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Micro-Computed Tomography Provides Accurate Measurement for Cardiac Function in Infarcted Rat Heart

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Abstract

Objective: High resolution image is required for accurate measurement of cardiac function for the analysis of detailed regional function especially in a small animal. Methods: Left ventricular function of rat hearts was measured using micro-computed tomography (micro-CT) with administration of contrast agent in a rat with normal heart as well as rats with mild and severe myocardial infarction (MI). Following the CT acquisition, the hearts were sectioned for pathological evaluation. Results: The volume plot per each disk of the normal heart revealed that contraction force peaked at the middle of the heart. In the heart with mild infarction, the volume plot curve clearly demonstrated that infarction was located only at the apex of the heart, whereas severe infarction was disturbed in larger area. The left ventricular ejection fraction of the normal, mild MI, and severe MI hearts were 68.6%, 40.0%, and 16.4%, respectively. In addition, volume analysis in severe MI demonstrated ventricular dilatation, although that in mild MI did not show any change in the ventricular volume. Histological results were consistent with the CT measurement. Conclusions: Micro-CT provided accurate measurement of cardiac function in rats, which is especially useful for the analysis of small animals with heterogeneous dysfunction of the heart.

Keywords

Regional Cardiac Function, Small Animal, Contrast Agent

1. Introduction

An animal model is crucial for the regenerative medicine to determine the safety or efficacy of a therapy. In the

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cardiac field, functional analysis of the heart is one of the most important information to be evaluated when determining the benefit of the therapy. Currently, the most widely used modality to measure cardiac function is echocardiography (echo) allowing repeat measurements to observe the time-course effect of the therapy, because it is easy to handle and non-invasive. However, functional analysis with echo, especially in small animals, has a potential risk of inaccurate measurement or a wide interobserver variability compared to those with magnetic resonance imaging (MRI) [1], which is known be the most reliable method [2] [3]. Accurate measurements and detailed analysis with high resolution imaging are becoming more important for the field of regenerative medicine of the heart, as it is nearly to be clinically applied. Nevertheless, MRI also has a disadvantage that its installation site is limited due to the need for magnetic shielding and a robust floor to support its heavy weight. The cost of the machine as well as the installation environment is comparatively more expensive than an echo or CT. In this paper, we measured the cardiac function of rats with both normal and infarcted hearts using a portable micro CT with contrast agent to determine its efficacy.

2. Methods

2.1. Animal Experiments Protocol

Experimental animals were treated in compliance with the institutional guidelines for animal experimentation of Institutional Animal Care and Usage Committee of Juntendo University, School of Medicine.

2.2. Functional Measurement of the Heart by Micro-Computed Tomography (Micro-CT)

A male Sprague-Dawley (SD) rat at 8 weeks of age was anesthetized by intra peritoneal injection of sodium pentobarbital (40 mg/kg). After anesthesia, body weight was measured to determine the volume of contrast agent to be administered. The rat was set in the micro-CT equipment (Latheta LCT-200, Hitachi-Aloka Medical, Tokyo, Japan) with a holder (120 mm in diameter), then iodized contrast agent (Bystage 370, Teva Pharma, Tokyo, Japan) was intravenously administrated (150 µL/min) through 24 gauge of angiocatheter. When the dose of contrast agent reached 20 mL/kg, image acquisition was started. The CT image was obtained in disks of 480 µm slice thickness and width using the "cardiac synchronized" mode. This rat was numbered as Rat#1.

2.3. Image Analysis

Two series of images, diastole and systole, were obtained from each disk by scanning. The image data was analyzed in "volume measurement" mode with Latheta image analysis software (Hitachi-Aloka). The left ventricle (LV) cavity, which can be detected separately from the muscle by contrast, was defined as the sum of all disk volume from the base to the apex of the heart. The area [μm^2] of each disk was measured as the "circle ROI (region of interest)" to include whole blood-pool contrast. Since the thickness of each disk was 480 µm, the regional volume [μm^3] of diastole or systole can be calculated by multiplying the area times the 480 µm of thickness. Once the diastolic volume (Vd) and systolic volume (Vs) of the whole heart was calculated by adding the value of all disk volume, stroke volume (SV = LVVd − LVVs) and ejection fraction (LVEF = SV/LVVd) of the left ventricle were determined.

