Evaluation of Concanavalin A-induced Acute Liver Injury in Rats using an Empirical Mathematical Model and Dynamic Contrast-enhanced MR Imaging with Gd-EOB-DTPA

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Purpose: We evaluated concanavalin A (Con A)-induced acute hepatic injury in rats using an empirical mathematical model (EMM) and dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) with gadolinium ethoxybenzyl diethylenetriamine penta-acetic acid (Gd-EOB-DTPA).

Materials and Methods: We allocated 18 rats into 3 groups of six each and intravenously injected them with either 10 mg/kg body weight (BW) of Con A (Con A [10] group), 20 mg/kg BW of Con A (Con A [20] group), or a single dose of of saline (4 mL/kg BW, normal control group). We performed the DCE-MRI studies using Gd-EOB-DTPA (0.025 mmol Gd/kg; 0.1 mL/kg BW) as the contrast agent 24 hours after injection of Con A or saline. We then sampled blood, measured serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and calculated the rate of contrast uptake ($a$), rate of contrast washout ($b$), area under the curve ($AUC$), time to maximum relative enhancement ($RE$ ($T_{\text{max}}$)), and elimination half-life of $RE$ ($T_{1/2}$) from the time-signal intensity curves using the EMM.

Results: $\beta$ values were significantly smaller in the Con A (10) and Con A (20) groups than the control group, but $\alpha$ did not differ significantly among the 3 groups. The $AUC$ value was significantly greater in the Con A (10) group than controls, and the $T_{\text{max}}$ and $T_{1/2}$ values were significantly greater in the Con A (20) group than controls. The $\beta$, $T_{\text{max}}$, and $T_{1/2}$ values correlated significantly with AST and ALT.

Conclusion: In conclusion, the EMM is useful for evaluating Con A-induced acute hepatic injury using DCE-MRI with Gd-EOB-DTPA.

Keywords: acute liver injury, concanavalin A-induced liver injury, DCE-MRI, empirical mathematical model, Gd-EOB-DTPA

Introduction

Acute hepatic failure (AHF) is a severe liver injury accompanied by hepatic encephalopathy that causes multiorgan failure and carries with it an extremely high rate of mortality even with the provision of intensive care. Management of severe AHF continues to be one of the most challenging problems in clinical medicine. Liver transplantation, the most effective therapy, is limited by a shortage of donor organs. Furthermore, different liver assist devices undergoing clinical trials do not significantly affect patient survival alone but are regarded rather as a means to bridge patients with AHF to liver transplantation. Thus, there remains a need for reproducible experimental animal models that resemble clinical conditions as well as a reliable method to evaluate the extent of liver injury and monitor the effect of therapeutic strategies.

The 3 main approaches to creating an animal model for AHF are surgical procedures, toxic liver
injury, and infective procedures. Most common models are based on surgical techniques or the use of hepatotoxic drugs, and a few satisfactory viral models are available. The model of liver injury induced by concanavalin A (Con A) is regarded as an appropriate model of immune-mediated liver diseases. Injection of Con A results in T-cell stimulation and release of tumor necrosis factor-α (TNF-α) and other cytokines, which lead to apoptotic and necrotic death of hepatocytes.

Various liver-specific contrast agents for magnetic resonance (MR) imaging have been introduced to increase the accuracy of hepatic imaging. One recently available hepatocyte-targeted contrast agent is gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA). Its partial accumulation in hepatocytes and bile via various transporters after intravenous injection increases signal intensity in the liver on T1-weighted images, with the signal intensity dependent on the uptake of the contrast medium by the hepatocytes and bile excretion. Gd-EOB-DTPA transport is known to correlate with liver function, so dynamic contrast-enhanced MR imaging (DCE-MRI) with Gd-EOB-DTPA appears to be a promising method for evaluating liver function.

The dual blood supply of the liver from the hepatic artery and portal vein increases the complexity of the pharmacokinetic model used in analyzing DCE-MRI data in the liver. A simple and reliable method that does not require vascular input functions is desired for this purpose because it is often difficult to measure the dual vascular inputs separately, especially in animal experiments. Recently, Fan and colleagues developed an empirical mathematic model (EMM) to describe contrast uptake and washout behavior without using vascular input functions, and the model has been applied to DCE-MRI data to distinguish metastatic from nonmetastatic transplanted rodent prostate tumors and benign from malignant breast lesions and to detect bowel inflammation in patients with Crohn’s disease.

