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Regular Article

Functional mechanisms of myocardial microcirculation in left ventricular hypertrophy
A hypothetical model of capillary remodeling post myocardial infarction

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Abstract

Objectives: Left ventricular (LV) remodeling after myocardial infarction (MI) is characterized by myocyte hypertrophy and a disproportional capillary growth. We developed a hypothetical model of capillary remodeling mechanisms based on quantitative data of microcirculation determined by magnetic resonance (MR) imaging techniques and histology.
Methods: Perfusion and regional capillary blood volume (RBV) were quantified 8 and 16 weeks after MI (mean 27.0±2.9% of the left ventricle 16 weeks post MI) or sham operation in rats using MR imaging and were correlated with morphometric data.
Results: Maximum perfusion (ml/(g min)) in the remote area decreased from 5.69±0.63 to 3.48±0.48 compared to sham animals (5.33±0.31, $p \leq 0.01$) and showed a close inverse relation to hypertrophy. In contrast, maximum RBV in the remote area was similar to that of sham animals (16.79±0.42% and 16.52±0.33%, respectively) and did not change over time. Thus, mean transit time (MTT) was longer in remote than in sham myocardium. Morphology revealed that hypertrophy was inversely related to capillary density which was associated with an increase in capillary cross-sections.
Conclusions: Perfusion data in synopsis with histological observations demonstrate that the functional capillary length increases during hypertrophy post MI which is consistent with the increase of the mean transit time. Despite a relative decrease in capillary density, RBV may be restored by an increase in the cross-sections. In the light of almost maximum oxygen extraction under normal conditions, this hypertrophy related remodeling may be deleterious for tissue supply.

Keywords: Infarction; Remodeling; Capillaries; Regional blood flow; Microcirculation

Introduction

Tissue loss after MI results in remodeling of the surviving myocardium with reactive myocyte hypertrophy (Pfeffer et al., 1979; Anversa et al., 1985). Morphometric studies revealed a disproportional growth of capillaries compared to the increase of myocyte cross-sectional areas (Xie et al., 1997) and, hence, an increase of the diffusion distance for oxygen (Anversa et al., 1986). Whereas the quantitative determination of capillary density is well established, assessment of the capillary length is more complex. Depending on an anatomical or functional point of view one distinguishes the length of a single capillary from its functional length. The first refers to the distance between arteriolar and venular cross-connections and is about 90 μm in the normal rat myocardium (Batra and Rakusan, 1990). The functional length is the distance between adjacent arteriolar and venular domains, i.e. it reflects the way blood flows from the arteriolar to the venular zone (Beard and Bassingthwaighte, 2000) and is about 600 μm (Batra and Rakusan, 1990). Thus this definition considers the capillary as a hemodynamic unit within the capillary network and it directly corresponds to the supply function of a capillary. Morphometric

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analysis in the hypertrophied rat heart revealed that the functional mean length was significantly longer compared to normal hearts (Batra et al., 1991).

However, it would be of paramount interest to correlate these morphological observations to functional non invasive data of microcirculation in order to assess its potential consequences for remodeling of myocardium. Recently, new magnetic resonance imaging (MRI) techniques have been developed for the quantitative measurement of myocardial perfusion and intracapillary blood volume (RBV) in the healthy rat heart (Kahler et al., 1999; Waller et al., 2000) and in rat hearts during chronic left ventricular remodeling after myocardial infarction (Waller et al., 2001). As an additional parameter of microcirculation the mean transit time (MTT) of blood within the capillary system may be determined as MTT=RBV/perfusion. Obviously this parameter corresponds to the functional capillary length.

The aim of the present study was to determine functional microcirculation in the remote myocardium during left ventricular (LV) remodeling using MRI and to correlate these data with morphometric analysis of the same tissue. From these data, a hypothetical model of capillary remodeling has been developed to elucidate the functional mechanisms of blood supply during LV remodeling. We anticipated that in hypertrophied LV myocardium, functional length of capillaries and hence, MTT, increases. As it is known from basic physiology of the coronary microcirculation, the oxygen extraction along the capillary length is of about 70–75% even under normal conditions. This cannot be further increased without jeopardizing the average intracellular oxygen pressure. Hence, an increase of capillary length in accordance with an increase of MTT in post infarction hypertrophy could result in a lack of oxygen supply.

