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Original Article

Chronic hibernating myocardium in sheep can occur without degenerating events and is reversed after revascularization

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ABSTRACT

Introduction: Our goal was to show that blunting of myocardial flow reserve is mainly involved in adaptive chronic myocardial hibernation without apparent cardiomyocyte degeneration.
Methods and results: Sheep chronically instrumented with critical multivessel stenosis and/or percutaneous transluminal coronary angioplasty (PTCA)-induced revascularization were allowed to run and feed in the open for 2 and 5 months, respectively. Regional myocardial blood flow (MBF) with colored microspheres, regional and global left ventricular function and dimensions (2D echocardiography), and myocardial structure were studied. In sheep with a critical stenosis, a progressive increase in left ventricular end-diastolic and end-systolic cavity area and a decrease in fractional area change were found. Fraction of wall thickness decreased in all left ventricular wall segments. MBF was slightly but not significantly decreased at rest at 2 months. Morphological quantification revealed a rather small but significant increase in diffusely distributed connective tissue, cardiomyocyte hypertrophy, and presence of viable myocardium of which almost 30 % of the myocytes showed depletion of sarcomeres and accumulation of glycogen. The extent of myolysis in the transmural layer correlated with the degree of left ventricular dilation. Structural degeneration of cardiomyocytes was not observed. Balloon dilatation (PTCA) of one of the coronary artery stenoses at 10 weeks revealed recovery of fraction of wall thickness and near normalization of global subcellular structure at 20 weeks.
Conclusion: These data indicate that chronic reduction of coronary reserve by itself can induce ischemic cardiomyopathy characterized by left ventricular dilatation, depressed regional and global function, adaptive chronic myocardial hibernation, reactive fibrosis and cardiomyocyte hypertrophy in the absence of obvious degenerative phenomena.
Summary: Reduction of myocardial flow reserve due to chronic coronary artery stenosis in sheep induces adaptive myocardial hibernation without involvement of degenerative phenomena.

1. Introduction

In the original concept of chronic myocardial hibernation a permanent state of myocardial hypoperfusion as the consequence of a severe coronary artery stenosis was accepted resulting in impairment of cardiac function [1–4]. Another concept postulated repetitive episodes of demand ischemia in patients with coronary artery disease [5]. The latter concept was based upon the observation that proximal coronary artery occlusion compensated by collateral development provides normal myocardial perfusion at rest but relative underperfusion during periods of increased oxygen consumption. This concept was also observed in experimental setups in dogs and pigs and suggested that episodes of demand ischemia resulted in repetitive myocardial stunning which finally led to a chronic state of hibernation of the myocardium [6–13].

Before the phenomenon of hibernation was recognized, typical morphological alterations such as necrotic cell death of cardiomyocytes (CMs), intracellular myolysis resulting in disintegration and loss of contractile material and concomitant increased glycogen accumulation within the remaining CMs and increased interstitial fibrosis were described in human myocardial biopsies taken from areas that were dysfunctional and recovered after surgical revascularization [14]. Since then, these morphological phenomena have been

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described in a number of additional studies [reviewed in Ref. 13]. Borgers and colleagues mainly focused on the adaptive and the surviving, reversible nature of the dedifferentiated fetal phenotype of hibernating CMs and proposed dynamic passive stretch as the underlying mechanism [15–19]. Schaper and co-workers also indicated the adaptive nature of some morphological changes and their reversibility up to a certain degree but emphasized the degenerative nature of more severe CM alterations resulting in necrotic and/or apoptotic cell death and increased replacement fibrosis upon the necrotic cell death [20–23]. In pigs, reversible ischemia in an area of chronically reduced coronary flow reserve was described to induce regional myocyte loss via an apoptotic mechanism [24] and hibernating myocardium as a result of chronic stenosis or occlusion always showed various degrees of interstitial fibrosis [25–28]. Although the latter studies seem to indicate that the adaptation of myocardial cells to the hibernating state by chronic flow reduction is accompanied by obvious changes such as necrosis, apoptosis and replacement interstitial fibrosis, it would be interesting to know whether the adaptation could occur in the absence of pronounced degenerative phenomena—which lead per definition to irreversible cell death—as well. Moreover, the study of reversibility of the hibernating state would possibly be easier to approach.

