European Journal of Radiology 74 (2010) 280–286

Contents lists available at ScienceDirect

European Journal of Radiology

journal homepage: www.elsevier.com/locate/ejrad

Quantitative assessment of early experimental diabetes in rats using dynamic contrast-enhanced computed tomography

Kenya Murase*, Akihiro Kitamura, Atsushi Tachibana, Yoshinori Kusakabe, Risa Matsuura, Shohei Miyazaki

Department of Medical Physics and Engineering, Division of Medical Technology and Science, Faculty of Health Science, Graduate School of Medicine, Osaka University, 1-7 Yamadaoka, Suita, Osaka 565-0871, Japan

ARTICLE INFO

Article history:
Received 13 January 2009
Received in revised form 14 February 2009
Accepted 6 March 2009

Keywords:
Dynamic contrast-enhanced CT
Experimental diabetes
Rats
Renal volume
Glomerular filtration rate

ABSTRACT

Purpose: To quantitatively assess the time course of changes of the renal volume and function in the early phase of streptozotocin (STZ)-induced diabetes in rats using dynamic contrast-enhanced computed tomography (DCE-CT).
Methods: The DCE-CT studies were performed in 24 male Sprague–Dawley rats ($n = 6$ for control and $n = 18$ for STZ-treated group) on days 0, 4, 7, 11, and 14 using a multi-detector row CT. The rats of an STZ-treated group were given intraperitoneally 65 mg/kg body weight of STZ on day 0, and were divided into two groups based on the blood glucose concentration on day 4 being less than 300 mg/dL [STZ-treated group (L), $n = 8$] or greater than 300 mg/dL [STZ-treated group (G), $n = 10$]. The contrast clearance per unit renal volume (K_1) was estimated from the DCE-CT data using the Patlak model. The renal volume (V_{CT}) was calculated by manually delineating the kidney on the contrast-enhanced CT image. The contrast clearance of the entire kidney (K) was obtained by $K_1 \times V_{CT}$.
Results: V_{CT} in the STZ-treated group was significantly enlarged on day 4 compared to that on day 0 and continued until day 14. Although there were no significant changes in the time course of K_1 in all groups, K in the STZ-treated groups (L) and (G) significantly increased on days 7 and 4, respectively, and continued until day 14, suggesting that hyperfiltration occurs in parallel with renal volume enlargement.
Conclusion: The present method appears useful for quantitatively evaluating the time course of STZ-induced diabetes in rats, because it allows repeated and simultaneous evaluation of renal morphology and function.

1. Introduction

Dynamic contrast-enhanced computed tomography (DCE-CT) has a great potential for measuring physiological parameters such as perfusion [1], because CT can measure changes in the concentration of contrast agent (CA) quantitatively, accurately, and with high spatial and temporal resolution by subtracting unenhanced from enhanced CT scans.

Glomerular filtration is the main function of the kidney. Measurement of the glomerular filtration rate (GFR) is important in the evaluation of renal function for the assessment of many renal diseases and their treatment [2]. The conventional iodinated X-ray CAs have pharmacokinetics comparable to those of inulin, generally regarded as the benchmark extracellular fluid marker in physiology [2]. Consequently, DCE-CT using iodinated CA has been applied to the measurement of GFR [3–5].

The diabetic kidney hypertrophy–hyperfunction syndrome is a well-established phenomenon [6], i.e., the kidney begins to grow and GFR increases at the onset of diabetes. Some time later, structural changes can occur in the glomerulus which forms the basis for progressive diabetic nephropathy [7]. Thus, it is important to detect the renal hypertrophy–hyperfunction state in the early phase of diabetes in order to prevent diabetic nephropathy.

The purpose of this study was to quantitatively assess the time course of changes of the renal volume and function in the early phase of experimental diabetes in rats using DCE-CT and to investigate the usefulness and feasibility of DCE-CT for detecting the diabetic renal hypertrophy–hyperfunction state.

2. Materials and methods

2.1. Kinetic model of CA in the kidney

The kinetic model used for analysis consists of two compartments, i.e., the blood and the kidney. When using this model, the differential equation describing the kinetic behavior of the CA in

* Corresponding author. Tel.: +81 6 6879 2571; fax: +81 6 6879 2571.
E-mail address: murase@sahs.med.osaka-u.ac.jp (K. Murase).

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doi:10.1016/j.ejrad.2009.03.006

the kidney is given by [8]

$$\frac{d[C_{\text{kidney}}(t) - f \cdot C_b(t)]}{dt} = K_1 \cdot C_b(t) - k_2 \cdot [C_{\text{kidney}}(t) - f \cdot C_b(t)], \quad (1)$$

where $C_{\text{kidney}}(t)$ and $C_b(t)$ are the concentrations of the CA at time t in the kidney and the blood, respectively. K_1 and k_2 represent the rate constants for the transfer of CA from the blood to the kidney via glomerular filtration and outflow of the CA from the kidney, respectively. f denotes the blood volume fraction.

Solving Eq. (1) for $C_{\text{kidney}}(t)$ with the assumption that the initial conditions are zero and k_2 is negligible during the sampling period yields

$$C_{\text{kidney}}(t) = K_1 \cdot \int_0^t C_b(u)du + f \cdot C_b(t), \quad (2)$$

where u is a variable of integration. Dividing both sides of Eq. (2) by $C_b(t)$ yields

$$\frac{C_{\text{kidney}}(t)}{C_b(t)} = K_1 \cdot \frac{\int_0^t C_b(u)du}{C_b(t)} + f. \quad (3)$$

K_1 and f can be estimated from Eq. (3) using the Patlak graphical method [9]. The clearance of CA in the entire kidney (K) is given by

$$K = K_1 \cdot V, \quad (4)$$

where V represents the renal volume. It should be noted that K is the GFR in terms of the whole blood.

