PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

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• LIVER CANCER •

Experimental and clinical assessment of percutaneous hepatic quantified ethanol injection in treatment of hepatic carcinoma

Li-Wu Lin, Xue-Ying Lin, Yi-Mi He, Shang-Da Gao, En-Sheng Xue, Xiao-Dong Lin, Li-Yun Yu

Li-Wu Lin, Xue-Ying Lin, Yi-Mi He, Shang-Da Gao, En-Sheng Xue, Xiao-Dong Lin, Li-Yun Yu, Fujian Provincial Ultrasonic Medicine Institute, Ultrasound Department, Union Hospital of Fujian Medical University, Fuzhou 350001, Fujian Province, China
Correspondence to: Li-Wu Lin, Ultrasound Department, Union Hospital of Fujian Medical University, Fuzhou 350001, Fujian Province, China. lxdghl@163.net
Telephone: +86-591-3357896 Ext. 8352 **Fax:** +86-591-3339732
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Abstract

AIM: To detect the relationship between absolute ethanol injection quantity, the interval and formation of fibreboard, the curative effect in treatment of hepatocarcinoma and to evaluate the clinical application of percutaneous hepatic quantified ethanol injection (PHQEI) in treatment of hepatic carcinoma (HCC).

METHODS: (1) Experimental study: Twenty-four human hepatic carcinoma SMMC-7721 xenografted nude mice were randomly divided into three groups: group A injected with quantified ethanol at short intervals (QESI), group B with quantified ethanol at long intervals (QELI) and group C with a small quantity of ethanol at long intervals (SQLI). The tumor tissues were sent for patho-histology and electron microscopic examinations. The diameters of tumors were measured with high frequency ultrasound before and after therapies and tumor growth index (TGI) was calculated. (2) Clinical study: Tumors of 122 cases of pathologically proved HCC were injected with quantified ethanol guided by ultrasound every 3-5 d 4-10 times per period of treatment. The quantity of ethanol was calculated according to the regressive equations where Y = 2.885X when the mass was ≤5 cm in diameter and Y = 1.805X when the mass was >5 cm in diameter (X is the maximal diameter of the mass with the unit cm, Y is the ethanol quantity with the unit mL). The survival rates of 1, 2, 3 and 4 years and recurrent rates *in situ* as well as dystopia in the liver were calculated.

RESULTS: (1) Experimental study: TGI of QESI group (0.072±0.018) and QELI group (0.094±0.028) was apparently lower than that of SQLI group (1.982±0.482) (P<0.01). TGI of QESI group seemed to be lower than that of QELI group, but it was not markedly different (P>0.05) between two groups. Severe degeneration and necrosis could be seen in QESI group by patho-histology examination. Coagulative necrosis could be seen in most tumors of QESI group and there were no residual cancer cells under electronic microscope, while the residual cancer and inflammatory cells and fibre tissues could be seen around the tumors of QELI group. Infiltration of inflammatory cells could be seen and fibre tissues were formed. (2) Clinical study: B mode ultrasound showed that 62.5% of tumors shrank after PHQEI. The survival rates of 1, 2, 3 and 4 years of the group with tumors ≤3 cm in diameter were higher than those of the group with tumors >3 cm in diameter. The recurrent rates of tumors *in situ* of the former group were apparently lower than those of the latter group. The recurrent rates of tumors in dystopia in the liver of the former group were markedly lower than those of the latter group. The 122 cases underwent a total of 1221 PEI. There were no complications such as hemorrhage and severe heart, liver and kidney functional injuries except for 1 case of melena and 4 cases of jaundice who recovered after 1-2 wk under common therapies.

CONCLUSION: The experimental study shows quantified ethanol at intervals of 3-5 d could improve the curative effect of hepatocarcinoma. The clinical study shows PHQEI is an effective therapeutic method for HCC with few side-effects, and a low-cost. The treatment efficacy is more remarkable for tumors ≤3 cm in diameter.

