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БІОФІЗИКА

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ASSESSMENT OF HEPATIC PERFUSION AND DIFFUSION IN CCl_4 TREATED RAT LIVER USING MAGNETIC RESONANSE IMAGING

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The liver is one of the most important organs of detoxification in the body. Use of diffusion-weighted (DWI) and dynamic contrast enhanced (DCE) ^{1}H MRI for the assessment of hepatic perfusion and diffusion parameters were evaluated in a CCl_4-induced rat liver injury model. Acute liver injury was produced by a single gavage of mixture of CCl_4 and corn coil. MRI experiments were performed before and 24 h after the CCl_4 treatment. CCl_4-induced liver injury caused decreases in both fast and slow apparent diffusion coefficient of water measured by DWI, as well as a decrease in contrast agent uptake measured by DCE ^{1}H MRI. DWI should prove useful in assessment of liver damage in diffuse liver diseases without the need for a contrast agent.

Keywords: liver toxicity, CCl_4, ^{1}H MRI, apparent diffusion coefficient, perfusion.

The liver is one of the most important organs in the body when it comes to detoxifying or getting rid of foreign substances or toxins. Many of the toxic chemicals that enter the body are fat-soluble, which means they dissolve only in fatty or oily solutions and not in water. This makes them difficult for the body to excrete. Fat soluble chemicals have a high affinity for fat tissues and cell membranes, which are composed of fatty acids and proteins. The liver detoxifies harmful substances by converting fat soluble toxins into water soluble substances that can be excreted in the urine or the bile.

Liver hemodynamics plays an important role in hepatic function including an excretion of toxins. Several methods have been proposed for the noninvasive quantification of hepatic perfusion, including clearance of xenobiotics, single-photon emission computerized tomography, positron emission tomography, and dynamic contrast enhanced (DCE) ^{1}H magnetic resonanse imaging (MRI) [8]. Measurements of water apparent diffusion coefficient of water (ADC) by diffusion weighted ^{1}H MRI (DWI) has potential values in characterizing hepatic pathological changes, differentiating between malignant and benign liver tumors, and monitoring response to therapy [7, 11, 12]. As a quantitative parameter calculated from DWI, water ADC may reflect not only diffusion that represents mostly the Brownian motion of the water molecules, but also perfusion in microvessels. Thoeny et al. [13] proposed the use of DWI for simultaneous estimation of both perfusion and diffusion without the use of an exogenous agent. Previous studies show that for low strength of the diffusion weighting (b-values < 100 s/mm^2) perfusion dominates diffusion by a factor of 10. However, by using high b-values (> 500 s/mm^2), the influence of perfusion is largely attenuated [13].

CCl_4-induced liver injury in animals is widely used model to study the mechanism underlying the hepatotoxic effects such as steatosis, hepatitis, fibrosis, and cirrhosis [3]. At high dose

and short term, acute CCl_4 toxicity results with hepatocellular necrosis and accumulation of inflammatory cells in centrilobular regions and also significant fat droplet deposition in hepatocytes [3]. The most clearly illustrated mechanism underlying the CCl_4 induced liver injury has been related to the production of reactive radicals and lipid peroxidation [5, 6, 14]. In hepatocytes, CCl_4 is converted into CCl_3* by cytochrome P450 in endoplasmic reticulum. CCl_3* is a free radical and can react with various proteins, nucleotides and lipids. Furthermore, in the presence of oxygen, this radical is transformed to the trichloromethyl peroxy (CCl_3OO*), which is a more reactive radical and can cause more extensive damage than CCl_3*. CCl_3OO* is more likely to extract a hydrogen from unsaturated poly fatty acids, e.g. the lipids in membrane, resulting to lipid peroxidation with the formation of reactive aldehydes, carbonyls and alkanes. These radicals will carry on the CCl_4 hepatotoxic effects by binding to a variety of biological molecules and disorder their normal functions [14].

The purpose of this study was to evaluate the use of DWI and DCE ^{1}H MRI for the assessment of hepatic perfusion and diffusion parameters in a CCl_4-induced rat liver injury model.

Materials and methods

CCl_4 model:

All animal studies were approved by the Indiana University Institutional Animal Care and Use Committee. MRI experiments were performed on male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) weighing 300–400 g (n=7). Acute liver injury was produced by a single intragastrical gavage of 2.5 ml/kg mixture of CCl_4 and corn coil (1:1).