2.4. Rat Myocardial Infarction (MI)

A SD rat, which underwent anesthesia as previously described, was intubated, then connected to a pulmonary ventilator (respiration rate = 90/minute, inhalation volume = 4.0 mL) on right lateral position. Following intercostal thoracotomy, the left coronary artery (LCA) was ligated by monofilament suture (7-0 prolene, Ethicon, Tokyo, Japan) to create an experimental myocardial infarction. The size of the infarction varied from mild (Rat#2) to severe (Rat#3) by changing the ligation site to distal or proximal LCA, respectively. The functional measurement by micro-CT was performed 7 days after the surgery.

2.5. Histological Analysis

To determine the infarcted area with histology, the heart was stained by Masson's trichrome method. After performing the micro-CT, the heart was excised, then perfused with 20 mL of phosphate buffer saline (PBS) containing heparin sodium (1000 U) via ascending aorta followed by 4%-paraformaldehyde solution for 30 minutes.

The heart was dehydrated using 10%, 15% and 20% of sucrose solution, then embedded into the OCT compound and stored at −80˚C. It was cut into 10 µm sections with a cryostat and immersed into Bouin's solution overnight, then stained using Masson's trichrome staining kit (Sigma-Aldrich Japan, Tokyo, Japan).

3. Results

The initial disk images of the normal rat heart obtained by CT are shown in **Figure 1**. Panels on the left (**Figure 1(a)**, **Figure 1(c)**) are plane images, and panels on the right (**Figure 1(b)**, **Figure 1(d)**) are the images used for LV volume analysis. The LV cavity was larger and the wall was thinner in the diastolic phase compared to the systolic phase. **Figure 2** shows the volume change in the disks from the base to the apex of the normal rat heart (Rat#1). The gap between diastolic and systolic LV volume peaked at the middle of the heart, where contractile force was strongest. The difference in volume was reduced toward the apex of the heart. The diastolic LV volume at point (a) was the following; LVVd(a) = 18.5 µL, LVVs(a) = 10.1 µL, and SV(a) = 8.4 µL. In contrast, the contraction volume was lower at point b; LVVd(b) = 8.9 µL, LVVs(b) = 2.3 µL, and SV(b) = 6.6 µL. In addition, LVEF of the whole heart was calculated as 68.6%.

Next, we measured the cardiac function of rats with MI (Rat#2 and #3 in **Figure 3** and **Figure 4**, respectively). In Rat#2, systolic volume acutely increased in five disks (2.4 mm) at the apex of the heart (upper panel), indicating systolic contraction failure. In fact, the diastolic and systolic volumes of the LV cavity shown in the CT images were similar at point (b) (**Figure 3**, lower panel). In Rat#3, the area of contractile failure was wider than Rat#2, on 12 disks (5.8 mm) at the apex. The summary of the measurement of animals is shown in **Table 1**. Notably, the rat with severe MI showed an enlargement of the LV cavity, (425.0 µL at diastole) whereas the rat with mild MI presented an LV smaller in size (291.9 µL) similar to the normal heart (283.6 µL). In the CT image, the LV free wall of rat #3 was thinner than those of the other two rats. These findings were consistent with the histological images (**Figure 5**). In addition, LVEF of the rat with mild MI was 40.0%, and that of a rat with severe MI deteriorated to 16.4%.

4. Discussion

In this paper, we demonstrated that micro-CT provided accurate measurements of cardiac function for both normal and infarcted rat heart.

