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— Original Article —

An experimental study of percutaneous absolute ethanol injection therapy for small hepatocellular carcinoma: Effects of absolute ethanol on the healthy canine liver

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Summary: As a basic study of percutaneous ethanol injection therapy (PEIT) under ultrasound guidance, 1 ml of absolute ethanol was injected into the healthy liver of adult mongrel. Ultrasonographic, macroscopic and histologic studies of the injection site were carried out chronologically and the mechanism for cell necrosis was evaluated. It was concluded that absolute ethanol injected to the healthy mongrel liver caused not only direct cell damage due to potent fixative effect through deprivation of fluid from cells, but also indirect cell damage due to impairment of tissue blood flow related to thrombus formation, which resulted in necrosis. Moreover, disappearance of acoustic shadow (AS) was considered to be due to absorption or removal of the gasses, which were the air in the PTC needle injected into the liver and the evaporated oxygen from denatured oxyhemoglobin (HbO_2), and the danatured red blood cells after improvement of blood flow at the surrounding area of the injection site. Gradual attenuation of the echogenicity might reflect the progression of necrosis at the injection site recognized histologically. *Gastroenterol Jpn 1989;24:663–669*

Key words: *HCC; percutaneous ethanol injection therapy; tissue blood flow; ultrasound*

Introduction

Recent advances in medical imaging techniques have made it possible to detect small hepatocellular carcinoma (HCC) as small as 2 cm in diameter. However, surgical treatment in such cases has been frequently limited because of the association of advanced liver cirrhosis or other significant complications. Though therapeutic devices such as transcatheter arterial embolization (TAE) have been tried for such cases, a new therapeutic method of percutaneous absolute ethanol injection therapy (PEIT) under ultrasound guidance has been recently advocated for treatment of small HCC, and its favorable effects and some experimental studies have been reported by several investigators[1-9]. However, detailed mechanisms of its anti-cancer effect and chronological changes of ultrasonographic and histologic findings after absolute ethanol injections have not been sufficiently investigated. In order to elucidate these points, we studied the changes of the healthy liver of adult mongrel after absolute ethanol injection.

Materials and Methods

1. Chronological changes of ultrasonographic, macroscopic and histologic findings in canine livers after ethanol injections

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After an intravenous injection of pentobarbital (26 mg/kg) as general anesthesia in total of twelve adult mongrels (13 to 16 kg), the liver was surgically exposed. Then 1.0 ml of absolute ethanol was injected into three or four sites through a 21G-PTC needle (150 mm). The needle was kept in place for several minutes after the injection.

The changes of these sites on ultrasonographic, macroscopic, and histologic examination were evaluated immediately after, one day after, three days after, and eight days after the injection. The ultrasonographic apparatus used was a Toshiba SAL-32B (5 MHz). Ultrasound images were obtained by scanning through a water-filled condom placed between the probe and the canine liver as an echo vehicle. After ultrasonographic evaluation, the animals were sacrificed by intravenous KCl injection and the livers were removed for macroscopic and histologic evaluation. The liver sections were stained with Hematoxylin-Eosin (H.E.) and Azan-Mallory stains.

2. Tissue blood flow at the injection sites and the non-injection sites

Eight adult mongrels (13 to 16 kg) were used for this experiment. After the liver was surgically exposed, tissue blood flow was measured by hydrogen gas clearance method (Digital UH-Meter Model MHG-DI, Unique Medical Co., Ltd. Tokyo, Japan). A wire type electrode (UHE-201) was punctured into the liver under inhalation of hydrogen gas at a flow rate of 2 to 3 kg/cm^3 for 90 seconds through the intratracheal tube. Measurements were performed at 12 injection sites and 15 non-injection sites at before, 10, 20, 30, 40, 50, and 60 minutes after the injection. Pulse rate and blood pressure were simultaneously recorded on a Polygraph MIC-9800 (Fukuda Denshi Co., Ltd. Tokyo, Japan) through a transducer P-231D (Fukuda Denshi Co., Ltd. Tokyo, Japan). Portal blood flow and hepatic arterial blood flow were measured at the hepatic hilus with an ultrasonic transit time flow meter, Transonic T201 (Transonic System Inc. Ithaca, New York, USA).

