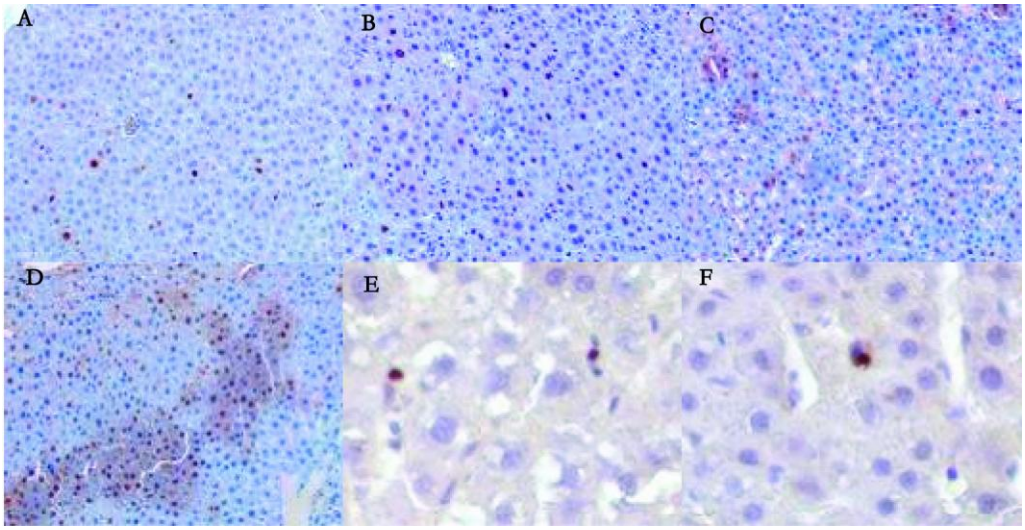

Figure 2. Ki-67-positive cells 24 h in controls(A) and PVA treatment(B), magnification 100× each. PCNA-positive cells 48 h in controls(C) and PVA treatment(D), magnification 100× each. Apoptosis cells 48 h in controls(E) and PVA treatment(F), magnification 400× each.

The number of PCNA positive cells increased in both groups on post-operative day 2 but the increase in the PVA group was more evident (P <0.01). At 4 days after surgery, the number of PCNA positive cells was similar between two groups (P>0.05) (Figure 2C, 2D, Figure 3B).

Hepatocyte apoptosis

In the present study, the number of apoptotic cells in both groups decreased in the initial stage and then increased. In the PVA group, the number of apoptotic cells was higher than that in the control group, but no significant difference was noted at different time points (P <0.05) (Figure 2E, 2F, Figure 3C).

DISCUSSION

Previous study suggested that the insufficient supply of nutrition to the liver following PVA was attributable to the changes in liver regeneration (Schleimer et al., 2008).Therefore, this technique usually achieves poor the long-term efficacy. However, clinical trials on liver transplants reported by Ringers et al (2006) and Bonnet et al(2010) showed the blood supply of the hepatic portal vein was mainly originated from the renal vein or artery. Soon after surgery, the liver experienced a rapid regeneration, and liver function remained favorable during the 1.5 to 6 years follow-up period. Particularly, for cases with blood supply of portal vein from the renal artery, symptoms related to portal hypertension were not observed after the blood flow was restricted(Ringers et al.,2006; Bonnet et al.,2010). The results from studies above not only pose a challenge to the theory of liver growth factors, but also support long-term effectiveness of PVA.

A couple of methods can be used to assess the liver regeneration such as the detection of liver weight, serum enzyme level, and immunohistochemistry for nuclear antigen (such as PCNA, Ki-67), etc(Fan et al.,2002; Schleimer et al.,2008; Assy and Minuk,1997). To detect the liver weight is the most convenient method, but the results are often affected by the amount of lipids, glycogen and blood in the liver. Thymidine kinase and fibronectin protein serve as important indicators in the