MRI experiments:

All *in vivo* MRI was performed on a 9.4 Tesla, 31-cm horizontal Varian bore system (Varian, Palo Alto, CA, USA). Animals were anesthetized with 1–1.5% isoflurane delivered in medical air at 1.0–1.5 L/min using a rat nose mask connected to a gas anesthesia machine (Vetland, Louisville, KY, USA). Warm air was blown through the magnet bore to maintain the temperature in the space surrounding the animal and the animal core body temperature at 26–28°C and 32–36°C, respectively. Animal respiration was examined with a physiological monitoring and gating system (SA Instruments, Stony Brook, NY, USA) using a pneumatic pillow located under the animal's abdominal area. DWI and DCE ^{1}H MRI of the liver were collected with a birdcage coil (ID = 63 mm, length = 190 mm) tuned to 400 MHz. MRI experiments were performed before and 24 h after the CCl_4 treatment.

DWI:

Multi-slice DWI was collected using a modified spin-echo sequence and the following parameters: repetition time (TR) = 1000 ms, echo time (TE) = 1000 ms /21 ms, δ = 6 ms, Δ = 11 ms, matrix size = 256 × 128, field of view (FOV) = 80 mm × 80 mm, number of slices = 12, slice thickness = 0.5 mm, slice gap =1.5 mm, and b = 0, 10, 20, 30, 100, 220, 350, 600, 1000, and 1600 s/mm^2. Total data collection time for a set of DWI at the ten b values was ~ 23 min.

DCE ^{1}H MRI:

After collecting a baseline of DWI, 0.2 mmol/kg of Gd-DOTA was manually injected over a 30 s interval through a 26-gauge catheter placed in the tail vein. All bolus injections were performed by the same investigator. DCE ^{1}H MRI was obtained using a gradient-echo sequence and the following parameters: TR/TE = 10 ms / 3.1 ms, matrix size = 256 × 128, FOV = 64 mm × 64 mm, number of slices = 1, and slice thickness = 4 mm. 200 images were collected over approximately 13 minutes, with 4.5 s acquisition time per image.

Data analysis and statistics:

^{1}H images were reconstructed using the Image Browser software (Varian, Palo Alto, CA, USA). PSI-PLOT software was used to analyze DWI and DCE ^{1}H MRI data. DWI signal inten-

sity (SI) versus *b* value data were fit to the following biexponential equation: where A_0 is signal intensity for $b = 0$ s/mm^2, ADC_{fast} and ADC_{slow} are the fast and slow ADC components which are related to tissue perfusion and random molecular diffusion of water, respectively, and A_f is the relative contribution of ADC_{fast} which is related to the relative vascular volume or the signal fraction of fast moving ADC. ^{1}H images were reconstructed using the Image Browser software. The kinetics of contrast agent uptake were estimated by measuring the area under the curve (AUC) over the first 60 s after the contrast agent arrival, as well as by fitting the DCE ^{1}H MRI signal intensity versus time data to a triexponential function [9].

All statistical data are presented as the mean ± standard error of mean (SEM) and represent the range across a cohort of animals. Analysis of the data was performed using Student's t-test. A *P* value ≤ 0.05 was used to define statistical significance.

Results

CCl_4 intoxication decreased body weight by 5% from 332±5 g (before CCl_4) to 314±5 g (24 h after CCl_4) ($P<0.0001$). After intoxication a liver color turned from red to mostly yellow with visible dots of the oil accumulation (Fig. 1, A). H&E stained histological sections proved that CCl_4 treatment caused moderate multifocal infiltration of fat in hepatocytes, as well as infiltration of lymphocytes around the portal triads, scattered or moderate hepatocellular degeneration, and mild vascular congestion (Fig. 1, B&C).

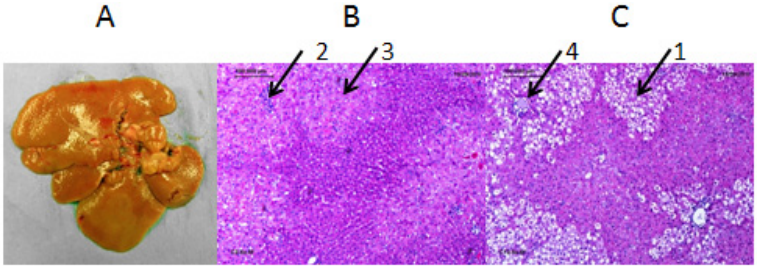

Fig. 1. Macroscopic appearance of the CCl_4 treated rat liver (A) and H&E stained histological sections (B and C) with infiltration of fat in hepatocytes (1), infiltration of lymphocytes around the portal triads (2), scattered or moderate hepatocellular degeneration (3), and mild vascular congestion (4). Original magnification, × 200.

