Hemodynamic Changes with Liver Fibrosis Measured by Dynamic Contrast-Enhanced MRI in the Rat

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Purpose: To evaluate the hemodynamic changes of liver cirrhosis in the rat and investigate the relationship between hemodynamic changes and properties of fibrotic change in the liver.

Materials and Methods: Three rats with cirrhosis induced by thioacetamide (TAA), three with disease induced by carbon tetrachloride (CCl4), and three with no treatment were measured on dynamic MRI using a 1.5T scanner. Compartment and moment analysis were used to quantitate hemodynamic changes.

Results: Compartment model analysis showed that increased transition speed from vessels to the liver correlated with grade of liver fibrosis. Moment analysis demonstrated that decrease of area under the curve (AUC), mean residence time (MRT), variance of residence time (VRT), half life (T1/2) and increased total clearance (CL) correlated with grade of liver fibrosis.

Conclusions: Hemodynamic changes in injured fibrotic liver may be influenced by the grade of fibrosis. Compartment model and moment analysis may be useful for evaluating hemodynamic changes in injured liver.

Keywords: hemodynamics, liver, fibrosis, dynamic MRI, pharmacokinetics

Introduction

The liver is essential in maintaining proper body functioning. Cirrhosis impedes the liver’s metabolism of nutrients, detoxification of the blood, bile production, and other normal functions. In cirrhosis, scar tissue replaces normal, healthy tissue, blocking the flow of blood through the organ and preventing it from working as it should. Damage is irreversible, and cirrhosis is frequently the last stage before liver failure. It is characterized by loss of the normal architecture and fibrosis. Inability to detect fibrotic change in tissue is a fundamental problem in clinical diagnosis; X-ray, ultrasound, and conventional MR imaging have not allowed successful differentiation between tissue fibrosis and other pathology.

Recently, perfusion imaging using contrast enhancement has been of increasing interest in MR examination. In liver imaging, it helps establish differential diagnosis between hemangioma and hepatocellular carcinoma or other liver masses. Although perfusion study is useful for detecting the flow of blood in the liver parenchyma, few studies have been done to detect degeneration in the liver. One reason for this is the absence of an optimal method to analyze liver perfusion study. The liver has multiple blood-input routes and a single output route, complicating detection of hemodynamic changes caused by fibrosis.

Pharmacokinetics is the mathematics of the time course of absorption, distribution, metabolism, and excretion (ADME) of drugs in the body. The biological, physiological, and physicochemical factors that influence the transfer processes of drugs in the body also influence the rate and extent of ADME of those drugs in the body. In many cases, pharmacological action, as well as toxicological action, is related to plasma concentration of drugs. Pharmacokinetic analysis can be adapted to hemodynamic analysis in perfusion study using sig-
nal intensity of MR imaging modifying the equation based on plasma concentration. Compartment model analysis is useful for analyzing pharmacokinetics in perfusion study in single photon emission computed tomography (SPECT), X-ray computed tomography (CT) and MR. Moment analysis is another method in pharmacokinetics, and few papers have applied this method to analyzing liver perfusion.

To the best of our knowledge, few papers have evaluated the hemodynamics of liver parenchyma on MRI. The purpose of this study was to quantitate the hemodynamic changes of liver cirrhosis using dynamic contrast-enhanced MRI with moment and compartment model analysis.

Materials and Methods

Animal model

Male Wistar rats 7 to 8 weeks old were used. Thioacetamide (TAA) and carbon tetrachloride (CCl₄) were used to induce cirrhosis. Three rats were injected intraperitoneally with TAA (200 mg/kg body weight) 3 times per week for 7 to 8 weeks; three rats received 2 mL/kg of 25% (V/V) of CCl₄-olive oil solution administered orally 2 times per week for 7 to 8 weeks; and three rats receiving no treatment were used as normal controls. No significant difference in the condition of the rats among the 3 groups was confirmed before treatment.

