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Effect of selective hepatic inflow occlusion during liver cancer resection on liver ischemia−reperfusion injury

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ABSTRACT

Objective: To study the effect of selective hepatic inflow occlusion during liver cancer resection on liver ischemia-reperfusion injury. **Methods:** A total of 68 patients with primary liver cancer who underwent left liver resection in our hospital between May 2012 and August 2015 were selected for study and divided into group A (selective hepatic inflow occlusion of left liver) and group B (Prignle hepatic inflow occlusion) according to different intraoperative blood occlusion methods, serum was collected before and after operation to determine liver enzyme content, the removed liver tissue was collected to determine energy metabolism indexes, inflammation indexes and oxidative stress indexes. **Results:** 1 d, 3 d and 5 d after operation, GPT, GOT, GGT, LDH and ALP content in serum of both groups were significantly higher than those before operation, and GPT, GOT, GGT, LDH and ALP content in serum of group A 1 d, 3 d and 5 d after operation were significantly lower than those of group B; ATP, ADP, AMP, PI3K, AKT, GSK3 β , T-AOC, PrxI and Trx content in liver tissue of group A were significantly higher than those of group B while PTEN, IL-12p40, MDA and MPO content were significantly lower than those of group B. **Conclusions:** Selective hepatic inflow occlusion during liver cancer resection can reduce the liver ischemia-reperfusion injury, improve the energy metabolism of liver cells and inhibit inflammation and oxidative stress in liver tissue.

1. Introduction

Liver cancer is one of the malignant digestive system tumors with highest incidence in our country, and surgical resection is an important method of clinical treatment of liver cancer. The blood supply to the liver tissue is rich, and effective intraoperative hemostasis is the key operation step. Pringle room-temperature hepatic inflow occlusion is a clinical common way to control hepatic inflow and reduce intraoperative bleeding, it doesn't need to anatomize the first porta hepatis and the operation is relatively simple[1,2]. However, the non-selective total hepatic vascular exclusion of the method will cause ischemia-reperfusion injury of the non-removed liver. Selective hepatic inflow occlusion of left liver is a way of selective hepatic inflow occlusion that is suitable for intraoperative blood occlusion of left liver resection and can avoid ischemia-reperfusion process in non-removed liver tissue, thus reducing the ischemia-reperfusion injury of overall liver tissue[3,4]. In the following study, the effect of selective hepatic inflow occlusion during liver cancer resection on liver ischemia-reperfusion injury was analyzed.

2. Materials and methods

2.1. Research subjects

A total of 68 patients with primary liver cancer who underwent left liver resection in our hospital between May 2012 and August 2015 were selected for study, were all diagnosed with liver cancer that was confined to the left lobe of liver by preoperative imaging

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and pathological examination, conformed to the indications of left liver resection, were with preoperative liver function Child-Pugh grade A and normal blood coagulation function, and did not receive radiotherapy and chemotherapy as well as interventional therapy. According to different intraoperative blood occlusion methods, they were divided into group A and group B, 34 cases in each group. Group A received selective hepatic inflow occlusion of left liver and included 22 male cases and 12 female cases that were (59.6±7.8) years old; group B received Prignle hepatic inflow occlusion. The two groups of patients showed no significant difference in general information (P>0.05).

2.2. Operation methods

Both groups received left liver resection under general anesthesia, abdominal median incision was made to enter the abdominal cavity, and group A received selective hepatic inflow occlusion of left liver according to the following method: the perihepatic ligament of left liver was freed, the first porta hepatis was anatomized, the Glisson sheath was opened, then the left hepatic artery and right hepatic artery were separated, ligatured and cut off, the left portal vein was separated from behind the hepatic artery, suspended and ligatured, the hepatic inflow of right liver is not occluded, then the liver ischemia line was observed, the liver was removed, and the abdominal cavity was closed after properly hemostasis; Group B received Prignle hepatic inflow occlusion: hepatoduodenal ligament was freed, No. 8 catheter was used for occlusion, each occlusion time was 10-15 min and opening time was 5 min, there were 3 times of occlusion at most, the liver was removed in the process of blood occlusion, and the abdominal cavity was closed after properly hemostasis.

2.3. Evaluation methods of indexes in serum

Before operation as well as 1 d, 3 d and 5 d after operation, peripheral venous blood was collected from two groups of patients to separate serum, and full-automatic biochemical analyzer was used to determine glutamate pyruvate transaminase (GPT), aspartate transaminase (GOT), γ-glutamine transferase (GGT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) levels.