After CCl_4 treatment, the liver ^{1}H signal intensity with $b = 0$ s/mm^2 was almost 1.5 times higher compared to untreated liver, suggesting an increase in T_2 relaxation. Plots of DWI signal intensity as a function of *b* value, before and 24 h after CCl_4 treatment, are shown in Fig. 2. The plots were biexponential in both cases. A_f was not affected by CCl_4: 0.56±0.06 (baseline) and 0.47±0.08 (CCl_4) (Table). However, 24 h after CCl_4 administration ADC_{fast} was drastically decreased by 71%, from 27.3 to 8.1 × 10^{-3} mm^2/s ($P<0.05$). Furthermore, ADC_{slow} was also significantly decreased 24 h post CCl_4 treatment, from 1.2 ± 0.2 × 10^{-3} mm^2/s to 0.4 ± 0.2 × 10^{-3} mm^2/s, $P<0.05$ (Table).

Fast and slow components of the water apparent diffusion coefficient (ADC) in the rat liver before and after CCl_4 treatment

Parameter	Baseline	CCl_4
A_0	98.3±1.6	99.6±0.6
A_f	0.56±0.06	0.47±0.08
ADC_{fast}	27.3±7.3	8.1±1.2
ADC_{slow}	1.2±0.2	0.4±0.2

Note. A_0 is signal intensity for b=0 s/mm^2, A_f is the relative contribution of ADC_{fast}. ADC values are in 10^{-3} mm^2/s. Mean ± SEM, n=7. * – P ≤ 0.05 (vs. Baseline).

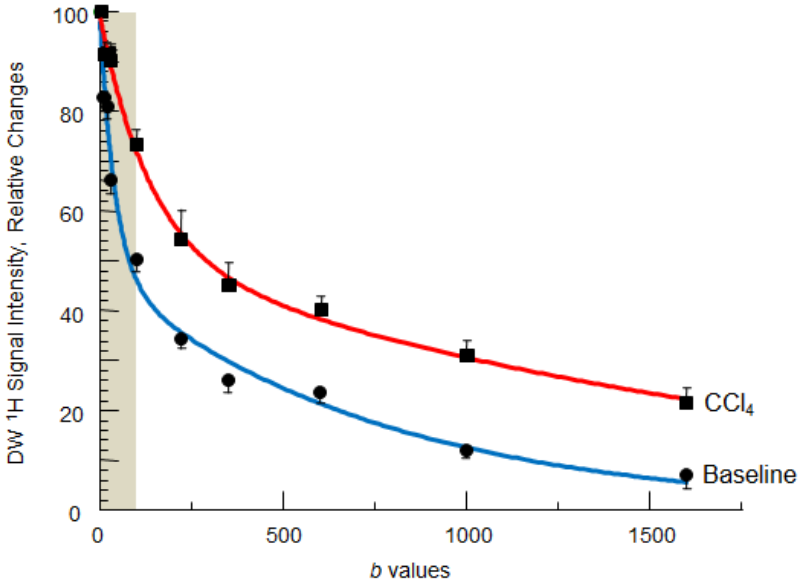

Fig. 2. Effect of CCl_4 on the diffusion-weighted imaging ^1H MRI signal intensity vs. *b* value plot. Shading indicates the perfusion component area. M ± SEM, n = 7.

Fig. 3 shows the representative tracks accumulation of gadolinium by liver and by subcutaneous muscle. The fit of DCE ^1H MRI liver signal intensity versus time data had the triexponential shape that contains inflow (first 60–70 s), fast outflow (~ 70–200 s), and slow outflow (~ 200–800 s) parts. At the same time muscle had the biexponential shape that contains inflow (first 100–110 s) and outflow (~ 110–800 s) parts. The gadolinium inflow represents mostly a development of perfusion system in liver whereas slow outflow most likely reflects a steady-state of inflow wash-out of the contrast agent. Both inflow and wash-out was significantly faster in liver compared to subcutaneous muscle.