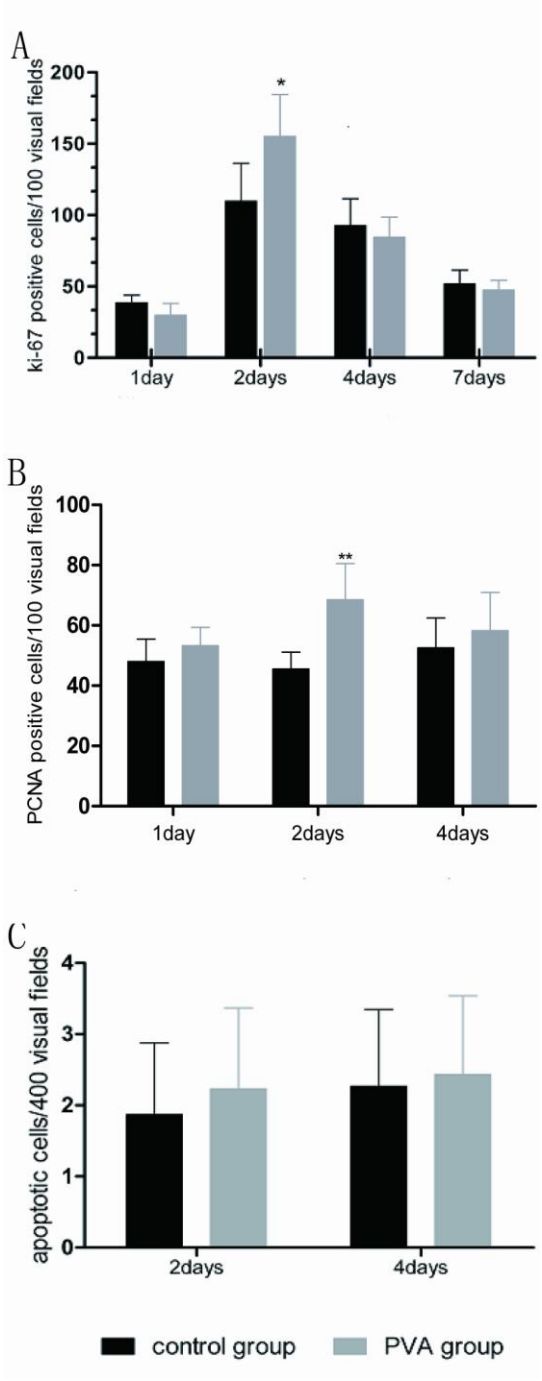

Figure 3. (A) Number of Ki-67-positive cells, at different time points,*P<0.05. (B) Number of PCNA positive cells at different time points, **P<0.01. (C) The number of apoptotic cells between the two groups at different time points had no significant difference.

detection of serum enzymes, but this method is not sensitive enough to assess the liver regeneration induced by PH.

Immunohistochemistry for nuclear antigens (Ki-67, PCNA) are acceptable and commonly used in studies，but those methods are subject to inter- and intra-observer variability(Assy and Minuk,1997). Therefore, in the present study, the combined methods were employed to evaluate the liver regeneration. At 2 days after surgery, immunohistochemistry showed the number of Ki-67 and PCNA positive cells in the PVA group was higher than that in the control group (P<0.05); but no difference was found between two groups at other time points. Moreover, the LRR was not markedly different between two groups at different time points. Our results revealed the liver regeneration was initiated and maintained after surgery in both groups. This finding was consistent with previously reported by Fan et al (2002) and Chen et al (2008).

Schleimer et al. (2008) detected the hepatocyte apoptosis using M30 Cytodeath immunohistochemistry Their results showed that animals undergoing PVA had post-operative day 2 (P<0.01); but no significant difference was found in the number of apoptotic cells between two groups at 4 days after surgery. In our study, TUNEL assay was employed to detect the hepatocyte apoptosis following hepatectomy. Our results showed the number of apoptotic cells between two groups were no significant different at different time points, which was different from what reported by Schleimer et al (2008). This might be attributed to different methods for the detection of apoptosis. TUNEL assay is a common method with high-specificity in the detection of apoptosis (Loo, 2002; Costa et al., 2010). In the present study, the right renal artery was anastomosed to the portal vein which declines the time of warm ischemia from 30 min to 10 min. Therefore, the liver ischemia-reperfusion injury is reduced and the hepatocyte apoptosis improved. This modified surgery may be an important cause of discrepancy in the results between this study and that of Schleimer et al. (2008).

Conclusion

Although our results showed the PVA could initiate and maintain liver regeneration in the early stage following PH, the exact mechanism by which PVA affect liver regeneration is not currently known. It has been postulated that proper portal blood supply is essential to initiate and maintain liver regeneration after PH. With an equivalent portal inflow rate of either venous or arterial source, the hepatic regeneration response can be sustained. PVA may also affect liver regeneration through the other possible mechanisms (Fan et al., 2002). PVA may significantly increase oxygen concentration which is thought to promote hepatocytes proliferation (Holecek et al., 1991; Nardo et al., 2006). However, the molecular mechanisms of that mentioned above have not been clarified and need to be studied further.

The present results are preliminary but meaningful, suggesting a possible new strategy for some special situation, such as ALF and orthotopic liver transplantation with portal vein thrombosis.

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