The purpose of this study was to evaluate Con A-induced acute liver injury in rats quantitatively using DCE-MRI with Gd-EOB-DTPA and the EMM.

Materials and Methods

Animals and treatments

We used 18 8-week-old specific pathogen-free male Wistar rats (229 to 271 grams) that we purchased from Japan SLC, Inc. (Hamamatsu, Japan) and maintained under standard conditions. The rats had free access to standard laboratory food and water; room temperature was kept at 23°C; and a 12-hour light/dark cycle was maintained. The animal ethics committee of Osaka University School of Medicine approved all animal experiments.

The rats were allocated to control (n = 6), Con A [10] (n = 6) and Con A [20] groups (n = 6). The rats in the Con A [10] and Con A [20] groups were injected intravenously from the tail vein with 10 mg/kg body weight (BW) and 20 mg/kg BW of Con A (Sigma Chemical Co., St. Louis, MO, USA), respectively, dissolved in normal saline (4 mL/kg BW). The rats in the control group were injected intravenously with saline of the same volume as that of Con A. Twenty-four hours after Con A or saline injection, we performed the DCE-MRI studies, first anaesthetizing the rats by intraperitoneal injection of chloral hydrate solution (4%, 400 mg/kg BW) and immobilizing each animal with surgical tape in the supine position to prevent unnecessary motion.

DCE-MRI studies

We conducted the DCE-MRI studies using an MR imaging system for animal experiments equipped with a 1.5-tesla permanent magnet (MRmini, DS Pharma Biomedical Co., Ltd., Osaka, Japan). We acquired the DCE-MRI data using fast low angle shot (FLASH) sequence with parameters: repetition time (TR), 50 ms; echo time (TE), 6.7 ms; excitation pulse flip angle (FA), 36°; field of view (FOV), 63 × 31.5 mm²; matrix size, 256 × 128; 4 axial slices with 6.4-mm thickness, non-gap; and number of excitations (NEX), 2. We set one axial scan of 4 slices for T₁-weighted images at intervals of 13 s. We continuously acquired 200 scans, including 6 scans as precontrast measurements with the same parameters, about 43 min. Two minutes after the start of the DCE-MRI study, we manually injected Gd-EOB-DTPA (0.025 mmol Gd/kg; 0.1 mL/kg BW) into the animal’s tail vein via a 26-gauge indwelling needle connected to a 50-cm extension tube and 1.0-mL syringe.

Empirical mathematical model and estimation of parameters

First, to obtain the signal intensity at time $t$ ($SI(t)$), one observer manually drew a region of interest (ROI) of approximately 1000 pixels on one slice that included the liver parenchyma without the vascular region. We estimated contrast agent concentration as a function of time after its injection by comparing $SI(t)$ from a selected ROI to the signal intensity before contrast administration ($SI(0)$):
It should be noted that \( SI(0) \) was obtained by averaging the signal intensities of one slice including the liver parenchyma in the first to sixth frames (precontrast frames). \( RE(t) \) is the relative enhancement at time \( t; A \), the upper limit of signal intensity; \( \alpha \), the rate of contrast uptake (min\(^{-1}\)); \( \beta \), the rate of contrast washout (min\(^{-1}\)); \( q \), the parameter related to the slope of early uptake; and \( t_0 \), the rising time point (minutes). It should be noted that we added \( t_0 \) to the EMM with 4 parameters\(^{12}\) as an additional parameter in this study.