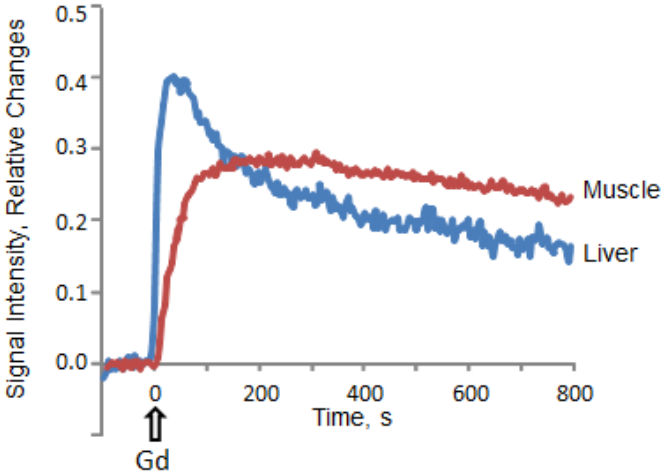

Fig. 3. The representative dynamic contrast enhanced ^1H MRI signal intensity vs. time curves in the rat normal liver and subcutaneous muscle.

Unlike ADC_{fast} (measured from DWI), AUC (measured from DCE ^{1}H MRI) did not change after CCl_4 treatment. In addition, there was no correlation ($R^2 = 0.55$) between ADC_{fast} and AUC values. Only the contrast agent inflow kinetics showed a decrease from 11±3 s^{-1} (baseline) to 5±1 s^{-1} (CCl_4, $P<0.05$) while both fast and slow outflow components did not show any significant difference (Fig. 4).

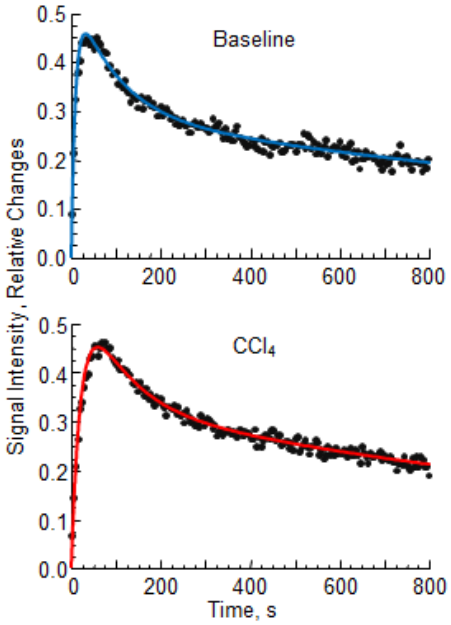

Fig. 4. Corresponding dynamic contrast enhanced ^{1}H MRI signal intensity vs. time curves in the rat liver before (baseline) and 24 h after CCl_4 treatment. Average of 7 experiments is presented.

Discussion

A noninvasive detecting and monitoring of the liver disease development and treatment efficacy are very important in hepatopathology. Traditional laboratory tests, such as measurements of aspartate aminotransferase to alanine aminotransferase ratio, aspartate aminotransferase to platelet ratio index, or FibroTest, a composite of five serum biochemical markers, are closely related to hepatocellular function [10]. However, all of the serum based tests have their limitations in specificity and sensitivity. Commonly used for liver studies imaging methods such as computerized tomography and DCE ^{1}H MRI traditionally require infusion of the contrast agents and do not properly differentiate the perfusion and diffusion processes in liver [10]. Thus, their specialties are more related to the gross morphologic or physical features of liver, like stiffness, shape or anatomic relationship to neighboring organs and are more targeted on assessment of well-developed liver fibrosis and have limited diagnostic utility in monitoring the whole progression of diffuse liver disease, especially in detection of liver disease at its early stage [10, 15].

In this work, the use of DWI and DCE ^{1}H MRI for the assessment of hepatic perfusion and diffusion parameters was evaluated in an acute CCl_4-induced rat liver injury model. The data presented here show that 24 h after CCl_4 treatment significantly increased the liver ^{1}H signal compared to untreated liver, suggesting an increase in T_2. Usually T_2 relaxation reflects the loss of phase coherence in the transverse plane and is associated with entropy of the spin system [2]. ADC_{fast} in the liver, which represents mostly perfusion [13], was significantly decreased 24 h after

CCl_4 treatment, while the relative contribution of ADC_{fast} (associated with the relative vascular volume) does not change. This decrease may be because of restricted perfusion in congested microvessels as was shown by histology (Fig. 1). A slight decrease in inflow slope detected using DCE ^1H MRI partly supports DWI and histology data. However, unlike ADC_{fast} (measured from DWI), AUC (measured from DCE ^1H MRI) widely used for estimation of perfusion did not change after CCl_4 treatment. Furthermore, a reproducibility and accuracy of non-invasive ADC measurement was higher than invasive DCE ^1H MRI experiments. In addition, more studies of the transition from vessels to the liver representing permeability, and the transition from the liver to vessels representing washout, need to be done.