Methods

Animal preparation

All experimental procedures with animals conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were approved by the local authorities. In adult female Wistar rats (Charles River, Sulzfeld, Germany, 250–290 g) myocardial infarctions were produced using a method described previously (Pfeffer et al., 1979). After oral intubation under ether anesthesia, left thoracotomy was performed and the pericardium was incised. The heart was exposed and the left coronary artery (LCA) was ligated. After reposition of the heart, the thorax was instantly closed. Animals recovered during the following 24 h after surgery. Mortality was 38% within this time period. In sham-operated rats, the ligature around the LCA was not tied and all animals survived after surgery.

For MR measurements, animals were anesthetized with sodium pentobarbital (Narcoren, Rhone Merieux GmbH, Laupheim, FRG; 40 mg/kg i.p.), were orally intubated and ventilated by a small rodent respirator (BAS-7025, FMI, FRG, tidal volume: 0.3 ml, respiratory rate: 65 cycles/min). Anesthesia was continued via a tail vein by intravenous infusion of sodium pentobarbital (10–30 mg/kg). After a stabilization period of about 30 min, MR images were acquired while respiration was automatically stopped by the pulse program. ECG signal was received via foreleg electrodes connected to a specially adapted ECG unit (Rapid Biomedical, Wuerzburg, Germany) to trigger the image acquisition. The intravascular contrast agent (CA) Gd-DTPA-albumin (0.75 μmol/kg, ~0.3 ml) for RBV measurements and the vasodilatatory drug (adenosine 2 mg/(kg min)) were administered via a tail vein.

MR imaging and data analysis

MRI of myocardial perfusion and relative intracapillary blood volume

All images were acquired on a 7.05-T Biospec 70/21 spectrometer (Bruker). A specially adapted double probe head for rat heart measurement was used (Rapid Biomedical, Germany). Quantitative T_1 measurements were acquired by using an inversion recovery snapshot fast, low-angle shot (FLASH) sequence (Haase et al., 1990). Twenty-four ECG-triggered snapshot FLASH images were recorded after global or slice-selective spin inversion. Each snapshot FLASH image (TR=2.25 ms, TE=1 ms, flip angle about 3°, slice thickness 3 mm, field of view 50×50 mm) had a spatial resolution in plane of 390×780 μm^2. Total acquisition time for one T_1 experiment was in the range of 2×24=48 FLASH images (1–2 min). Images were obtained in a short axis slice perpendicular to the long axis of the left ventricle 4–6 mm below the valvular plane. Infarcted myocardium was regularly visible in the imaging slice. Blood flow was measured with a slice-selective and a global T_1 experiment, RBV was determined by slice-selective T_1 experiments before and after application of Gd-DTPA-albumin. The acquisition time for four perfusion or RBV maps was about 15 min. The duration of the whole perfusion or RBV measurement was therefore about 30–35 min. Values for T_1 in the blood of global T_1 experiments before and after CA were 1.61±0.05 s and 0.52±0.03 s, respectively. The calculated RBV has to be corrected by a factor which is determined by the ratio of hematocrit in the ventricle and the capillaries. Since hematocrit (Hct) of the capillary blood is 63–75% of the blood of larger vessels the ratio is found to be $(1-Hct_{capillaries})/(1-Hct_{ventricle})=1.34$. Further methodological details are described elsewhere (Belle et al., 1998; Bauer et al., 1997).

MR cine imaging

An ECG-triggered fast-gradient echo sequence (FLASH) (Haase et al., 1990) was performed with a flip angle of 40°, echo time of 1.2 ms and a repetition time of 4.3 ms. 16 contiguous ventricular short-axis slices of 1 mm thickness with no interslice gap were acquired to cover the entire range of the ventricles. With a field of view of 30–35 mm and an acquisition matrix of 128×128, the resolution in-plane was 270×310 μm^2. Depending on heart rate, acquisition time for one cine image was in the range of 40 to 50 s. Signal-to-noise ratio (SNR) was increased by averaging the images four times.

Data analysis

For quantification of perfusion and RBV, a mid-myocardial region in the surviving myocardium of the infarcted animals covering the posterior, lateral and septal left ventricular regions remote from the infarct (160–180 pixels) was selected as region of interest (ROI). In the sham-operated group, a mid-myocardial ROI covering the whole left ventricle (210–250 pixels) was manually delineated. Mean values for perfusion and RBV were obtained by averaging the pixel data in the ROI. Mean capillary transit time