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INTRODUCTION

Because ultrasound-guided percutaneous ethanol injection (PEI) has the advantages of easy operation, definite curative effect, little damage and few side-effects, it has been widely used in clinical practice in recent years since the report by Japanese scholars in 1983[1-10]. Though more ultrasound-guided methods have been applied since then[11-20], PEI is still one of the most widely used non-operative methods in the treatment of hepatic carcinoma. Because there is no common standard for ethanol injection quantity and interval, the efficacy is affected apparently. In order to improve the efficacy, according to the pathological biological characteristics of HCC that there are capsula and tiny satellitic foci of cancer cells in the periphery of HCC, we once conducted the study of percutanous hepatic quantified ethanol injection (PHQEI) in treatment of HCC (Ethanol was injected until the diffusing area was 1-2 cm more than the maximal diameter of tumor ≤5 cm in diameter. According to the endurance of patients the ethanol was injected from several directions and diffused throughout the nodules >5 cm in diameter as fully as possible.) and we put forward the regressive equations[21]: Y = 2.885X (X≤5 cm), Y = 1.805X (X>5 cm) (X is the maximal diameter of the tumor with the unit cm, Y is the ethanol quantity with the unit mL). Recently, some scholars have reported that it could cause cancer cells to remain as the fibreboard in tumors affected the wide infiltration of ethanol[22]. So we carried out experimental studies to prove the relationship between absolute ethanol injection quantity and the formation of fibreboard. We have followed up 122 cases of hepatocarcinoma treated by PHQEI for 1-4 years to assess its clinical application.

MATERIALS AND METHODS

Experimental studies

Experimental animals For the experiment, we used 24 BALB/CA nude mice, which were 5-8 wk old, provided by the Medical Experiment Animal Unit of Anti-cancer Center of Xiamen University, China. The average weight was 18±2.1 grams. They were raised in the layer drift shelves under aseptic conditions. The cages, cushion, drinking water and standard forage provided by Shanghai Bikai Company were changed periodically.

Experimental methods Twenty-four nude mice with human HCC-transplanted hypodermically were randomly divided into three groups: group A injected with quantified ethanol (the ethanol diffused throughout the tumor) at short intervals (5 d) (QESI), group B with quantified ethanol at long intervals (10 d) (QELI) and group C with a small quantity of ethanol (half quantity of ethanol as used in QESI) at long intervals (SQLI). Each group contained 8 nude mice.

Three dimensional diameters of the tumors were measured by high frequency ultrasound (Aloka-5500 with 10 MHz probe) after 10 d of transplantation of HCC and the volume was calculated. Then ethanol was injected into the center of the tumor with No.5 needles. All the mice were injected twice. The three dimensional diameters of the tumors were measured again 5 d after the last injection and then the mice were killed. The center and peripheral tissues of tumors were sent for pathologic histology and electron microscopic examination. The experiment was carried out with double blind method.

Observation indexes Tumor growth index (TGI) was calculated by the formula: volume of the tumor (after treatment-before treatment)/volume of the tumor (before treatment). The degree of degeneration and necrosis, inflammatory response of tissues and fibre tissue were observed by pathologic histology examination. The microstructure of tumor tissues, inflammatory cells and fibre cells, fibreboard were observed under electron microscope.

Statistical methods

The data of each group were exporessed as mean±SD. Necrosis area of each group was calculated and analyzed with ANOVA. $P<0.05$ was considered statistically significant. All statistical analyses were performed using SPSS for windows version 8.0 software package.

Clinical studies

Patients One hundred and twenty-two cases of HCC, including 96 cases recurring after operation, consisted of 105 males and 17 females were enrolled in the study. The mean age was 54.8 years (range, 28-81). The diagnosis was established by pathocytology and/or histology. One hundred and twenty-two cases had 168 nodules including 62 cases with single nodules. The mean diameter of tumor was 3.8 cm (1.2-7.2 cm) and 122 cases comprised 64 cases with tumors <3 cm in diameter and 58 cases with tumors >3 cm in diameter (27 cases with tumor >5 cm in diameter). Seventy-eight cases had AFP >20 ng/mL.

Instruments Real-time ultrasonography (Aloka-650, 1700, Japan) was performed with a 3.5 MHz probe. The puncture needles were 22G PTC fine-needles and 15-20 cm in length. The platelet count of the patients was $>50\times10^9$/L.