MR imaging was performed for all rats within 1 week after the end of treatment with TAA or CCl₄. The animals were anesthetized with 50 mg/kg Nembutal, and a 25-gauge butterfly needle was inserted into the tail vein for bolus injection of contrast agent, Gd-DTPA (0.2 mmol/kg). Bolus injection was done manually at the beginning of the MR data acquisition period.

MRI experiments

MR study was performed using a 1.5T clinical scanner (Signa® Horizon, GE Medical Systems) with a 3-inch surface coil. The surface coil was fixed on the bed of the MR scanner, and each rat was lain on the surface coil. Dynamic contrast-enhanced MRI was obtained using spin-echo sequence (repetition time [TR] = 120 ms; echo time [TE] = 18 ms) with a single, 3-mm-thick slice for each animal. Other acquisition parameters were: field of vision (FOV) = 12 cm; matrix (MTX) = 256 × 128; number of excitations (NEX) = 0.75; and acquisition time = 15 s per phase. One phase required 18 s and seventy phases were obtained for dynamic kinetic analysis of Gd-DTPA, which totally took 21 min measurement.

Quantitative analysis of liver fibrosis

To quantitate collagen content in liver tissues, deparaffinized liver sections were incubated for 15 min in a solution of saturated picric acid containing 0.1% Fast Green FCF, then incubated 40 min in 0.1% sirius red in saturated picric acid. Stained slides were washed in running distilled water, dehydrated, mounted, and examined by light microscopy. Microscopic pictures of the stained liver specimens were taken (×40), and positive signals were analyzed with freeware image analysis program, ImageJ. Using ImageJ, the signals from the color scale (red, green and blue) were quantified, and the mean area of fibrosis was calculated as an average percentage for 5 fields (×40) from each animal.

Quantitative analysis of MR images

Using the ImageJ program, the region of interest (ROI) on the liver (right and left parenchyma), abdominal aorta, and muscle (right and left parenchyma) was localized manually in each animal to avoid the vessels (in the liver) and artifacts (Fig. 1). The mean value of the right and left position was adopted as a measurement of the liver and muscle. Aorta/muscle signal intensity ratio and liver/muscle signal intensity ratio were adapted to avoid signal intensity inhomogeneities that were caused by using surface coil. Smoothing of 5-point time-course were operated in each ratio to eliminate noise component. The pharmacokinetics of Gd-DTPA was conducted for each subject. For quan-
titative analysis, 2-compartment model analysis and moment analysis were adopted.

1) Two-compartment model analysis
For our study, 2-compartment analysis was adopted (Fig. 2). Plasma and tissue compartment were divided, and the speed indexes were defined as $K_1$ and $k_2$. $C_a(t)$ and $C_B(t)$ were the concentration in each tissue. This model was solved using the following equation:

$$dC_B(t)/dt = K_1C_a(t) - k_2C_B(t)$$

where $K_1$ represents the inflow rate constant from plasma to tissue, $k_2$ represents the outflow rate constant from tissue to plasma, and $C_a(t)$ and $C_B(t)$ represent the respective concentrations from the plasma and tissue.

Resolution was performed by Microsoft Excel using self-made worksheets with solution function.

2) Moment analysis
Moment analysis was done using the changes in signal intensity of the liver. The following parameters were calculated using a shareware analysis system, (http://www.de3.co.jp/com/MomWEB/MMTWEB.htm):

AUC: area under the plasma concentration-time curve

$$AUC = \int_0^\infty Cdt$$

MRT: mean residence time

$$MRT = \int_0^\infty t \cdot Cdt/\int_0^\infty Cdt$$

VRT: variance of residence time

$$VRT = \int_0^\infty (t - MRT)^2Cdt/\int_0^\infty Cdt$$

where $C$ represents the concentration from the plasma, and $t$ represents the observation time of $C$.

