study of portal vein embolization with absolute ethanol injection in cirrhotic rats

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Abstract

AIM To investigate the effects of portal vein embolization (PVE) with absolute ethanol injection on the cirrhotic livers.

METHODS Absolute ethanol was injected intraportally into normal and cirrhotic SD rats and the changes of the animals in anatomy, pathology, liver function as well as portal hemodynamics were observed.

RESULTS At a dose of 0.05mL/ 100g of ethanol, the survival rate was 100% in normal rats compared with 40.9% in cirrhotic rats. PVE in the cirrhotic rats with 0.03mL/100g of ethanol, caused significant hypertrophy in non-embolized lobes, mild or moderate damage to the hepatic parenchyma, slight and transient alterations in liver function, portal pressure and portal flow.

CONCLUSION PVE with absolute ethanol injection in the setting of liver cirrhosis could be safe at an appropriate dose, and precautions aimed at preserving liver function were preferable.

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INTRODUCTION

Portal vein embolization (PVE) plays an important role in the management of hepatocellular carcinoma (HCC). We modified the conventional method of transcatheter embolization and developed a new PVE technique with ethanol injection via a fine needle in experimental study[1] and subsequent clinical application under guidance of portoechography[2]. To further elucidate the therapeutic basis of this technique, particularly its effects on the cirrhotic liver, we observed the alterations in liver anatomy, pathology, biochemistry and portal hemodynamics in cirrhotic rats undergoing PVE with ethanol injection.

MATERIAL AND METHODS

Reproduction of cirrhotic rat model

Normal Sragues-Dawly (SD) rats with a body weight between 200 g - 250 g obtained from The Laboratory Animal Center of our university were used. Based on our previous method of producing cirrhotic dog model[3], a dose of 0.3 mL/ 100 g of 60% CCl_4 solution was injected subcutaneously at abdominal wall of rats once every 4 days. Throughout the period, the rats were fed with ordinary food and 5% ethanol as drinking water. Pathohistological examination confirmed the development of cirrhosis at 60 days after initial administration of CCl_4 solution.

Measurement of weight ratio of rat liver lobes

Livers of 20 SD normal rats were resected under anaesthesia and weighed, and then the right, middle and left lobes of each liver were weighed individually. The mean weight ratios of right, middle and left lobes to the whole liver were 40.5%, 36.5% and 23.0%, respectively.

Test of rat tolerance to ethanol

Laparotomy was performed in normal rats (n = 10) and cirrhotic rats (n = 22) under intraperitoneal anaesthesia with penobarbitol. After exposure of the hepatic hilum, portal vein was punctured with a 3-gauge needle and a dose of 0.05 mL/ 100g of absolute ethanol was injected into the vessel. The test showed that all rats in the normal group kept alive and only 9 (40.9%) of 22 in the cirrhotic group were alive 4 days after operation.

PVE

According to the method of PVE described above

and the results of tolerant test, a dose of 0.05 mL/100g of absolute ethanol was used to embolize the portal branches of left and middle lobes in normal rats (NE group, $n = 24$), in which the embolized tissue accounted for 77% of the whole liver. In cirrhotic rats (ME group, $n = 24$), the portal branch of middle lobe (accounting for 36.5% of the liver) was embolized with a dose of 0.03mL/ 100g of absolute ethanol. In addition, the same quantity of normal saline was injected into portal vein in 10 normal rats (NC group) and 10 cirrhotic rats (MC group).

Investigations after PVE

The following aspects were investigated at day 1, 3, 7 and 14 after PVE, respectively: patency of the portal system by X-ray portography; the weight ratio of hepatic lobes; blood biochemical profile (ALT, TBIL, ALP, ALB, A/G); pathohistological changes of liver tissue microscopically; and portal blood flow (Qpv) and portal pressure (Ppv) measured by MRF-1200 electromagnetic flowmetry (NIKON, Japan).

RESULTS

The survival rate after PVE was 95.8% in both NE and ME groups. Postoperative X-ray portographies demonstrated filling defects at injection sites and the corresponding portal branches were embolized. Dissection of the intrahepatic portal system revealed that in the sites of filling defects on portographies, there were intraluminal red thrombi, which varied from 10 mm to 20 mm in length. The thrombi were soft in consistency and easily separated from the vascular wall at day 1 and 3 after PVE, became less bright at day 7, and then brightless, fragile and tightly adhered to the wall at day 14.

The embolized hepatic lobes gradually atrophied and the non-embolized lobes became hypertrophical with the time after PVE. The weight ratio of the embolized lobes to the non-embolized lobes at the 14th post-PVE day was significantly higher than the physiological value (Table 1) statistically.

Pathohistological examination revealed focal intimitis with development of thrombi at the site of ethanol injection, degen eration of hepatocytes, infiltration of inflammatory cells and focal coagulation necrosis at the regions surrounding the portal triads of the embolized lobes. With respect to the ratio of total necrosis area to embolized area, ME group (30% - 40%) was more severe than NE group (10% - 20%). One week after PVE, organization or calcification of thrombi with partial recanalization, as well as hyperplasia of fibrotic tissue in the embolized lobes, was noted. In addition, hepatocytes of the non-embolized lobes became hypertrophic and proliferative, which was more remarkable in ME group than in NE group.