2.4. Evaluation methods of related indexes in liver tissue

After intraoperative removal of liver tissue, appropriate amount of liver tissue with normal incisal margin (about 50-60 mg) was collected, wash with normal saline, then added in 0.5 mL of PBS buffer, fully homogenized and then centrifuged, the supernatant was kept, enzyme-linked immunosorbent assay kit was used to determine PTEN, IL-12p40, PI3K, AKT and GSK3β content, immunoprecipitation kit was used to determine MDA, MPO, T-AOC, CAT and PrxI-Trx content, high-pressure liquid chromatography was used to determine ATP, ADP and AMP content, and finally BCA kit was used to determine the content of total protein and calculate the content of target molecules per mg protein sample.

2.5. Statistical methods

SPSS20.0 software was used to input and analyze data, measurement data analysis was performed by t test and P<0.05 indicated statistical significant differences.

3. Results

3.1. Liver function indexes at different points in time before and after operation

Before operation, GPT, GOT, GGT, LDH and ALP content in serum were not significantly different between two groups (P>0.05); 1 d, 3 d and 5 d after operation, GPT, GOT, GGT, LDH and ALP content in serum of both groups were significantly higher than those before operation (P<0.05), and GPT, GOT, GGT, LDH and ALP content in serum of group A 1 d, 3 d and 5 d after operation were significantly lower than those of group B (P<0.05), shown in Table 1.

3.2. Energy metabolism in liver tissue

Energy metabolism indexes ATP, ADP and AMP content in liver tissue of group A were significantly higher than those of group B

Table 1
Comparison of liver function indexes between two groups at different points in time before and after operation (U/L).

Groups (Case No.)	Time	GPT	GOT	GGT	LDH	ALP
Group A	Before operation	34.5±5.3	27.5±3.6	15.6±2.4	103.5±15.2	31.9±5.2
(n=34)	1 d after operation	154.8±19.7△	114.6±15.7△	68.7±9.3△	194.5±25.7△	93.5±10.4△
	3 d after operation	183.2±23.1△	156.5±20.4△	114.5±15.2△	254.4±33.4△	165.3±20.5△
	5 d after operation	134.5±17.8△	129.5±17.9△	92.4±10.4△	217.8±24.8△	121.4±17.7△
Group B	Before operation	35.1±4.9	29.1±3.8	16.1±1.9	105.1±14.9	32.4±5.7
(n=34)	1 d after operation	242.1±29.7△	189.4±22.6△	103.4±14.5△	289.5±33.7△	168.6±22.1△
	3 d after operation	315.4±37.8△	243.2±34.6△	186.5±22.4△	401.5±64.5△	245.7±31.6△
	5 d after operation	267.4±33.9△	215.4±27.6△	136.6±17.8△	337.6±38.7△	193.4±22.8△

△: compared with same group before operation, differences were statistically significant, P<0.05; ▲: compared with group B at same point in time, differences were statistically significant, P<0.05.

(P<0.05), shown in Table 2.

Table 2
Comparison of energy metabolism indexes in liver tissue (ng/mg).

Groups	Case No.	ATP	ADP	AMP
Group A	34	57.81±7.93	17.25±2.14	30.56±4.52
Group B	34	33.52±5.65	9.33±1.05	13.42±1.94
t		8.928	9.482	15.582
P		<0.05	<0.05	<0.05

3.3. PTEN/PI3K/AKT-mediated inflammation in liver tissue

PTEN and IL-12p40 content in liver tissue of group A were significantly lower than those of group B while PI3K, AKT and GSK3 β content were significantly higher than those of group B (P<0.05), shown in Table 3.

3.4. Oxidative stress products and antioxidant capacity in liver tissue

MDA and MPO content in liver tissue of group A were significantly lower than those of group B while T-AOC, PrxI and Trx content were significantly higher than those of group B (P<0.05), shown in Table 4.

Table 4
Comparison of oxidative stress products and antioxidant capacity in liver tissue.