Unlike DCE ^1H MRI, DWI provides information about water diffusion in liver tissue. CCl_4 significantly decreased a diffusion component of ADC (ADC_{slow}) which can be explained by compartmental changes in liver tissue, such as cellular swelling and hepatocellular degeneration leading to a coagulative type of necrosis. The cellular swelling may also result in a decrease in extracellular space and restriction of water diffusion. In addition, our previous data [4] show that the acute effect of CCl_4 in rat liver is associated with a significant decrease in the ATP/P_i ratio in hepatocytes from 1.24 to 0.94 ($P<0.01$) and a drastic increase in intracellular Na^+ from 17 to 49 mM ($P<0.0005$). A decrease in bioenergetic status after CCl_4 treatment may lead to a decrease in intracellular water diffusion that plays an important role in total tissue ADC as well [1].

Thus, a biexponential model for analysis of non-invasive DWI provides important information about toxic transformation in the capillary liver tissue perfusion and water molecular diffusion. Recognition of both perfusion and diffusion components of water ADC may be important for monitoring response to therapy of liver disease, such as steatosis, fibrosis, hepatitis, and cirrhosis.

Conclusion

CCl_4 treatment caused decreases in both fast and slow apparent diffusion coefficient of water in rat liver measured by DWI, as well as a decrease in contrast agent uptake measured by DCE ^1H MRI. DWI should prove useful in assessment of liver damage in diffuse liver diseases without the need for a contrast agent.

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ДОСЛІДЖЕННЯ ПЕРФУЗІЙНИХ ТА ДИФУЗІЙНИХ ПРОЦЕСІВ ЗА ДОПОМОГОЮ ЯМР У ПЕЧІНЦІ ЩУРІВ, ІНТОКСИКОВАНИХ КАРБОНТЕТРАХЛОРИДОМ

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Для дослідження перфузійних процесів у печінці щурів, інтоксикованій карбонтетрахлоридом (CCl_4), використані дифузійно-градієнтний і динамічно-контрастний методи ядерного магнітного резонансу (ЯМР). Пошкодження печінки викликали одноразовим внутрішньошлунковим введенням суміші CCl_4 і кукурудзяної олії. CCl_4 викликав зменшення швидкого (перузійного) та повільного (дифузійного)

компонентів коефіцієнта дифузії води, який вимірювали дифузійно-градієнтним ЯМР. Одержані дані були частково підтверджені динамічно-контрастним ЯМР, що зафіксував зменшення швидкості нагромадження контрастної речовини гадолінію в інтоксикованій печінці. Отже, метод дифузійно-градієнтного ЯМР дає змогу неінвазивно досліджувати пошкодження печінки без використання контрастних препаратів.

Ключові слова: печінка, CCl_4, ЯМР, перфузія, дифузія води.

ИССЛЕДОВАНИЯ ПЕРФУЗИОННЫХ И ДИФФУЗИОННЫХ ПРОЦЕССОВ С ПОМОЩЬЮ ЯМР В ПЕЧЕНИ КРЫС, ИНТОКСИЦИРОВАННЫХ КАРБОНТЕТРАХЛОРИДОМ

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Для исследования перфузионных процессов в печени крыс, интоксицированной карбонтетрахлоридом (CCl_4), использованы диффузионно-градиентный и динамически-контрастный методы ядерного магнитного резонанса (ЯМР). Повреждение печени вызывали внутрижелудочным одноразовым введением смеси CCl_4 и кукурузного масла. CCl_4 вызывал уменьшение быстрой (перфузионной) и медленной (диффузионной) составляющей коэффициента диффузии воды, который измеряли с помощью диффузионно-градиентного ЯМР. Полученные данные были частично подтверждены методом динамично-контрастного ЯМР, который зафиксировал уменьшение скорости накопления контрастного вещества гадолиния в интоксицированной печени. Таким образом, метод диффузионно-градиентного ЯМР позволяет неинвазивно исследовать повреждения печени без применения котрастных веществ.

Ключевые слова: печень, CCl_4, ЯМР, перфузия, диффузия воды.