Injection methods The point of puncture was determined by ultrasonography. Under local anesthesia, the fine needle punctured from the point into the posterior of the axis of nodules. Then ethanol was injected into the nodule gradually and the needle was withdrawn slowly. The injection quantity was calculated according to the regressive equations: Y = 2.885X (X<5 cm), Y = 1.805X (X>5 cm) (X is the maximal diameter of the tumor with the unit cm, Y is the ethanol quantity with the unit mL). All the cases were injected every 3-5 d 4-10 times per period of treatment. The tumor>5 cm in diameter could also be injected 10-20 times per period of treatment.

Follow-up time The follow-up period lasted for 12-48 mo (mean period 34 mo). The survival rates of 1, 2, 3 and 4 years and recurrent rates *in situ* as well as dystopia in the liver of each group were calculated and compared using ANOVA. The 14 nodules of 9 cases were resected after quantified ethanol injection 2-4 times and sent for pathologic examination.

RESULTS

Experimental studies

Tumor growth index Table 1 shows that TGI of QESI and QELI groups was apparently smaller than that of SQLI group ($P<0.01$). TGI of QESI group seemed to be smaller than that of QELI group, but not different markedly ($P>0.05$).

Table 1 Comparison of TGI between groups (mean±SD)

Groups	Cases (*n*)	TGI	*P*
A: QESI	8	0.072±0.018	>0.05[1]
B: QELI	8	0.094±0.028	<0.01[b]
C: SELI	8	1.982±0.482	<0.01[d]

[1]$P>0.05$ *vs* group B, [b]$P<0.01$ *vs* group C, [d]$P<0.01$ *vs* group A.

Table 2 Comparison of the percentage of tumor necrosis between groups

Necrosis area	QESI (A)	QELI (B)	SELI (C)	*P*
80%-	87.5 (7/8)	50 (4/8)	0	<0.01[b] <0.01[d]
30%-	12.6 (1/8)	50 (4/8)	100 (8/8)	<0.01[f]

[b]$P<0.01$ between groups A and C, [d]$P<0.01$ between groups A and B, [f]$P<0.01$ between groups B and C.

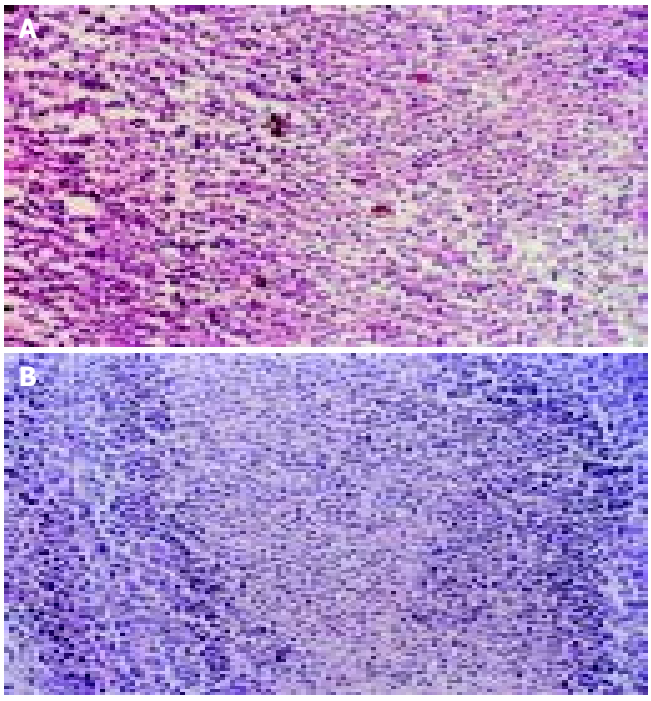

Figure 1 Coagulation necrosis in the center to periphery of the tumors of QESI after injection. (A, HE×10). The center of the tumor of SELI appeared coagulation necrosis, but in the periphery cancer cells grew luxuriantly after injection (B, HE×10).