For analysis, we used the EMM with 5 parameters\(^{13}\) to fit the time-signal intensity curves obtained using DCE-MRI with Gd-EOB-DTPA:

\[
RE(t) = \begin{cases} 
0 & 0 \leq t < t_0 \\
A \cdot [1 - e^{-\alpha(t-t_0)}]^q \cdot e^{-\beta(t-t_0)} & t_0 \leq t.
\end{cases}
\]

We calculated the above 5 parameters using the nonlinear least squares method (simplex method).\(^{13}\) Appropriate choices of initial guesses of the parameters were important; poor initial guesses resulted in long computation times or occasional failure to converge on a good fit. Based on our experiences, the initial guesses were taken to be as follows: \( A \) = maximum value of relative enhancement, \( \alpha = 0.2 \) min\(^{-1}\), \( \beta = 0.03 \) min\(^{-1}\), \( q = 0.3 \), and \( t_0 = 2 \) min. We calculated the area under the curve (AUC) by integrating Eq. (2) from \( t_0 \) to the last time point using the trapezoidal rule.\(^{13}\) We calculated the time to maximum relative enhancement (\( T_{\text{max}} \)) by setting the derivative of Eq. (2) equal to zero:

\[
T_{\text{max}} = \frac{1}{\alpha} \cdot \ln \left( 1 + \frac{\alpha q}{\beta} \right).
\]

We obtained the maximum relative enhancement (\( RE_{\text{max}} \)) by substituting Eq. (3) into Eq. (2) as:

\[
RE_{\text{max}} = A \cdot [1 - e^{-\alpha T_{\text{max}}}]^q \cdot e^{-\beta T_{\text{max}}}.
\]

We also calculated the elimination half-life of relative enhancement (\( T_{1/2} \)) by solving the following equation numerically using the Newton-Raphson algorithm\(^{13}\):

\[
A \cdot [1 - e^{-\alpha(T_{\text{max}} + T_{1/2})}]^q \cdot e^{-\beta(T_{\text{max}} + T_{1/2})} = \frac{RE_{\text{max}}}{2}.
\]

Biochemical and histopathological analyses

We obtained blood by cardiac puncture after the DCE-MRI study, centrifuged each sample for 10 min at 2000 rotations per minute, and determined the serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). SRL Inc. (Tokyo, Japan) tested the blood samples.

The liver was removed immediately after acquisition of blood samples and fixed in 7.5% formaldehyde neutral buffer solution. The tissues were dehydrated, embedded in paraffin, sectioned at 2-\( \mu \)m thickness, and stained by hematoxylin and eosin (HE) for histopathological analysis.

Statistical analysis

The estimated parameter values, AST values, and ALT values were expressed as mean \( \pm \) standard error (SE). We analyzed differences in the estimated parameter values, AST values, and ALT values among groups by one-way analysis of variance (ANOVA) and determined statistical significance by Tukey’s multiple comparison test. \( P < 0.05 \) was considered statistically significant.

Results

Figure 1 shows examples of curve fitting in the normal control (a), Con A (10) (b), and Con A (20) groups (c). After Gd-EOB-DTPA administration, a continuous increase and slow reduction of \( RE(t) \) after peaking at \( T_{\text{max}} \) were induced in all groups, but signal profiles differed among the 3 groups.

Figure 2a shows the AST values in the normal control, Con A (10), and Con A (20) groups, and Fig. 2b, the ALT values. Both AST and ALT differed significantly between the normal controls and Con A (10) group and between the controls and Con A (20) group.

Figure 3 shows the comparison among the normal control, Con A (10), and Con A (20) groups for (a) contrast uptake (\( A \)), (b) rate of contrast washout (\( \beta \)), (c) area under the curve (AUC), (d) time to maximum relative enhancement (\( RE \)) (\( T_{\text{max}} \)), and (e) elimination half-life of \( RE \) (\( T_{1/2} \)). Contrast uptake did not differ significantly among the 3 groups (Fig. 3a). We observed significant differences in: the rate of contrast washout between the normal control group and both the Con A (10) and Con A (20) groups (Fig. 3b); AUC between the normal control group and the Con A (10) group (Fig. 3b); and \( T_{\text{max}} \) and \( T_{1/2} \) between the normal control group and the Con A (20) group (Fig. 3d, e). There were no significant differences in \( A \), \( q \), and \( RE_{\text{max}} \) among groups (plots not shown).

