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Quantitative Assessment of Hepatic Tissue Perfusion by Contrast Ultrasonography

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Abstract：Objective：The purpose of this study was to evaluate the efficacy of contrast ultrasonography using the ultrasound contrast agent Levovist® for assessing hepatic circulation in chronic liver disease. Methods：Twenty-seven patients who had been admitted to our department for the treatment of hepatobiliary disease were investigated. The degree of liver disease was classified as normal liver (NL), chronic hepatitis (CH), or liver cirrhosis (LC). Flash echo imaging (FEI) was performed to obtain the echo from Levovist®. The parameters of the time-intensity curve, maximum peak intensity (Max), total cumulated intensity of the ascending segment (Total), ascending slope to the peak (Slope), and time to peak (Time), were compared between the groups. Results：Of the 27 patients, 8 had NL, 13 CH, and 6 LC. The Total and Time parameters increased with the progression in liver disease. The mean value and standard deviation of the Total were 35.9±12.0 (units) for NL, 40.0±34.1 (units) for CH, and 55.4±43.4 (units) for LC；and those of the Time were 43.3±16.3 (sec) for NL, 55.3±34.0 (sec) for CH, and 60.0±30.3 (sec) for LC. In contrast, the Slope in NL was greater than that in CH and LC：0.57 ±0.60 (units/sec) for NL, 0.48±0.29 (units/sec) for CH, and 0.47±0.40 (units/sec) for LC. However, this difference did not reach statistical significance. Conclusion：The change in time intensity curve after bolus Levovist® administration may correlate with the progress of liver disease, although the correlation is not sufficiently reliable to make a diagnostic quantitative assessment in the small study group. Further investigations in the larger population group are prompted.

Key words：Contrast ultrasonography, Chronic liver disease, Levovist®, Time-intensity curve

Introduction

Hepatic failure is a dire complication of liver surgery and liver transplantation. The postoperative liver function is closely related to the hepatic functional reserve. Therefore, an evaluation of the hepatic functional reserve is essential for predicting the safe limit of a liver resection or graft viability after transplantation.

The condition of the hepatic circulation plays an important role in the hepatic functional reserve. In chronic liver disease, progressive fibrosis develops in the liver tissue. This fibrosis has been show to be correlated to a reduced portal blood flow due to an increased portal vascular resistance.[1)-4)] These changes result in an impaired microcirculation in the liver, thus decreasing the liver's functional reserve.

Several modalities have been proposed to evaluate the hepatic circulation. The indocyanine green (ICG) test is the most widely used test to assess the hepatic circulation and liver function.[5)6)] This method is influenced, however, by the presence of an intrahepatic portasystemic shunt. Scintigraphy with technetium-99 m labeled galactosyl serum

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albumin (GSA) is also a useful method, to evaluate not only liver perfusion but also the liver's functional reserve.[7)8)] CT hepatic arteriography and CT during arterial portography can independently evaluate the contributions of the artery and portal vein to the liver blood flow and perfusion.[9)] Recent advances in magnetic resonance imaging (MRI) also enable assessment of liver tissue perfusion.[10)] All of these radiology-based modalities are either invasive or complicated, however.

Conventional Doppler ultrasound can measure the portal blood flow. For the hepatic arterial flow, however, hepatic artery resistance is the only measurable parameter.[11)-13)] Although ultrasound contrast agents are effective for imaging blood vessels, perfusion in the hepatic parenchyma remains difficult to evaluate because of the slow blood velocity in the hepatic sinusoids.[14)]

In recent studies, the ultrasound contrast agent Levovist produced a strong echo when it was disrupted by the irradiation of strong acoustic power.[15)-20)] Flash echo imaging is a new technique of contrast ultrasonography in which a strong echo is obtained from the collapsing microbubbles induced by transmitting acoustic power.[15)] Using this method, the distribution of the microbubbles in the hepatic parenchyma can be visualized. If the liver circulation can be accurately and conveniently evaluated by flash echo imaging, then ultrasonography will be useful for the pre and postoperative monitoring of patients with liver disease.