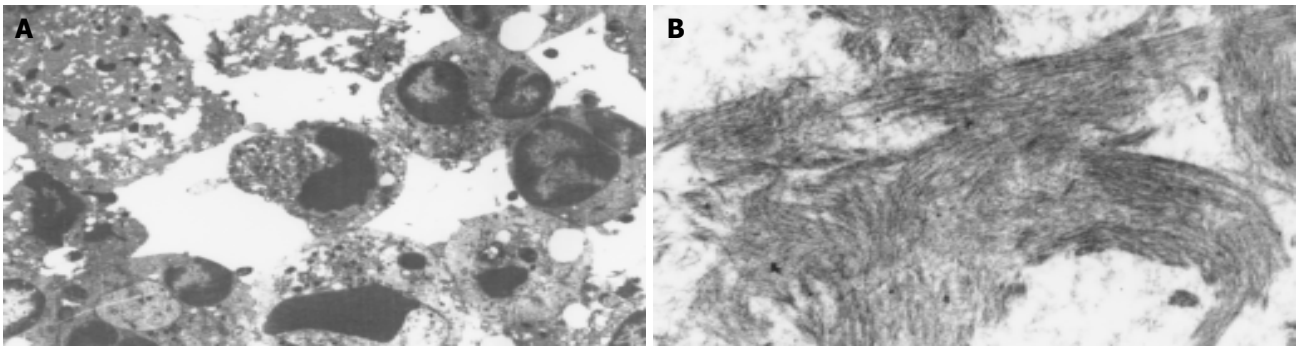

Figure 2 Inflammation cell infiltration (A) and faciculus (B) in the periphery of tumors of SQLI group. electron microscope examination.

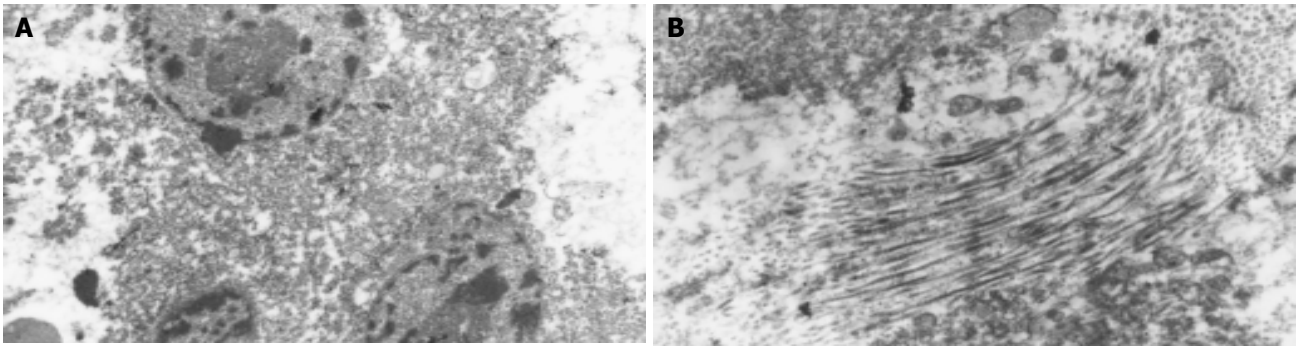

Figure 3 Degeneration cancer cells (A) and fasciculus (B) in the periphery of the tumors of QELI. Shown in electron microscope examination after injection.

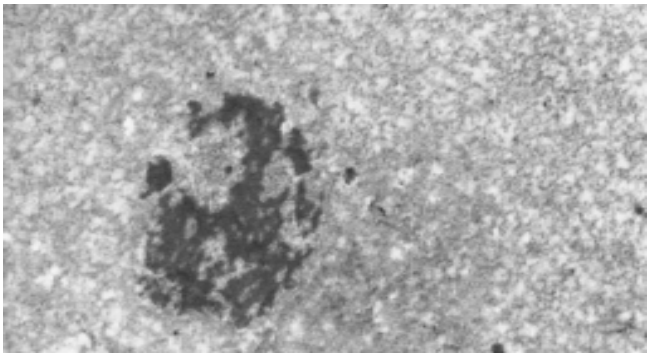

Figure 4 Necrosis changes such as karyalysis, karyorrhexis and cell membrane disappearance in the tumors of QESI after injection under election microscope.