Blood biochemical investigations showed that hepatic functional parameters were significantly higher in MC group than in NC group (Table 2). ALT, TBIL and ALP in both NE and ME groups were elevated from their own baseline values in small amplitude after PVE, began to fall at the day 3, and returned to the baseline values at week 1 after injection. In contrast, there was no obvious change of ALB and A/G in both groups after PVE. ME group had significantly higher portal blood flow and portal pressure than NE group before PVE. At the day 1 after PVE portal blood flow and portal pressure declined slightly in both groups, thereafter elevated in a limited degree, and returned to the baseline values at 1 week after PVE.

Table 1 The weight ratio of embolized lobe to the whole liver

		Time after PVE (days)				
		1(n=6)	3(n = 6)	7(n = 6)	14(n = 6)	Baseline value(n = 20)
A. nonembolized lobe (g)	NE	3.04±0.29	2.75±0.20	4.04±0.42	5.25±0.38	1.86±0.42
	ME	8.04±0.22	7.71±0.17	8.63±0.24	9.58±1.10	5.13±0.53
B. whole liver (g)	NE	10.62±0.32	8.63±0.28	8.12±0.33	7.63±0.14	8.08±0.51
	ME	12.29±0.31	12.09±0.21	12.13±0.21	11.04±0.72	8.08±0.51
A / B (%)	NE	30.0	31.9	49.8[a]	68.8[a]	23.0[a]
	ME	65.5	63.8	71.7	86.8[b]	63.5[b]

[a],[b]P<0.01, as compared with each own baseline value by Student's t test.

Table 2 Changes in portal pressure and portal blood flow after PVE

		Time after PVE (days)				
		1(n = 6)	3(n = 6)	7(n = 6)	14(n = 6)	Baseline value(n = 20)
FPP (kPa)	NE	1.27±0.23	1.82±0.20	1.82±0.32	1.52±0.59	1.67±0.48[a]
	ME	2.82±0.50	3.67±0.37	3.96±1.65	3.17±0.50	2.97±0.33[a]
PVI (mL / min)	NE	13.30±2.16	14.50±2.07	14.30±1.75	13.40±2.20	13.50±3.03[b]
	ME	14.50±4.04	16.80±2.48	18.30±9.05	17.17±7.49	18.20±4.16[b]

[a],[b]P<0.01, as compared with each own baseline value by Student's t test.

DISCUSSION

In comparison with transcatheter embolization, the technique of PVE with absolute ethanol injection via fine needle has the advantages of easy attainment of hyperselective embolization, simple manipulation and avoidance of radiation exposure. Studies of PVE with absolute ethanol injection in normal dogs have been reported[1,4]. However, the fact is that 80% of patients with HCC in our country have underlying cirrhosis, therefore it is necessary to research into its effects on cirrhotic liver so as to better orientate its clinical application. The present study demonstrated that the cirrhotic rats became much less tolerant to ethanol than the normal rats, so the dose of ethanol used for PVE should be strictly controlld. On the other hand, the results shoerd that once PVE is undertaken with a tolerant dose of ethanol, the changes in hepatic histology and anatomy, liver function and portal hemodynamics in cirrhotic rats were no more severe and persistent than those of normal rats, even though these aspects were significantly different between the two groups before PVE. It indicated that a good postoperative course could also be achieved in cirrhotic rats with an appropriate dose of ethanol.

Like other methods of portal vein (PV) blockade, PVE with ethanol injection could effectively lead to proliferation and hypertrophy of non-embolized lobes. It was due to the fact that nourishing factors carried by PV blood were increasingly drained to the non-embolized lobes after PVE, which caused proliferation of endoplasmic reticulum, increase in number of mitochondria and synthesis of ATP, speeding up synthesis of DNA and RNA[5], and thus improving the liver function. This may not only account for that abnormality of hepatic function after PVE in cirrhotic rats could recover over a short period of time, but suggest that indications of hepatectomy for HCC could be broadened as well. In addition, it was found that PV thrombus induced by ethanol injection was often subject to organization or calcification with partial recanalization 2 weeks after PVE, which suggested that resection of HCC should not be delayed too long in clinical practice.

After being injected into PV, ethanol mainly mixed with blood and gradually led to formation of thrombus. The part entering into the hepatic parenchyma was usually diluted and induced a relatively mild damage to the tissue. It was advantageous to the patients with liver cirrhosis. Meanwhile, our results showed that PVE caused more severe hepatic necrosis in cirrhotic rats than normal rats, which may be related to a reduced resistance of cirrhotic liver to injury. Prophylactic administration of antibiotics was necessary to prevent development of postnecrotic hepatic abscess.

In addition to the mechanic obstruction, some other factors could affect the portal pressure. Ligation of PV led to an elevated portal pressure and reduced portal blood flow, which returned to the normal values within 1-4 weeks[5]. In contrast, PVE with ethanol injection caused a slightly different pattern of portal hemodynamic changes, in which portal pressure and blood flow rose after an transient initial decline, and returned to the baseline values within 2 weeks. Two reasons were presumed to contribute to the difference. One was that blood flow was not blocked completely at the beginning after ethanol injection. The other was due to the instant injury of ethanol to liver tissue and accumulation of acid metabolites, which induced a lowered PV pressure. With reference to this regularity, for those patients with remarkable portal hypertension undergoing PVE, it is advisable to administer β-blocker in short term for lowering PV pressure from the 3rd day after PVE.

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