Groups	Case No.	Oxidative stress products (nmol/mg)		Antioxidant capacity (U/mg)		
		MDA	MPO	T-AOC	PrxI	Trx
Group A	34	1.83±0.25	1.04±0.12	15.48±2.17	5.46±0.74	12.36±1.56
Group B	34	3.37±0.51	2.27±0.35	7.15±0.89	3.41±0.45	5.76±0.71
t		8.308	11.485	13.048	7.684	12.348
P		<0.05	<0.05	<0.05	<0.05	<0.05

4. Discussion

Hepatic inflow occlusion during liver cancer resection can reduce the intraoperative blood loss, but the ischemia and reperfusion process can cause liver damage[5,6]. The most basic pathological changes of ischemic injury and ischemia-reperfusion injury are ischemia hypoxia-induced glycogen consumption and ATP depletion, and the liver parenchyma cell death and rupture occur[7,8]. Liver cell rupture can cause many kinds of enzymes in the cytoplasm released into the blood circulation, the determination of liver enzyme levels in serum can reflect the degree of liver ischemia-reperfusion injury, and the analysis of serum liver enzyme levels at different points in time before and after operation confirmed that serum liver enzyme levels of both groups significantly increased after operation and GPT, GOT, GGT, LDH and ALP content in serum of group A after treatment were significantly lower than those of group B. This means that both selective hepatic inflow occlusion and Prignle hepatic inflow occlusion can cause different degree of liver damage, and after selective hepatic inflow occlusion, serum liver enzyme levels were lower and the degree of liver function damage is less. Further analysis of the energy metabolism in liver tissue between two groups of patients showed that ATP, ADP and AMP content in liver tissue of group A were significantly higher than those of group B. It shows that selective hepatic inflow occlusion has less influence on energy metabolism of liver cells, and after occlusion, energy substance consumption is relatively small in liver tissue.

In the liver ischemia-reperfusion process, in addition to the damage caused by ischemia hypoxia and ATP depletion, the inflammation and oxidative stress activated by ischemia factors and reperfusion factors are also associated with liver damage. PTEN/PI3K/AKT is the important signaling pathway that regulates cell function in the body, and the function of the pathway is affected by the phosphorylation and dephosphorylation[9]. The oxidative phosphorylation process is apparently unusual in ischemia-reperfusion liver tissue, which will affect the function of PTEN/PI3K/AKT signal pathway. PTEN can cause the dephosphorylation of PI3K, AKT, GSK3 β and other signal molecules, thereby inhibiting the expression and activation of inflammatory cytokine IL-12p40[10]. Studies have shown that the PTEN inhibitor can increase the expression of PI3K/AKT and inhibit the expression of IL-12p40, and the expression level of PTEN significantly increases in the liver ischemia-reperfusion process[11,12]. In the study, analysis of inflammation mediated by PTEN/PI3K/AKT in the liver tissue between two groups showed that PTEN and IL-12p40 content in liver tissue of group A were significantly lower than those of group B while PI3K, AKT and GSK3 β content were significantly higher than those of group B. This means that the selective hepatic inflow occlusion can inhibit the high expression of PTEN-mediated inflammatory factors, thus reducing the inflammatory damage of the liver.

Oxidative stress is also the important pathologic factor causing liver ischemia-reperfusion injury. PrxI-Trx antioxidant enzyme system is the most important antioxidant enzyme in the liver tissue, and it is of great significance for the scavenging of oxygen free radicals and oxidative stress products[13,14]. In the liver ischemia-reperfusion process, the expression of MPO significantly increases, and it can

Table 3
Comparison of PTEN/PI3K/AKT-mediated inflammation in liver tissue (ng/mg).

Groups	Case No.	PTEN	PI3K	AKT	GSK3 β	IL-12p40
Group A	34	1.95±0.22	5.65±0.76	2.44±0.37	0.92±0.10	20.33±3.52
Group B	34	3.42±0.51	3.04±0.55	1.21±0.18	0.44±0.06	37.69±4.74
t		9.181	7.868	11.039	12.475	8.958
P		<0.05	<0.05	<0.05	<0.05	<0.05

change H_2O_2 and Cl- into hypochlorous acid and form oxygen free radicals, leading to oxidative damage of liver cells and massive generation of lipid oxidation product MDA. In the continuous generation process of oxygen free radicals, antioxidant enzymes PrxI and Trx are constantly consumed and the antioxidant capacity of liver tissue decreases significantly[15,16]. In the study, oxidative stress products and antioxidant capacity in liver tissue of two groups were analyzed, and the results showed that MDA and MPO content in liver tissue of group A were significantly lower than those of group B while T-AOC, PrxI and Trx content were significantly higher than those of group B. This means that the selective hepatic inflow occlusion can reduce the oxidative stress in liver tissue, reduce the consumption of antioxidant enzymes and enhance the antioxidant capacity of the tissue.

To sum up, selective hepatic inflow occlusion during liver cancer resection can reduce the liver ischemia-reperfusion injury, improve the energy metabolism of liver cells and inhibit inflammation and oxidative stress in liver tissue.

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