We found that AST was in inverse proportion to \( \beta \) and in direct proportion to \( T_{\text{max}} \) and \( T_{1/2} \). Similarly, we found that ALT was in inverse proportion to \( \beta \) and in direct proportion to \( T_{\text{max}} \) and \( T_{1/2} \). Figure 4 shows the statistical correlations between (a) AST and \( \beta \) (\( r = 0.862 \)), (b) AST and \( T_{\text{max}} \) (0.872), and (c) AST and \( T_{1/2} \) (0.936). On the other hand, there
Fig. 1. Examples of curve fitting in the (a) normal control, (b) Con A (10), and (c) Con A (20) groups; open circles represent measured data and lines represent fitted data. Con A (10) denotes the group of rats intravenously injected with 10 mg/kg body weight (BW) of concanavalin A (Con A), and Con A (20) denotes the group injected with 20 mg/kg BW of Con A.

were no correlations between AST and: \( A (r = 0.441) \); \( \alpha (r = 0.023) \); \( q (r = 0.212) \); \( AUC (r = 0.257) \); and \( RE_{\text{max}} (r = 0.413) \) (plots not shown). Figure 5 identifies the statistical correlations between (a) ALT and \( \beta (r = 0.816) \), (b) ALT and \( T_{\max} (r = 0.891) \), and (c) ALT and \( T_{1/2} (r = 0.936) \). There were no correlations between ALT and: \( A (r = 0.425) \); \( \alpha (r = 0.109) \); \( q (r = 0.162) \); \( AUC (r = 0.315) \); and \( RE_{\text{max}} (r = 0.324) \) (plots not shown).

Figure 6 shows typical HE-stained liver tissues obtained from the (a) normal control, (b) Con A (10), and (c) Con A (20) groups. We observed hepatic necrosis and infiltration in the portal areas in the Con A (10) and Con A (20) groups and prominent alterations of hepatic tissue in the Con A (20) group compared to the Con A (10) group (Fig. 6b, c).

Discussion

In this study, we evaluated Con A-induced liver injury in rats by analyzing the DCE-MRI data obtained with Gd-EOB-DTPA using the EMM with 5 parameters. As Fig. 1 shows, the EMM provided excellent fits to data from all rats despite large variations in their uptake and washout behaviors, suggesting that use of the EMM is feasible for analyzing DCE-MRI data in Con A-induced liver injury.
Fig. 3. Estimated parameter values in the normal control, Con A (10), and Con A (20) groups for (a) rate of contrast uptake ($\alpha$), (b) rate of contrast washout ($\beta$), (c) area under the curve (AUC), (d) time to maximum relative enhancement ($RE$) ($T_{max}$), and (e) elimination half-life of $RE$ ($T_{1/2}$). Error bar represents the standard error (SE). *$P<0.05$. Con A (10) denotes the group of rats intravenously injected with 10 mg/kg body weight (BW) of concanavalin A (Con A), and Con A (20) denotes the group injected with 20 mg/kg BW of Con A. Con A (10) denotes the group of rats intravenously injected with 10 mg/kg body weight (BW) of concanavalin A (Con A), and Con A (20) denotes the group injected with 20 mg/kg BW of Con A.

Fig. 4. Correlations between aspartate aminotransferase (AST) and (a) rate of contrast washout ($\beta$), (b) time to maximum relative enhancement ($T_{max}$), and (c) elimination half-life of $RE$ ($T_{1/2}$).

Our results (Figs. 3–5) demonstrated that of the parameters extracted by the EMM approach, $\beta$, $T_{max}$, and $T_{1/2}$ are the parameters that are sensitive and useful for evaluating Con A-induced liver injury.