The following second parameters were calculated by former parameters; factor $b$ of exponent function $B \cdot \exp (-b \cdot t)$ and prerequisite condition as administration dose was set as one.

T1/2: half life

$$T_{1/2} = \frac{\ln 2}{b} = \frac{0.693}{b}$$

CL: total clearance

$$CL = \frac{Dose}{AUC(\infty)}$$

Results
In this study, 3 rats with cirrhosis induced by TAA, 3 rats with cirrhosis induced by CCl4, and 3 rats without treatment underwent dynamic MR imaging. The body weights of the rats treated by each method were: 293.9 ± 5.3 g, TAA; 363.4 ± 22.6 g, CCl4; and 405.0 ± 41.9 g, no treatment. Significant differences were shown among each group ($P < 0.05$ with Kruskal-Wallis test). The percentages of liver fibrosis induced by each method were: 5.5 ± 3.4% by TAA; 20.3 ± 8.8% by CCl4; and 0.8 ± 0.4% by no treatment. Strong fibrosis was induced by CCl4 and mild fibrosis by TAA, and significant differences in degree of fibrosis were shown among each method ($P < 0.05$ with Kruskal-Wallis test).

Good-quality dynamic images of rat livers were acquired using clinical equipment. All dynamic studies were successful, and a time-signal intensity curve was obtained in each rat. An example of time-signal intensity ratio curves for the abdominal aorta and liver of control rats is shown in Fig. 3, and MR images for each timing (a, b, c) are shown in the left column. Time-signal intensity ratio curves of abdominal aortas and livers of rats treated with TAA and with CCl4 are shown in Figs. 4 and 5; MR images for each timing (a, b, c) are shown in the left column. The correlations between the index of $K_1$, $k_2$ and the percentage of area of fibrosis are shown in Fig. 6. Although no correlation was observed between $k_2$ and the percentage of fibrosis, correlation between $K_1$ and the percentage of fibrosis correlated well ($r = 0.63$).

The results of moment analysis are shown in Fig. 7. The correlations between indexes (below) and the percentage of fibrotic area are shown in each figure: a) AUC; b) MRT; c) VRT; d) T1/2; and e) CL. Negative good correlations are shown in AUC ($r = 0.87$); MRT ($r = 0.84$); VRT ($r = 0.78$); and T1/2 ($r = 0.84$), and positive good correlation is shown in CL ($r = 0.88$).
Fig. 3. Pharmacokinetics of Gd-DTPA in control rat. The time-ratio curve of the abdominal aorta and liver of control rat are shown. Dynamic images (a, b, and c) are shown in each timing.

Fig. 4. Pharmacokinetics of Gd-DTPA in rat with thioacetamide (TAA)-induced cirrhosis. The time-ratio curve of the abdominal aorta and liver of control rat are shown. Dynamic images (a, b, and c) are shown in each timing.

Discussion
In this study, liver fibrosis was evaluated quantitatively and noninvasively in rats using pharmacokinetic techniques and dynamic MR imaging. Compartment model and moment analysis were adopted as pharmacokinetic techniques. Although compartment model analysis is well adopted for perfusion study in some modalities, the optimal compartment model corresponding to the target organ should be applied adequately. Recently, dual-input, one-compartment model analysis was
adopted for dynamic CT perfusion analysis in liver disease.\textsuperscript{9–11} This method may be more reliable than single-input, one-compartment model analysis. However, in either case, it is very difficult to apply an optimal model for the target organ or disease. Moment analysis is another method in pharmacokinetics. It is unique because it is model free, or model independent, and no specific model is assumed. Both techniques were adopted to analyze the dynamics of contrast enhancement medium in the liver, especially in components, such as blood plasma, normal liver cells, and fibrotic cells.