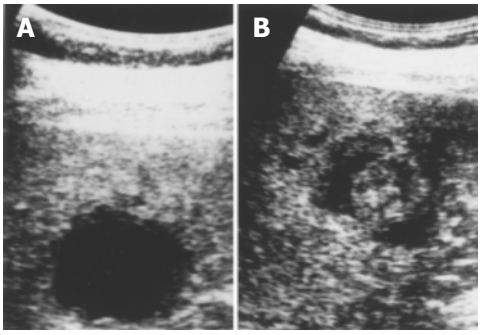

Figure 5 Reduction of the average diameter of HCC nodules from 3.6 cm (before injection, A) to 2.6 cm (two years after injection, B) and hyperechogenic rings in the periphery with haloes surrounding them after injection.

Patho-histology examination Table 2 shows the percentage of tumor necrosis of each group. Necrosis of QESI group was most severe. Coagulative necrosis could be seen from center to periphery of the tumor of QESI group. Eighty-seven point five percent of QESI group showed necrosis area >80%, markedly higher than those of QELI and SELI groups. None of SELI group showed necrosis area >80%. The center of the tumor of SELI group showed necrosis but in the periphery cancer cells grew actively (Figure 1).

Electron microscopic observation (1) SELI group: Cancer cells showed coagulation necrosis in the center only and most cancer cells grew luxuriantly, especially in the periphery. Most cancer cells only showed orbicular karyotheca, nuclei in different size, abundant euchromatin, orbicular cytomembrane, many inflammatory cells, fibrocytes and fasciculus (Figure 2).

(2) QELI group: Coagulation necrosis was comparably severe, but in the periphery orbicular cancer cells, some degenerative cancer cells, inflammatory cells and fasciculus could be seen (Figure 3).

(3) QESI group: Necrotic changes, such as karyalysis, karyorrhexis and cell membrane disappearance could be seen in most cancer cells (6/8) (Figure 4). There were severe degenerative cancer cells in which heterochromatin agglutinated under the nuclear membrane. No fasciculus formed.

Clinical studies

Ultrasonography, CT or MRI showed that 168 nodules in 122 cases of HCC shrank 2-8 wk after the last PHQEI. The average diameter reduced from 3.8 cm to 2.9 cm. Ultrasound showed the echoes of tumor nodules enhanced after PEI therapy. Hyperechogenic rings could be seen in the periphery of 25% (42/168) cases of haloes surrounding them (Figure 5). The diameters of 62.5% (105/168) tumors shrank and the internal echo enhanced and facula or light dot formed (Figure 6). Tumor nodules disappeared in 12.5% cases. CT showed that the nodules showed homogeneous hypodensity or/and non-enhanced changes in 62 cases after PHQEI. MRI showed that 38 nodules of 26 cases showed low T1 and T2 signals. fine-needle or/and

thick-needle biopsies were taken 1-3 times in all the cases and no cancer cells appeared in 88.5% (108/122) cases and degenerative cancer cells appeared in 11.5% (14/122) cases with nodules >5 cm in diameter. After therapy AFP reduced in 78 cases with high AFP before therapy and 82.1% (64/78) cases turned negative.

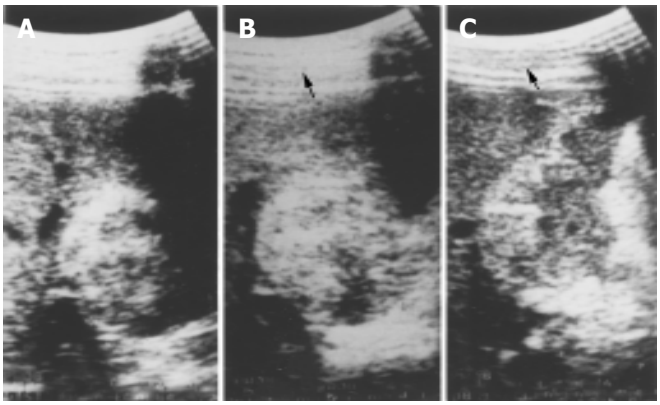

Figure 6 A: Average diameter of HCC nodule was 4.4 cm. B: It was 4.8 cm after 10 injections of absolute ethanol and the echogenic enhanced. C:After 42 mo of therapy the tumor shrank to 2.9 cm in diameter and formed facula.