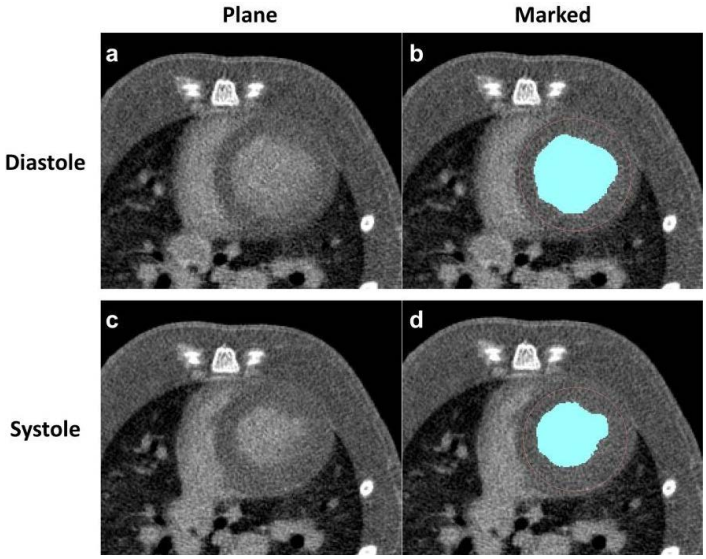

Figure 1. An example image of measuring slice volume inside left ventricle (LV). Two images, diastolic (a) and systolic (c), are provided per each disk when micro-CT is acquired (left panels). To analyze the volume, LV cavity is encircled by the "circle ROI" tool (red circle on right panels). The blue mark indicates the LV cavity in diastolic (b) and systolic (d).

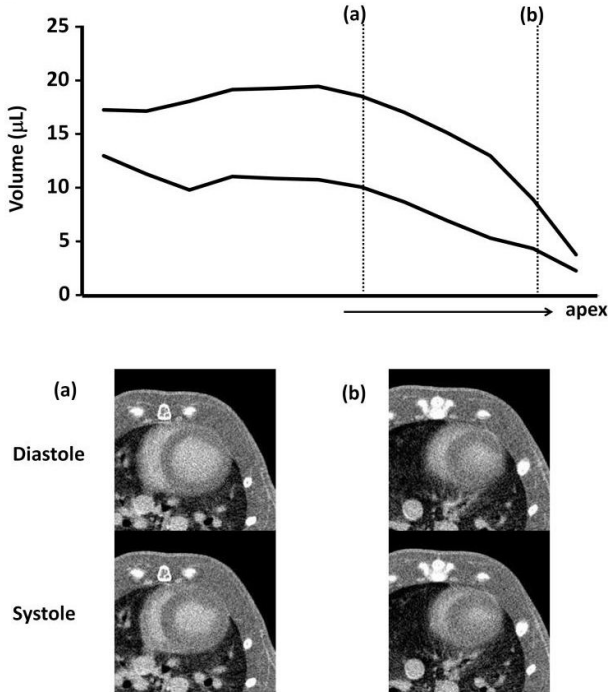

Figure 2. Volume analysis in normal heart (Rat#1). In the upper graph, contractile force is the largest in the middle of the heart and attenuates on the apex of the heart. The lower panel clearly indicates the LV cavity is smaller in systole both in the middle (left) and in the apex (right) than in diastolic.

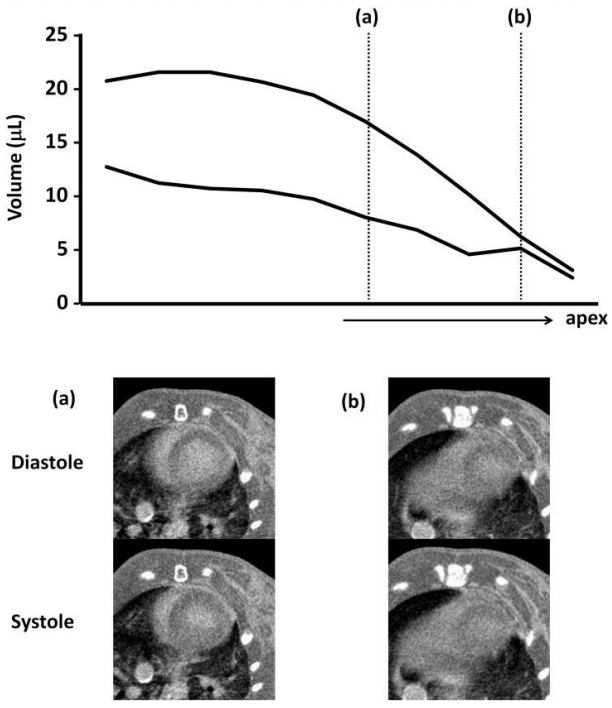

Figure 3. Volume analysis in the heart with mild myocardial infarction (Rat#2). Heart contraction is disturbed in the apex. Heart images also show the LV cavity is similar in both systole and diastole (lower right panels).