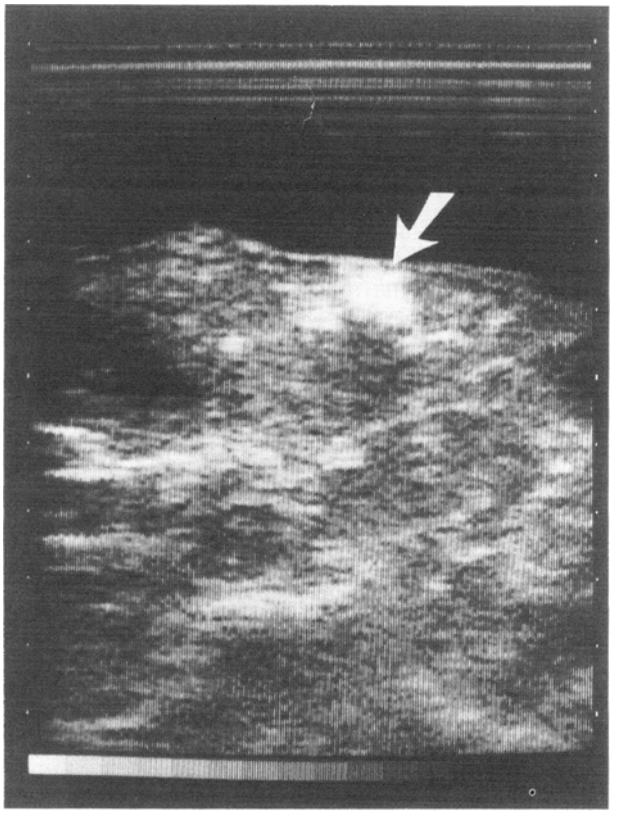

Fig. 1a Hyperechoic region with AS was observed at the injection site (arrow)

Results were expressed as means±standard deviation. Paired and unpaird t-tests were used for statistical analysis.

Results

1. Chronological changes of ultrasonographic, macroscopic and histologic findings of canine livers after ethanol injections

a) Immediately after injection

The hyperechoic region with acoustic shadow (AS) was observed at the injection site on ultrasonography (**Fig. 1a**). The cut surface of the liver appeared spherical or dendrite-shaped, white liver parenchyma with surrounding congestion macroscopically. Thrombus formation due to aggregated blood cells in the vessels and atrophy of hepatic cell cords with dilated sinusoids were seen at the injec-

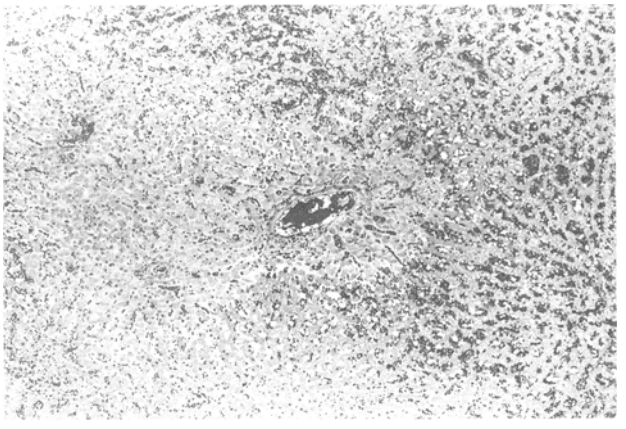

Fig. 1b Thrombus formation in the vessels and atrophy of hepatic cell cords with dilated sinusoids were seen at the injection site. Denatured red blood cells were observed in the surrounding sinusoids. (H.E. stain, medium power view.)

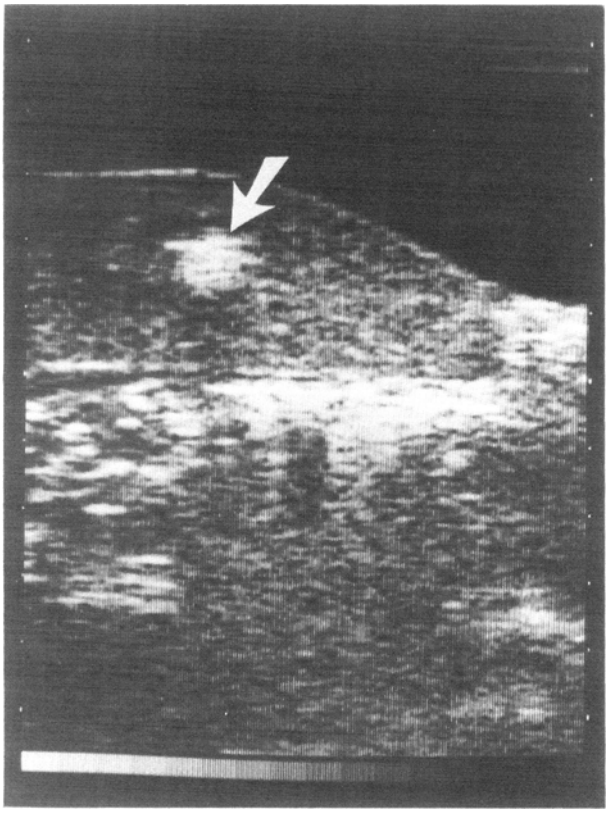

Fig. 2a Hyperechoic region without AS was still observed at the injection site (arrow).