Table 3 Efficacy of PEI reported in literature

Scholar	Time	Cases	Diameters of nodules (cm)	Survival rate (%)				
				1 yr	2 yr	3 yr	4 yr	5 yr
Ebara	1992	112	≤3	94	84	63	49	39
Castells	1992	162	<5	90	80	63		
Li Po	1996	188	4.6	85	44	19		
This article	2002	58	>3	84	64	58	52	
This article	2002	64	≤3	94	85	72	63	

Table 3 shows the survival rates of 1, 2, 3 and 4 years of each group with tumors <3 cm (64 cases) and >3cm in diameter (58 cases) and the treatment efficacy reported by different scholars.

Table 4 shows that the recurrent rate of tumors *in situ* and dystopia of the group with tumor ≤3 cm in diameter was apparently lower than that of the group with tumor >3 cm in diameter.

None of 9 cases with 14 nodules but 1 case showed cancer cells in pathologic examination of the resected specimens after 2-4 PHQEIs.

The 122 cases underwent a total of 1 221 PHQEIs. There were no complications such as hemorrhage and severe heart, liver and kidney functional injuries except that 1 case of melona after the injection of 8 mL ethanol and 4 cases of jaundice after 2-3 PHQEIs recovered after 1-2 wk under common therapies. Different degrees of pains occurred in 33.6% (41/122) cases after 1-2 PHQEIs and disappeared in 30 min without any therapy. Low-fever appeared in 56.6% (69/122) cases and disappeared after 2-4 d. GPT increased in 16.4% (20/122) cases and reduced to normal in 1 mo. There were no apparent changes of kidney function and ECG examination in all cases before and after therapy.

DISCUSSION

HCC is one of the most common cancers throughout the world. In order to surmount the obstinate disease scholars at home and abroad have conducted a great number of studies from many aspects, such as causa moli, pathology, diagnosis and therapy, and have acquired some accomplishments, especially in therapy[23-35]. Operation is the most common method in early stage, while HCC has the biologic characteristics of early invasion to blood vessels and multiple origins, which cause low resection rate and high recurrent rate (90%) of 5 years. With the advantages of definite efficacy, low complication and easy operation, PEI is still one of the most appropriate non-operative methods for small HCC which is unsuitable for resection and recurrent hepatic carcinoma. The mechanism of PEI is to make cancer cells incur dehydration and coagulative necrosis by injecting ethanol into tumors. But there is not a unified standard for total injection quantity, quantity per time and injection interval. At present, some scholars have adopted the formula[36]: $V = 4/3 \pi (r+0.5)^3$ (V is the total quantity, r is the radius of focus.) and others used the calculation[37]: Injection quantity (mL) = diameter (cm) or quantity (mL) = diameter (cm) +1 (tumor ≤5 cm in diameter) and quantity (mL) = diameter (cm) + 2 (tumor >5 cm in diameter). Liu *et al*.[38] reported that 238 cases of HCC were given PEI with 1-20 mL ethanol per injection and 1-2 times per week. The injection area involved the center as well as periphery of tumor and the part adhering closely to the capsule. Excessive ethanol increases side-effects and unnecessary liver injury, while too little ethanol could not kill cancer thoroughly and cause tumors to recur and metastasize. We once conducted the study of percutaneous hepatic quantified ethanol injection in treatment of HCC and put forward the regressive equation based on tumor diameter and injection quantity of ethanol[21]. The initial clinical application and following observation showed PHQEI had apparent efficacy. This experimental study was to further probe the pathologic basis of ethanol injection quantity, interval and the formation of fibreboard and to assess the clinical application value of PHQEI by follow-up observations.

Experimental study showed that tumor growth of QESI group (The ethanol diffused throughout the tumor and was injected every 5 d) was markedly inhibited after 2 injections. Extensive coagulative necrosis could be seen from the center to periphery of tumors and the necrosis area of 87.5% (7/8) cases reached more than 80% by pathologic histology. Electron microscopy showed the majority had coagulative necrosis and some had severe degenerative tumor cells. Though the growth of tumor of QELI group (The injection quantity was the same as that of QESI, but the interval was prolonged twice.) was also inhibited, the necrosis area of only 50% (4/8) cases was >80%. There were more residual cancer cells in the periphery of tumors and fibre tissue and inflammatory cells could be seen under electron microscope. Because ethanol injection was only half of that of QESI group and the injection interval was prolonged twice, growth inhibition and necrosis degree of tumors of SQLI group