Figure 6 shows the correlation between the index of $K_1$ and $k_2$ and the percentage of fibrotic area. Although no correlation was observed between $k_3$ and percentage of area of fibrosis, $K_1$ and percentage of fibrosis correlated well ($r>0.63$). This result suggested that the increase of transition speed from vessels to the liver correlated with grade of liver fibrosis. It also suggested a small tendency of reduction in transition speed from the liver. The transition from vessels to the liver shows permeability, and transition from the liver to vessels shows washout. The increase of permeability may show that the blood remains longer from vessels in cirrhotic liver tissue than in normal liver tissue.

Figure 7 shows the correlations between indexes of moment analysis and the percentage of area of fibrosis. These results suggest that the decrease of AUC, MRT, VRT, and $T_{1/2}$ and the increase of CL correlated with the grade of liver fibrosis. Good correlations were observed in these results ($r>0.78$). The AUC shows the volume of contrast enhancement medium in the liver parenchyma. The blood volume of liver parenchyma observed by AUC decreased with increase in area of fibrosis (Fig. 7a). It has been suggested that the blood volume of the liver parenchyma is dependent on the severity of fibrosis. The MRT shows the mean retention time of contrast enhancement medium in the liver parenchyma. The retention time of blood in the liver was shorter with the increase in area of fibrosis. This shows the rapidity of blood transition time caused by fibrosis. The VRT shows the
Fig. 7. Results of moment analysis show: a) AUC, the area under the curve shows the volume of contrast enhancement medium in the liver parenchyma; b) MRT, the mean residence time shows the mean retention time of contrast enhancement medium in the liver parenchyma; c) VRT, the variance of residence show the duration time of contrast enhancement medium in the liver parenchyma; d) T1/2, the half life shows the half life of contrast enhancement medium in the liver parenchyma; and e) CL, clearance shows the clearance of contrast enhancement medium from the liver parenchyma. All indices were correlated with the percentage of area of fibrosis \((r > 0.78)\).

duration of contrast enhancement medium in the liver parenchyma. The function of blood pooling in the liver parenchyma observed by VRT decreased with the increase in area of fibrosis. The CL shows the clearance of contrast enhancement medium from the liver parenchyma. The clearance of blood in the liver was increased with the increased area of fibrosis. The results of VRT and CL show the rapidity of disappearance from liver parenchyma. It has been suggested that the increase of clearance speed was not so much by liver function as by increased blood transition time. The T1/2 shows the half-life of contrast enhancement medium in the liver parenchyma. The half-life of blood in the liver parenchyma was decreased with increased area of fibrosis. This shows the rapidity of blood transition time, and it has been suggested that the function of hepatic blood pooling decreased in the hepatic cirrhosis induced by TAA or CCl4.

These results suggest that decreased blood
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volume and reduced retention time in the liver parenchyma lead to increase clearance of the liver. However, the increased transition speed from blood plasma to tissue suggested increased permeability from blood vessel wall to liver parenchyma and decreased blood washout from liver parenchyma in the fibrotic liver. Previously, it was reported that vascular endothelial growth factor (VEGF) was expressed in the hepatocellular hypoxia or necrotic liver parenchymal cell and induced increase of permeability.\(^\text{12–14}\) These papers suggested that increased permeability may contribute to the remodeling of liver architecture. Increased permeability in our results may correspond to these reports. It was suggested that the relationship between the permeability and the grade of fibrosis may be explained using VEGF expression.

These results may show that the progression of liver fibrosis may increase caused by undernourishment with decrease of substantial blood volume in the liver parenchyma and maintain blood uptake for a long time. Compartment model analysis and moment analysis may be useful in evaluating the function of liver parenchyma, including normal and fibrotic cells, as well as hemodynamics changes from injured tissue of the liver. The results from both analysis methods were applicable in injured liver and complemented each other. In particular, moment analysis may be a universal analysis method for liver perfusion despite any disease. Both analyses appeared useful to analyze function of the injured liver and to quantitate the grade of injury.

**Conclusion**

Our results suggested that compartment modeling analysis and moment analysis may be useful to evaluate hemodynamic changes in liver cirrhosis.

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**References**