The time-attenuation curve (TAC) in the aorta was used as an arterial input function (AIF) instead of that in the renal artery because it was difficult to directly obtain the TAC in the renal artery. The transit time of the CA from the aorta to the renal artery was corrected as follows. First, we manually drew the region of interest (ROI) on the aorta and entire kidney as illustrated in Fig. 1, and then generated the TACs in the aorta and entire kidney ROIs. Second, the transit time between the aorta and the kidney was determined by visually finding the rise points of these TACs. Finally, the transit time was corrected by shifting the TAC in the aorta to that in the entire kidney. The dispersion in AIF between the aorta and the kidney ROIs was not corrected in this study.

The partial volume effect (PVE) on $C_b(t)$ was corrected according to Cenic et al. [10] Briefly, a calibration curve was generated by phantom experiments containing background and contrast tubes with various diameters [8]. From the calibration curve, the recovery coefficient (RC) value was determined, knowing the standard deviation (SD) of the gaussian fit to the background-subtracted image profile of the aorta from which the AIF was obtained [8]. The AIF corrected for the PVE was obtained by multiplying the AIF by the RC value obtained above.

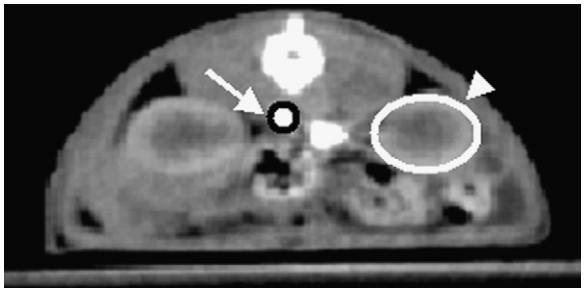

Fig. 1. Example of the regions of interest (ROIs) drawn on the aorta (arrow) and entire kidney (arrowhead) to obtain the time-attenuation curves (TACs). The TAC in the aorta ROI was used as an arterial input function. The CT image shown here was acquired 20 s after the start of scanning.

Fig. 2 shows the typical examples of $C_b(t)$ and $C_{\text{kidney}}(t)$ (a) and Patlak plot (b).

2.2. Animal experiment protocol

A total of 24 male Sprague–Dawley rats (8 weeks old) weighing 309 ± 31 g [mean ± SD] were used. They were purchased from Charles River Japan (Yokohama, Japan), and were maintained under standard conditions. The rats were allowed free access to standard laboratory food and water. All animal experiments were approved by the animal ethics committee at Osaka University School of Medicine.

The rats were randomly allocated into control (n = 6) and STZ-treated groups (n = 18). The rats of an STZ-treated group were intraperitoneally injected with STZ (65 mg/kg body weight). STZ was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Day 0 was defined as the day of STZ injection. The DCE-CT studies were performed on days 0, 4, 7, 11, and 14. When performing the DCE-CT studies, the animal was anaesthetized using pentobarbital (Nembutal; Dainippon-Sumitomo Seiyaku Co., Ltd., Osaka, Japan) (50 mg/kg body weight) and was placed on the CT table in a prone position. To reduce physiological motion such as respiratory motion, we immobilized the animal with surgical tape.

Blood glucose concentration was measured using a blood glucose meter (Medisafe-mini GR-102; Terumo Co., Tokyo, Japan). Depending on the blood glucose concentration on day 4, the STZ-treated rats were allocated into two groups. One was the group of STZ-treated rats with blood glucose concentration less than 300 mg/dL [STZ-treated group (L)], and the other was the group of STZ-treated rats with blood glucose concentration greater than 300 mg/dL [STZ-treated group (G)].

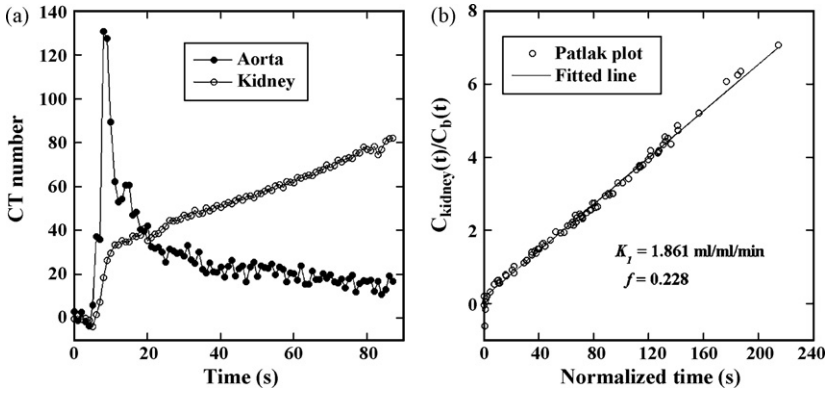

Fig. 2. (a) Typical example of the TACs in the aorta [$C_b(t)$] and entire kidney [$C_{\text{kidney}}(t)$]. (b) Patlak plot in which the normalized time given by $\int_0^t C_b(u)du/C_b(t)$ was taken as the x axis and $C_{\text{kidney}}(t)/C_b(t)$ was taken as the y axis. The rate constant for the transfer of contrast agent (CA) from the blood to the kidney via glomerular filtration (K_1) and blood volume fraction (f) were obtained from the slope and y-intercept of the fitted line (solid line) of the Patlak plot, respectively.