Table 4 Comparison of the recurrent rates between groups

Group	Total Cases	Recurrent rate *in situ* (%)				Recurrent rate in dystopia (%)			
		1 yr	2 yr	3 yr	4 yr	1 yr	2 yr	3 yr	4 yr
>3 cm (A)	58	6.9 (4/58)	7.1 (3/42)	24.2 (8/33)	28.6 (6/21)	15.5 (9/58)	21.4 (9/42)	36.4 (12/33)	52.4 (11/21)
≤3 cm (B)	64	0 (0/64)[a]	2.1 (1/48)	5.1 (2/39)[a]	4.2 (1/24)[a]	9.0 (6/64)	10.4 (5/48)	12.8 (5/39)[a]	20.8 (5/24)[a]

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

were apparently slighter than those of the former two groups. Except for necrosis in the center, cancer cells grew luxuriantly, inflammatory response appeared and fasciculus formed in the periphery of the tumor.

Tumors in 122 cases of pathologically proved HCC, including 58 cases with tumors >3 cm in diameter and 64 cases with tumors ≤3 cm in diameter, were given PHQEL. We followed up the cases for 12-48 mo and proved that PHQEI had good efficacy, especially for tumors ≤3 cm in diameter. The 1-4 year survival rates of the group with tumors ≤3 cm in diameter were markedly higher than those of the group with tumors >3 cm in diameter and *in situ* tumor as well as dystopia recurrent rates were lower than those of the latter group. The reason might be that the ethanol quantity was not enough to meet the therapy standard for tumors >5 cm in diameter of 27/58 cases for the patients were weak and their liver function was bad. Giorgio *et al*.[39] reported that 112 cases were given single-session percutanous large dosage ethanol infection therapy with 16-120 mL ethanol per injection in panplegia. Five patients died after 7-10 h, and the 1, 2 and 3 year survival rates of the remaining 107 cases were 88%, 76% and 76%, respectively. The result showed that large amount ethanol injection could improve the treatment efficacy, while the side-effect also increased. So patients with advanced HCC of large tumors should be closely observed and an appropriate injection quantity should be chosen[40].

Recently, some scholars have proposed that fibreboard also appeared in HCC of 3 cm in diameter and the infiltration capacity of absolute ethanol was bad. In order to improve the treatment efficacy, ethanol should be injected from different points repeatedly[22]. Some scholars used drugs with a stronger infiltration capacity, for example, 50% acetic acid injection (PAAI)[41]. Ohnishi *et al*.[42] reported that the 1 and 2 year survival rates of PAAI were 100% and 92%, markedly higher than those of PEI which were 63% and 53%, respectively. The recurrent rate was 8%, lower than that of PEI which was 37%. But our study showed that the survival rate of HCC ≤3 cm in diameter was not lower and the recurrent rate was not higher than those of PAAI. The 4 year recurrent rate *in situ* was only 4.2% (1/24). Except for 1 case with a tumor of 5.1 cm in diameter none of the 9 cases with 14 nodules showed cancer cells in pathologic examination of resected specimens after 2-4 PHQEIs. Okuda *et al.* reported that about 90% of small HCCs had different degree of gross fibre pseudo-capsule and tumors <1 cm in diameter seldom had fibre capsule due to the gradually enhanced immune response of the body during the growth of tumor[22]. Our experimental study showed that SQLI injection could stimulate strong immune response of organisms and cause inflammatory cell infiltration and fibreboard formation even if the tumor was less than 1 cm in diameter.

In conclusion, the experimental study shows that, in order to improve the efficacy of PEI adequate quantity must be adopted to maintain a strong diffusing capacity of ethanol in tumor and the interval must be short (The appropriate interval is 3-5 d) because small dosage and long interval will cause incomplete coagulated necrosis and formation of fibreboard and/or fasciculus which will influence the infiltration of ethanol and cause cancer cells to remain and metastasize.

The clinical study also shows that PHQEI has a high application value. So PEI is still the method with advantages of convenience, few side-effects and effectiveness. To further improve the treatment efficacy, studies on the quantity per injection and injection interval are needed.

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