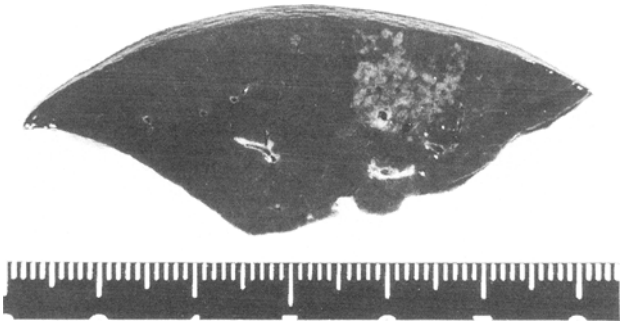

Fig. 2b Cut surface of the injection site showed homogenous, faintly white regions with less prominent congestive changes.

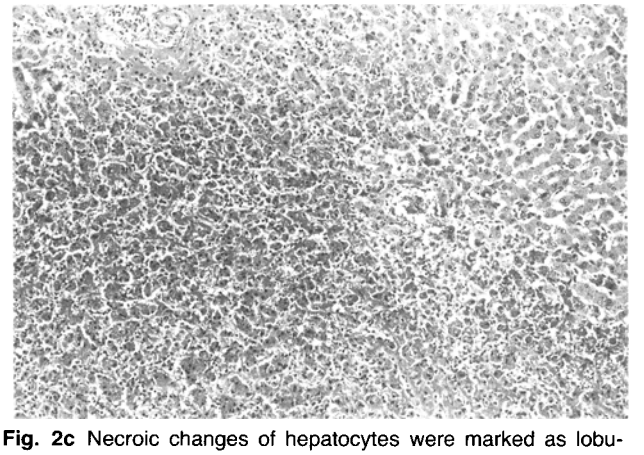

Fig. 2c Necroic changes of hepatocytes were marked as lobular distribution, but inflammatory changes were not observed. (H.E. stain, medium power view.)

tion site. Marked congestion and deformed red blood cells were present in the surrounding sinusoids (**Fig. 1b**).

b) One day after injection

The hyperechoic region (without AS) was still observed on ultrasonography (**Fig. 2a**). The cut surface appeared homogenous, with faintly white regions with less prominent surrounding congestive changes (**Fig. 2b**). Necrotic changes of hepatocytes were marked corresponding to hepatic lobules but inflammatory changes were not observed at the injection site and surround-

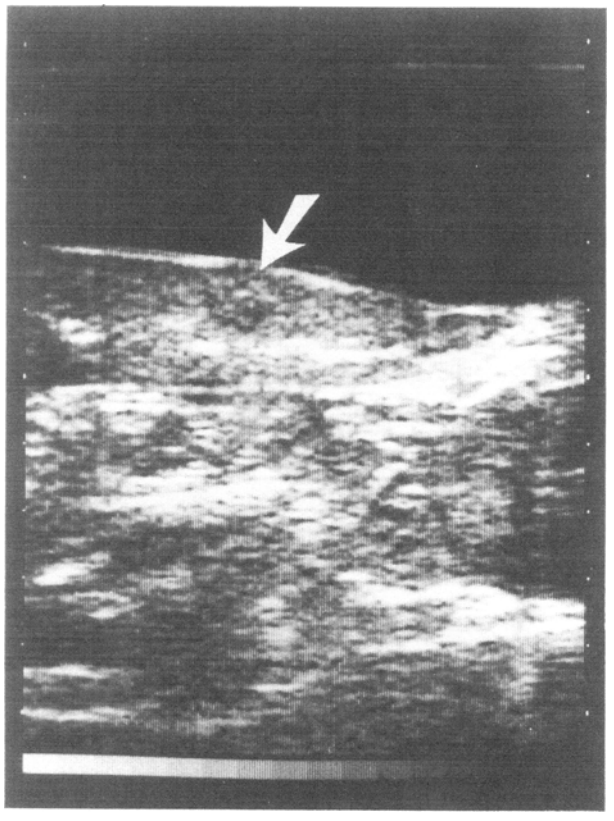

Fig. 3a The hypoechoic region was seen at the injection site (arrow).