The purpose of this preliminary study was to determine whether the changes in hepatic circulation that are known to correlate with advancing chronic liver disease can be evaluated by contrast ultrasonography using the contrast agent Levovista.

Materials and Methods

Patients

Twenty-seven patients in our hospital with hepatobiliary diseases were studied (mean age, 62.7 yr; range, 47-79). Seventeen had hepatocellular carcinoma (HCC), 2 had bile duct cancers, 4 had alveolar echinococcosis, 1 had a gallbladder polyp, 2 had cholangiocellular carcinoma (CCA), and 1 had metastatic liver cancer.

Informed consent was obtained from each patient. Conventional ultrasound and contrast ultrasonography were scheduled before any treatment was attempted. Each ultrasound examination was conducted in the morning after a 9-hour fast to avoid any influence of a meal on the portal blood flow.[18)]

Classification of Liver Disease

The patients were classified into 3 groups : normal liver (NL) (n=8), chronic hepatitis (CH) (n=13), or liver cirrhosis (LC) (n=6) (Table 1). Histopathological findings were obtained in 8 of the 27 cases (3 NL, 4 CH and 1 LC). Gross findings were obtained from open or laparoscopic surgery in 12 cases. The other 7 cases with HCC were not diagnosed based on the gross findings because they were treated by transcatheter arterial embolization, and microwave or radio frequency ablation. Along with macro- and microscopic observations, the ICG R-15, serum hyaluronic acid level and type Ⅳ collagen level were used to assign patients to the 3 study groups (Table 1).[21)-23)]

Ultrasound Contrast Agent

The ultrasound contrast agent Levovista (Nihon Schering K. K., Osaka, Japan) consisting of galactose (99.9%) and palmitic acid (0.1%) was used for this study. Before administration, distilled water was added to Levovist® according to the manufacturer's instructions, to achieve a final concentration of 300-mg/ml. In the suspension, Levovist consists of microbubbles of air encapsulated by galactose with a mean diameter of 1.3 microns. Flash echo imaging (FEI) examination was conducted with 0.1 ml/kg×body weight (BW) (30 mg/kg×BW) of Levovist® at an injection speed of 0.5 ml per second through an intravenous catheter into the forearm.

Ultrasound equipment and FEI

A Toshiba SSA-370A ultrasound system (Toshiba, Tokyo, Japan), equipped with a convex shaped transducer, was used for this study. In the harmonic mode, the transmitting (fundamental) frequency from the transducer was 2.5 MHz, and the receiving (harmonic) frequency was 5 MHz.

FEI was used to obtain the microbubble-emphasized tissue perfusion image. The details of FEI

Table 1. Liver fibrosis parameters of three tissue types: normal liver(NL), chronic hepatitis (CH), and liver cirrhosis (LC)

Tissue Type	NL (n =8)	CH (n =13)	LC (n =6)
Disease category (number of cases)	Alveolar echinococcosis (4) HCC (2) GB polyp (1) Bile duct cancer (1)	HCC (9) CCA (2) Bile duct cancer (1) Metastatic liver cancer (1)	HCC (6)
ICG R15 (%)	9.49±4.57◆	14.24±6.03◆	30.38±5.23◆
HA (mg/ml)	83.4±21.8*	225.6±153.7*	537.8±300.0*
Type IV collagen (μg/ml)	4.46±1.23n.s.	5.88±2.09n.s.	7.30±4.16n.s.

*◆ : $p < 0.01$
n.s. : nonsignificant
HCC : Hepatocellular carcinoma
GB : Gallbladder
CCA : Cholangiocarcinoma
ICG : Indocyanine green
HA : Hyaluronic acid

have been reported elsewhere.[15] In brief, FEI was performed every 10 seconds after the injection of the Levovist®. Flash echo images obtained from 10 to 300 seconds after injection were analyzed using pre-installed software on the ultrasound machine. Regions of interest (ROIs) were placed in the peripheral part in the liver in order to measure the enhancement in the hepatic parenchyma, and the mean echo intensity within each ROI was calculated and represented the amount of microbubbles perfused in the liver. The shape of the ROIs was circular ; each was 10 mm in diameter. The major hepatic vessels were excluded from the ROIs to avoid any artifact from these structures (Figure 1).