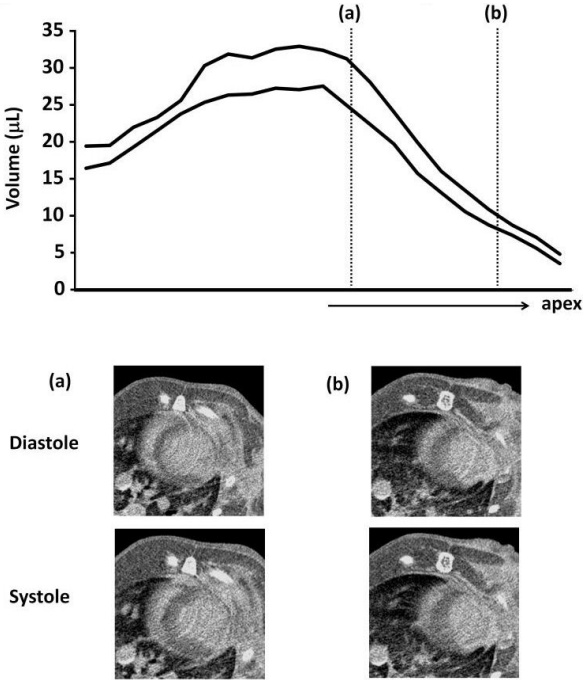

Figure 4. Volume analysis in the heart with severe myocardial infarction (Rat#3). Heart contraction is disturbed from the middle to the apex of the heart. LV free wall has become thin, whereas the septal side wall still shows differences in the LV mass between diastole and systole in the middle of the heart (Lower left panels).

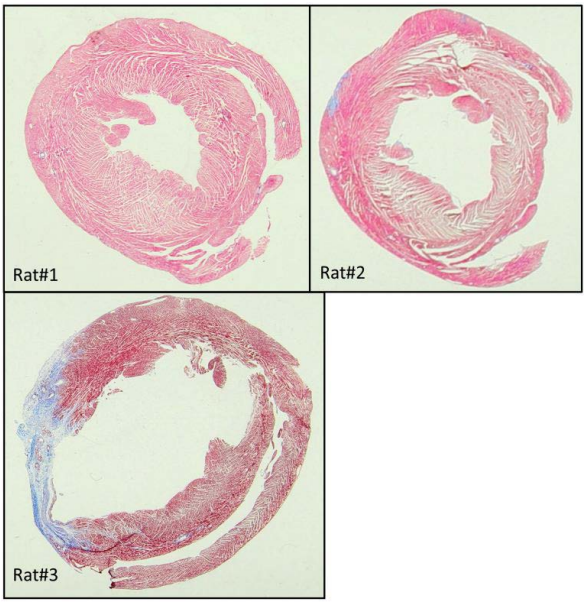

Figure 5. Histological evaluation of the rat hearts. Left side of each panels indicates free wall of the left ventricular and right sides indicates right ventricular. The wall thickness of the heart with severe infarction (Rat#3) is thinner and the ventricular cavity is enlarged, whereas those of the mild myocardial infarction (Rat#2) were preserved as in the control heart (Rat#1).

Table 1. Functional data of rat hearts.

	Rat#1	Rat#2	Rat#3
Body Weight, g	295.8	294.9	309.6
Size of MI	-	mild	severe
LVDV, µL	283.6	291.9	425.0
LVSV, µL	168.2	175.0	355.2
SV, µL	115.4	116.9	69.8
LVEF, %	68.6	40.0	16.4

MI: myocardial infarction, LVDV: left ventricular diastolic volume, LVSV: left ventricular systolic volume, SV: stroke volume, LVEF: left ventricular ejection fraction.