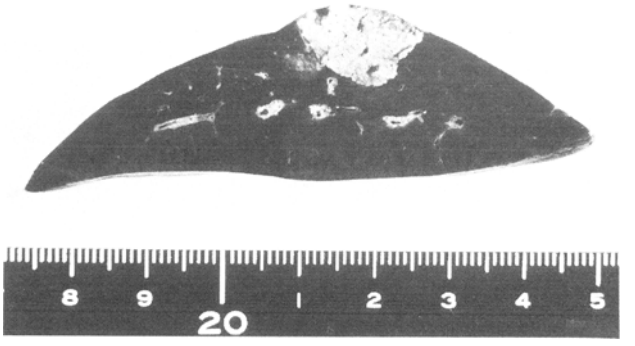

Fig. 3b Cut surface of the injection site showed clearly demarcated, white regions.

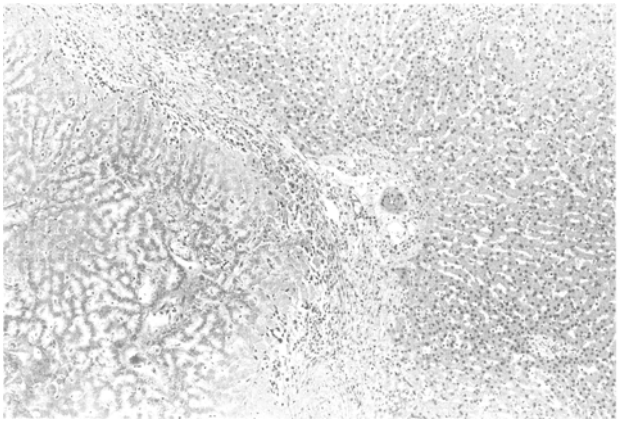

Fig. 3c A clearly demarcated massive necrosis of hepatic cell cords bordered with capsule-like connective tissue was seen. (H.E. stain, medium power view.)

ing congestive changes were less prominent (**Fig. 2c**).

c) Three days after injection

The echogenicity at the injection site decreased compared to that at one day after. A more clearly demarcated white region with no surrounding congestive change was observed macroscopically. Massive necrosis of hepatocytes in the perivascular region was present, but neither congestive changes nor inflammatory cells were observed in surrounding areas.

d) Eight days after injection

The echogenicity became further attenuated, demonstrated as a rather hypoechoic region on ultrasonography (**Fig. 3a**). The injection site appeared as a clearly demarcated, white region with no surrounding congestive change (**Fig. 3b**). Large arterial thrombus formation resulted in a clearly demarcated region bordered with capsule-like connective tissue at the area where blood was supplied through the artery and massive necrosis was seen (**Fig. 3c**).

2. Tissue blood flow difference between injection and non-injection sites

Tissue blood flow was 100.9±17.7 ml/min/100 g at zero minutes, changing to 16.1±15.3 ml/min/100 g at 10 minutes after the absolute ethanol injection ($P<0.01$). A similar tendency was noted at every 10 minutes interval for 60 minutes after the injection. Chronological

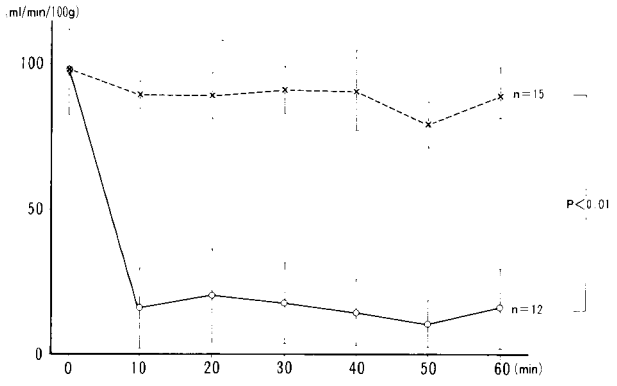

Fig. 4 Chronological changes of tissue blood flow between the injection sites and the uninjection sites. (○: injection site, x: non-injection site.)

measurements of tissue blood flow at non-injection sites showed no significant changes (**Fig. 4**). Pulse rate (147.5±13.4 /min), blood pressure (143.4±13.8/96.9±18.2 mmHg), portal blood flow (333.6±37.3 ml/min) and hepatic arterial blood flow (91.1±18.5 ml/min) measured simultaneously did not significantly change during the observation period (data not shown).