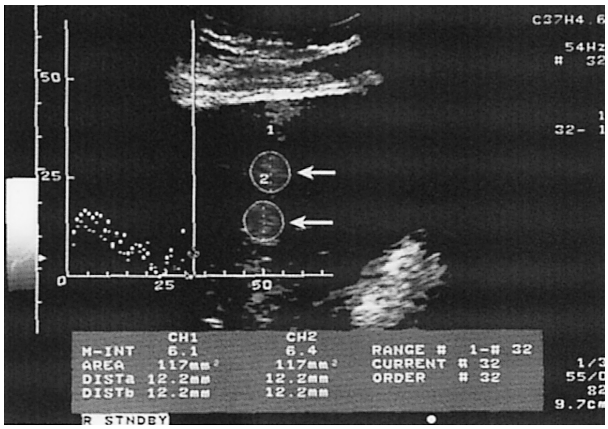

Figure 1. A flash echo imaging and time intensity curve analysis. The regions of interest (ROIs, arrows) were placed on the peripheral side of the liver. The flash echo intensity was calculated using a software package provided by the manufacture.

Time intensity curve (TIC) and parameters

The time intensity curve (TIC)—a plot of the echo intensity as a function of time— was obtained from the calculated intensity of the ROIs in the liver. To distinguish the difference in the echo intensity from the liver tissue, the preinjection value (baseline) of flash echo intensity was subtracted from the actual value of the obtained TIC. The ascending segment of each TIC, which represents the change in echo intensity to the peak, was evaluated, because previous studies have shown that the ascending segment of the TIC correlates with hepatic inflow.[19][20] The peak intensity (Max), the total cumulated intensity of the ascending segment (Total) that corresponded to the area under the curve until the peak, the time to peak after the injection of the Levovist® (Time), and the ascending slope to the peak (Slope) were all obtained for the analysis.

Statistics

The TIC parameters were expressed as the mean and standard deviation. Differences in the parameters between the tissue groups were tested by the Kruskal-Wallis test. Differences in parameters between the each 2 groups were analyzed by the Tukey-Kramer test.

Results

The average TIC for each group is shown in Figure 2. The enhancement in the liver tissue showed an acute increase followed by a decrease with a gradual slope, and back to baseline at about 300 seconds after injection of Levovist®. As discussed previously, differences in the ascending segment of TIC were compared among the 3 groups.[19)20)] Table 2 shows the TIC parameters for each group. The mean value of the total cumulated intensity of the ascending segment (Total) and the time to maximal intensity (Time) increased with the progression in liver disease. In contrast, the mean value of the ascending slope to the peak (Slope) decreased with the progression in liver disease. There was no statistical difference among the tissue types,