Currently, echo has been most commonly used for the measurement of cardiac function in the clinical area due to the following advantages: less invasive, unnecessary to use contrast agent, no exposure to radiation, portability of the machine which allows prompt measurement at bedside, relatively inexpensive cost, and less restrictions on the installation site. However, it has been reported that echo has less reproducibility of regional functions than MRI, indicating less accuracy when representing the heart with regional disturbance such as myocardial infarction [4]. LVEF, the index of global heart function, is generally measured with echo and analyzed with the modified Simpson's rule. In this method, LV volume is determined by the summation of discs using a measurement of the LV length as well as the LV area at the base, middle and apex of the left ventricle. The LV area of a small animal may often be determined by a single disk because of its small heart. This method is reliable if the regional contraction is similar to the remaining area. However, in a heart with regional disturbance, the global function calculated by this method is affected by the site of measurement; measurement taken at the middle of the infarction will result in underestimation of the global function, and measurement taken at the normal area will cause the infarcted area to be omitted from the result. In addition, the heart contraction was not uniform within the heart, but it was strong at the middle of the heart and weakened towards the base and the apex. This indicates the potential risk of failure to reproduce LVEF measurements by echo, especially in small animals.

As another method to measure the cardiac function, a conductance catheter system is also used. The catheter is intravascularly inserted to place the edge into the heart chamber. This method is reported to be useful for the measurement of cardiac function of the rat with myocardial infarction [5]. However, catheter inserting not only requires a certain skill, but it might be too invasive for the animal when repeat measurement is needed to assess chronological change of cardiac function. Moreover, LV volume is often overestimated in conductance catheter measurement, because pressure-volume conductance device is unable to continuously measure parallel conductance [6].

Alternatively, positron emission tomography (PET) or single photon emission computed tomography (SPECT) has been reported to be less invasive than catheterization [7]-[9]. These methods, especially SPECT, demonstrated shorter time of imaging and improved spatial resolution by introducing the multitracer imaging system. However, the maximum resolution is still more than 1 mm [10], compared to that of the micro-CT used in this paper of 24 - 480 µm, which might be a huge disadvantage of PET/SPECT for small animal study.

Since both MRI and CT capture the whole image of the heart, complex movements such as regional disturbance can be assessed more accurately than other "single-slice" modality. In fact, recent studies revealed that the image analysis with CT is more reliable and reproducible than 2-D or 3-D echo [1] [4]. In our analysis, micro-CT clearly represents the volume and functional differences among normal heart, mild infarction and severe infarction. However, exposure to radiation and administration of contrast agent still remain to be huge disadvantages of CT imaging. In this study, larger amount per body weight of contrast agent was required to obtain sufficient contrast of the LV cavity than in human use. Currently, MRI is considered as the most reliable modality to measure the cardiac function [2] [3]. However, not only is the cost of the system itself expensive compared to a CT, but there are also certain environmental requirements for its installation; the room must have magnetic shielding, and the floor must be monolithic to sustain the heavy weight of the system of over 1,000 kg. In contrast, the weight of the micro-CT system used in this study was only 220 kg including radiation shielding. It also had wheels, allowing easy movement when necessary. In addition, the scanning time of the CT was much shorter

than that of an MRI. Importantly, despite of the recent development of MRI, the resolution of CT imaging is still superior, allowing more detailed analysis of both structure and function [11]. Furthermore, the image data obtained by single scanning of the CT can be reconstructed into short axis, longitudinal, or even 3-D images. Some of them have been reported to be useful for structural and functional analysis [12] [13]. Moreover, recent report presented a more accurate imaging of functional changes using four dimensional (4-D) imaging of CT [14] [15]. In conclusion, micro-CT imaging is useful for accurate and detailed analysis of the whole heart function in both normal rats and rats with myocardial infarction.

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Disclosure

No disclosure.

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