Discussion

Regarding anti-cancer action, absolute ethanol has no specific cell tropism, which is distinct from other anti-cancer drugs. Therefore we undertook this experiment using the healthy liver of mongrels based upon the assumption that the necrotizing effect of absolute ethanol on mongrel livers would be similar to that on HCCs in humans. Macroscopic and histologic findings immediately after the injection showed that the injected ethanol spread to the surrounding tissue through sinusoidal spaces or vessels where tissues were less compact. At the injection sites the hepatocytes became atrophic and the sinusoids dilated. These findings were caused by the potent fixative action of absolute ethanol through deprivation of fluid from the cells. This direct action of ethanol on the hepatocytes attenuated in accordance with dilution of ethanol after spreading to surrounding area. As denatured red blood cells were observed at the peripheral area where atrophic hepatocytes were no longer observed, it seemed that denatured red blood cells at the injection site were pushed away to surrounding tissue along the sinusoids or vessels in relation to increase of the injection pressure. Subsequent impairment of tissue blood flow at the injection site might occur due to the presence of denatured red blood cells in the sinusoids and the vessels.

It has been also pointed out in the histologic studies of Takashimizu et al.[8] and Doon et al.[10] that impairment of blood flow may occur at the injection site immediately after PEIT. However, no reports on tissue blood flow at the injection site have been made. In this study, direct measurement of tissue blood flow was done by hydrogen gas clearance method. It was found that the ethanol injection actually decreased tissue blood flow at the injection site with no significant effects on any other parts of the liver or other hemodynamics. Our result might suggest that an HCC on the liver surface could be safely treated by PEIT.

One of the complications after PEIT is severe pain at the injection site. It was confirmed that lodging the needle at the site for several minutes after the injection could prevent shedding of absolute ethanol on the liver surface in this experiment. This might reduce severe abdominal pain. It is also reasonable that longer stagnation of absolute ethanol injected into the tumor could cause a more potent anti-cancer effect.

Injection of absolute ethanol caused impairment of tissue blood flow in surrounding areas of the injection site for at least for one day as shown by the histologic findings. Therefore to obtain more potent anti-cancer effect, PEIT should be completed in the shortest period possible if the patient can tolerate it. If repeated PEIT were feasible every other day, it could be expected that more potent anti-cancer effects may be obtained by diffusion of ethanol to surrounding areas without any significant influence on blood flow.

Eight days after the injection in this experi-

ment, the size of necrotic area was 15.5±5.7 mm (n=12), which was significantly larger than that of our previous study[11] using excised livers. Such differences might be due to the period after the injection and the presence of the relevant tissue blood flow. Sugiura et al.[1] report that the necrotic area three days after injection of 1 ml absolute ethanol reached 1.0 to 1.5 cm in diameter at maximum in an experiment using the healthy rabbit liver. In the present study, the necrotic area immediately after the injection was 11.5±3.8 mm (n=12), which was also smaller than that of eight days after ($P<0.01$). In other words, the extent of necrosis immediately after the injection was defined as the effect of direct cell damage of ethanol mainly. On the other hand the mechanism for increase in the extent of necrosis of eight days after might be considered to be due to indirect cell damage due to thrombus formation in larger arteries in the perfused area in addition to direct cell damage.

Hyperechoic region with AS immediately after PEIT was seen at the injection site, and subsequent disappearance of AS within one or two days and gradual attenuation of the echogenicity are observed in human HCC. We previously reported[11,12] that many factors might be related to the image immediately after injection, including atrophic hepatocytes, dilated sinusoids, denatured red blood cells, injection of air in the PTC needle into the liver tissue or generation of oxygen caused by evaporation of chemically soluble oxygen with denatured HbO_2. However, disappearance of AS and attenuation of the echogenicity remained controversial. The ultrasound images during this experiment seemed similar to those of HCCs in humans. However, a variety of ultrasound images are recognized about one week after PEIT in HCCs in clinical practice. These discrepancies may derive primarily from the difference of the objects, one being the healthy mongrel liver and the other human HCC, and secondly from a variety of the ultrasound images of HCC before PEIT. Two investigators[13,14] report that the ultrasound images of HCCs one or two weeks after PEIT show hypoechoic lesions. However, those images are not compared with the corresponding histologic findings in their reports. In this study, the similar ultrasound images results to their reports were obtained and we further made a comparison between these images and the histologic findings at the injection sites. Consequently the mechanism of the chronological changes of ultrasound images might be explained as follows: 1) Disappearance of AS was considered to be due to absorption or removal of the gasses and the denatured red blood cells after reperfusion of blood flow in the surrounding areas. 2) Gradual attenuation of the echogenicity reflected progression of necrosis recognized histologically at the injection site.

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