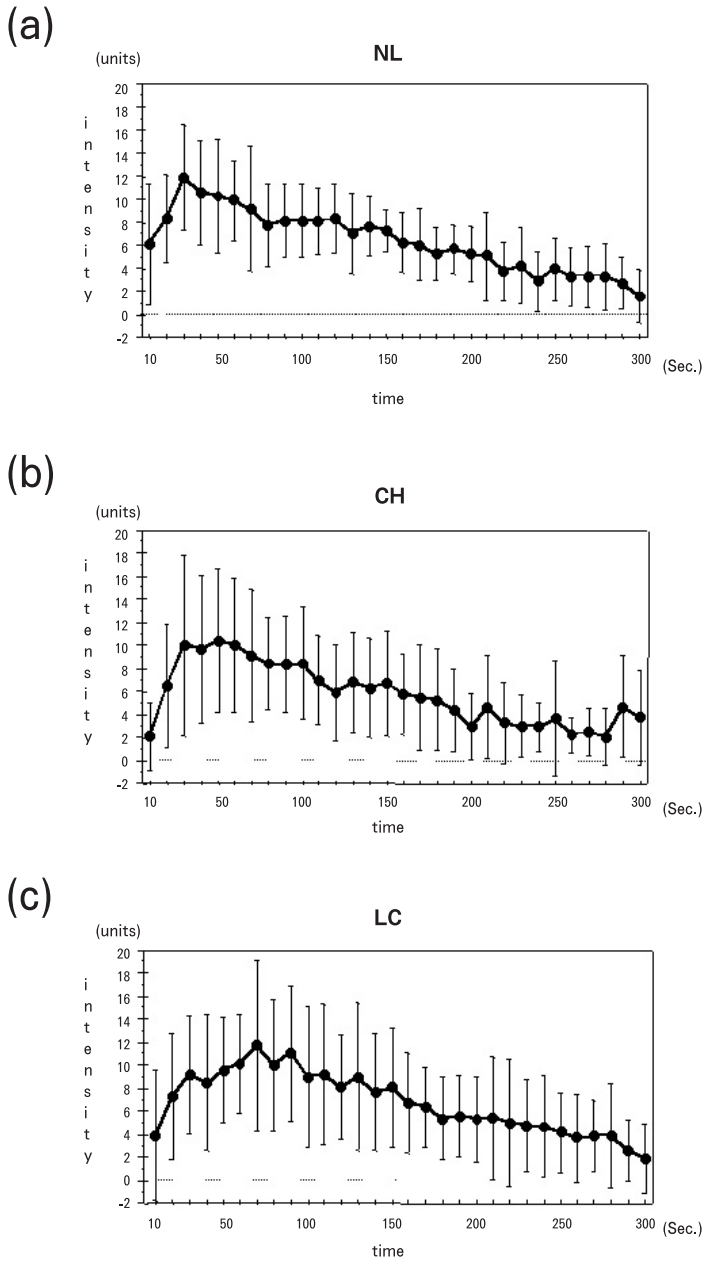

Figure 2. The change in the flash echo intensity for each tissue class. The averaged values for each time point were plotted: (a) normal liver (NL); (b) chronic hepatitis (CH); (c) liver cirrhosis (LC); error bars: standard deviation.

Table 2. TIC parameters for three groups : NL : normal liver, CH : chronic hepatitis, LC : liver cirrhosis

	NL (n=8)	CH (n=13)	LC (n=6)
Total (units)	34.1±13.4$^{n.s.}$	41.7±36.0$^{n.s.}$	55.4±43.4$^{n.s.}$
Time (seconds)	38.8±18.1$^{n.s.}$	60.0±33.7$^{n.s.}$	60.0±30.3$^{n.s.}$
Slope (units/second)	0.57±0.60$^{n.s.}$	0.48±0.29$^{n.s.}$	0.47±0.40$^{n.s.}$
Max (units)	13.4±4.3$^{n.s.}$	12.9±5.9$^{n.s.}$	13.4±6.9$^{n.s.}$

Table 3. TIC parameters for the cases in which a histopathological diagnosis was made : NL : normal liver, CH : chronic hepatitis, LC : liver cirrhosis

	NL (n=3)	CH (n=4)	LC (n=1)
Total (units)	36.0±19.5$^{n.s.}$	32.7±3.5$^{n.s.}$	94.7
Time (seconds)	33.3±20.8$^{n.s.}$	40.0±18.3$^{n.s.}$	70
Slope (units/second)	1.10±0.80$^{n.s.}$	0.59±0.32$^{n.s.}$	0.37
Max (units)	16.5±2.5$^{n.s.}$	14.5±50.6$^{n.s.}$	23.1

however. Furthermore, no trend was found in the peak intensity (Max).

The data from the 8 cases for which a histological diagnosis was available are shown in Table 3 (3 NL, 4 CH, 1 LC). The Max and the Total parameters of LC were greater than those of NL and CH. The Time parameters tended to increase with more advanced disease. In contrast, the mean value of the ascending slope to the peak tended to decrease with the progression in liver disease. There was no statistically significant difference among the tissue types.

Discussion

In this study, we investigated the correlations between various degrees of chronic liver disease and the hepatic circulation as assessed by contrast ultrasonography with Levovist®.