Various surgical and/or pharmacological methods have been used to create liver injury models for small animal experiments, of which Con A-induced liver injury is commonly used to mimic various aspects of human liver disease.\textsuperscript{1,2} Although the precise mechanisms of Con A-induced liver injury are not fully understood, there is direct evidence that activation of T cells and neutrophils is essential.\textsuperscript{3,4} In this study, AST and ALT increased 24 hours after injection depending on the injected dose of Con A (Fig. 2), and confirmation of liver damage by histopathological observation (Fig. 6) indicated the usefulness of this model of acute liver injury.
To obtain results that allowed quantitative analysis, we used the raw data to calculate the concentration of contrast agent as a function of time following its injection based on the change in $T_1$ (longitudinal relaxation time) signal. Ideally, this requires accurate $T_1$ measurements before and after contrast agent injection, which can be time consuming. In heavily $T_1$-weighted images, that is, with TR and TE much less than $T_1$ and $T_2^*$, Medved and associates\textsuperscript{14} demonstrated the relative enhancement as approximately proportional to the concentration of contrast agent. To avoid lengthy $T_1$ measurement and/or the use of phantom calibrators, we adopted the relative enhancement rather than concentration of contrast agent for analysis.

It is well known that organic anion transporting polypeptide 1 (OATP1) mediates the uptake of Gd-EOB-DTPA by hepatocytes and that multidrug resistance protein 2 (MRP2) mediates the biliary excretion of Gd-EOB-DTPA.\textsuperscript{15-17} In addition, the expression of OATP1 and MRP2 has been reported to decrease in hepatitis and cirrhosis.\textsuperscript{18,19} It is thought that the hypofunction of OATP1 induces the reduction of Gd-EOB-DTPA uptake by hepatocytes, and the hypofunction of MRP2 results in the accumulation of Gd-EOB-DTPA and/or the reduction of Gd-EOB-DTPA washout. As shown in Fig. 3, the rate of contrast washout ($\beta$) in the Con A (10) and Con A (20) groups significantly decreased compared to that in the normal control group. On the other hand, there were no significant differences in the rate of contrast uptake ($\alpha$) among groups. These results may suggest greater damage of the MRP2 than OATP1 in the Con A-induced acute liver injury. Immunocytochemistry\textsuperscript{20} and RNA extraction and real-time polymerase chain reaction (RT-PCR)\textsuperscript{21} enabled assessments of the expression of transporters, such as OATP1 and MRP2. Therefore, further study was needed to clarify fully the relationship between the uptake and washout of Gd-EOB-DTPA and expression of transporters in the hepatic injury. As shown in Fig. 3, the time to maximum relative enhancement

![Fig. 5](image1.png) Correlations between alanine aminotransferase (ALT) and (a) rate of contrast washout ($\beta$), (b) time to maximum relative enhancement ($T_{\max}$), and (c) elimination half-life of RE ($T_{1/2}$).

![Fig. 6](image2.png) Light micrographs of the liver tissue stained by hematoxylin and eosin (HE) in the (a) normal control, (b) Con A (10), and (c) Con A (20) groups. Calibration bar (100 $\mu$m) is also shown. Con A (10) denotes the group of rats intravenously injected with 10 mg/kg body weight (BW) of concanavalin A (Con A), and Con A (20) denotes the group injected with 20 mg/kg BW of Con A.
(T_{\text{max}}) and the elimination half-life of relative enhancement (T_{1/2}) were significantly prolonged in the Con A (20) group compared to the normal control group. On the other hand, AUC differed significantly between the control and Con A (10) groups. Seemingly, it is difficult to evaluate dose-dependent liver damage using AUC, T_{\text{max}}, and T_{1/2} compared to the rate of contrast washout (β) in Con A-induced acute liver injury.

As described, the EMM does not require vascular input functions to analyze DCE-MRI data. Neither does it require making assumptions about the underlying physiology or anatomy as other model-based approaches do. However, as Fan and associates pointed out, the primary disadvantage of the EMM approach is that there is no direct correspondence between the parameters obtained by this approach and identifiable physiological or anatomic features. Nevertheless, Fan’s group addressed this problem by deriving equations that connect the parameters obtained by the EMM approach to physiological and anatomic parameters associated with various pharmacokinetic models. Of the pharmacokinetic models, the 2-compartment model originally of Brix and colleagues has been most often applied to DCE-MRI data. Hayton’s team reported that the equation describing the time-signal intensity curve in Brix’s model became similar to that for the EMM when an instantaneous injection of contrast agent was assumed.

In conclusion, this study demonstrated that the EMM is useful for evaluating Con A-induced acute hepatic injury using DCE-MRI with Gd-EOB-DTPA.

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