The greater value of Total parameter in CH and LC means that the total number of microbubbles perfused in the livers with chronic liver disease was greater than that in the normal livers. In the 8 histologically diagnosed cases, the Max and the Total parameters in CH and LC were greater than in NL. These findings suggest that the total hepatic circulation increased in the livers with chronic liver disease. This effect may be caused by a local factor such as hepatic vascular resistance and by the systemic circulation. The longer time parameter in CH and LC may be correlated to the vascular resistance in the liver, as reflected by the delayed distribution of contrast medium, because hepatic microvascular and cellular derangements were observed in the rats developing cirrhosis.[24]

The hepatic circulation correlated with the systemic circulation. In liver cirrhosis, hepatic artery flow is increased. The mechanisms of this increase are the hyperdynamic circulation and the hepatic arterial buffer response,[13)25)-28)] explained below. In a hyperdynamic circulation, there is vasodilatation with decreased arterial pressure, as well as increased cardiac output and increased regional organ blood flow.[25)] In chronic hepatitis, the hepatic arterial flow increases due to the dilatation of the hepatic artery, while the portal blood flow shows no change.[13)] When the portal blood flow decreases in liver cirrhosis, flow in the hepatic artery increases to maintain the total hepatic blood flow. This effect is called the hepatic artery buffer response.[27)28)] By means of these mechanisms, the total blood flow in chronic liver disease is maintained, or even increases, due to the increased arterial flow that is reflected in our study by the maximal and total cumulated intensity.

Uchimoto and co-workers investigated the kinetics of Levovista in rabbits. After the bolus intravenous administration of Levovist®, the time to peak intensity in the aorta was less than 5 seconds, whereas it was greater than 10 seconds in the portal vein.[29)] Therefore, the peak intensity in the liver parenchyma from the hepatic artery may appear earlier than that from the portal vein. Since the liver parenchyma has a dual blood supply, from

the hepatic artery and the portal vein, the TIC of liver tissue may represent the sum of the arterial and portal components. As a result, the maximum peak intensity (Max) and total cumulated intensity (Total) may correlate with the sum of arterial and portal flow. The time to peak (Time) and the slope of the ascending segment of the TIC (Slope) may correspond to the arterial factor and the dominant portal venous factor.

Ugolini et al. reported that parameters of the intensity curve of the Doppler signal showed a good correlation with the flow rate of contrast medium *in vitro*.[19] In their study, the onset time, time to maximal enhancement, peak intensity, area under the curve, and maximal ascending slope closely correlated with the flow rate of the contrast medium.

Albrecht et al. measured the echo enhancement in the hepatic vein a bolus injection of Levovist® into the peripheral vein[20] and observed an earlier time to peak enhancement and a greater peak enhancement value in cirrhotic patients. They attributed their findings to the arterializations of the liver, pulmonary arteriovenous shunt and the systemic hyperdynamic circulation state. In our study a similar result was found in the Total value (total cumulated intensity of the ascending segment of TIC). Our results regarding the Time parameters were opposite to theirs, however. This difference may be due to the fact that Albrecht et al. measured the ultrasound intensity at the hepatic vein, whereas we measured the enhancement in the hepatic parenchyma. In other words, we analyzed the presinusoidal or sinusoidal hepatic microcirculation, while they measured the postsinusoidal circulation. In our study, the Slope values of CH and LC, which partly correlated with the Time value, were smaller than those of NL. Particularly, in our 8 histologically diagnosed cases, the Slope value decreased with the progression in liver disease. These data suggest that the peak saturation with contrast medium in the hepatic sinusoids was delayed in more advanced disease. The diffusion of the microbubbles to the hepatic sinusoids may be obstructed by fibrosis in the peripheral liver tissue and the reduction of sinusoidal permeability. It is reported that sinusoidal permeability is reduced in proportion to liver disease.[30] Therefore, our method may have more precisely reflected the fibrotic state of the liver than their method.

In conclusion, the TIC obtained from bolus administration of Levovist® might correlated with the hepatic circulation. In particular, A TIC analysis in the liver parenchyma may thus successfully evaluate the presinusoidal status of the hepatic circulation. This study found no statistical difference between the groups in this small population. Further investigations in larger sized population groups are thus called for.

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