During our study on critical multivessel stenosis in sheep, it appeared that chronic reduction of coronary flow reserve could induce ischemic cardiomyopathy upon repetitive stress, i.e., repetitive episodes of critical shortage of blood supply. This critical, repetitive shortage of blood supply resulted in cardiomyopathy which was characterized by left ventricular dilatation, depressed regional and global function and subcellular evidence of myocardial hibernation with a small increase in connective tissue formation. Percutaneous transluminal coronary angioplasty (PTCA) of one of the two stenoses was used to verify the influence of revascularization on regional and remote areas.

2. Methods

2.1. Experimental preparation and protocol

Experiments were carried out in compliance with the "Guide for the Care of Laboratory Animals" published by the National Institute of Health. Experimental protocols were approved by the Ethical Committee for Animal Experiments of the Katholieke Universiteit Leuven.

2.2. Acute experiments

The five animals in this acute study were premedicated with ketamine 10–20 mg/kg intramuscularly and anesthesia was induced with a halothane-oxygen mixture. The sheep were ventilated and the chest was opened. Catheters were inserted in the left atrium and the descending aorta for pressure monitoring and the administration and withdrawal of colored microspheres. A high-fidelity micromanometer (Micro Transducer Catheter, Drager, Germany) was introduced in the left ventricle via the left atrium. The left circumflex coronary artery (LCX) was instrumented with a pulsed Doppler flow probe (Pulsed Doppler 20 MHz module Baylor College of Medicine, Houston, TX, USA). For induction of the coronary stenosis C-shaped plastic rings of appropriate size were placed around left anterior descending (LAD) and LCX. The experimental protocol was as follows: (1) All baseline measurements were recorded after stabilization including a first injection of colored microspheres. (2) The LAD was occluded for 1 minute and at peak reactive hyperemia (30 seconds) another injection of colored microspheres was given. (3) After stabilization (15 min) the LCX was occluded for 1 minute and reactive hyperemia was recorded. (4) After stabilization of LCX, flow baseline measurements were performed and dobutamine infusion was started at incremental doses from 5 μg kg^{-1} min^{-1} at 5-min steps up to 15 μg kg^{-1} min^{-1}. During the infusion of 10 μg kg^{-1} min^{-1} of dobutamine, a third injection of microspheres was given. During a 30-min period, the recovery phase was studied after cessation of dobutamine administration. (5) Then the coronary stenoses were induced and the experimental protocol was repeated.

2.3. Eight-week chronic coronary artery stenosis

Eight sheep (body weight of 60±13 kg) were premedicated with ketamine 10–20 mg/kg intramuscularly, whereafter a two-dimensional echocardiography (Sonotron Vingmed CFM Horten Norway with 2.5 Mhz transducer) was performed to evaluate the left ventricular function from the parasternal long- and short-axis view of the mid-papillary muscle level of the left ventricle (LV). The echocardiograms were analyzed by two independent observers. End-diastolic (ED) and end-systolic (ES) wall thickness were measured at the mid-papillary level. LV fraction of wall thickness (FWT) was calculated as end-systolic minus end-diastolic wall thickness divided by end-diastolic wall thickness, expressed as a percentage. Global left ventricular systolic function was measured by calculating the fractional area change (FAC) in the parasternal short axis view. ED and ES areas were defined after manual delineation of the endocardium and were measured for three consecutive beats and averaged. FAC was calculated as ED minus ES area, divided be ED area times 100.

Then induction of anesthesia was performed with a halothane-oxygen mixture. The sheep were intubated and ventilated. A sterile left thoracotomy was subsequently performed and 30 min before opening of the pericardium 100 mg lidocain was administered to avoid ventricular arrhythmias. Catheters were inserted in the left atrium and the descending aorta for pressure monitoring and the administration of colored microspheres. After stabilization, baseline hemodynamic parameters were measured. Then colored microspheres were administered before dissection of the coronary arteries as a baseline value. Plastic rings of appropriate size (<2.5 mm) were placed around the LAD and the LCX to induce coronary stenosis. Approximately forty minutes later hemodynamic measurements were repeated as well as microsphere injection. Then the thoracotomy was closed and the anesthesia was stopped. The animals remained sedated for thirty minutes to perform the last echocardiographic examination. At Day 2 postoperatively the animals were transported to the farm where they were allowed to run in the open. At 2-week intervals the animals were again sedated with Ketalar 10 mg/kg intramuscularly and the echocardiogram was repeated.

Finally, the animals were again sedated and a last echocardiographic examination was performed. Then the sheep were anesthetized, hemodynamic variables were measured and another injection of colored microspheres was administered. After termination of the experiments, the hearts were prepared for light- and electron microscopic examination and postmortem angiography (see below).

2.4. Chronic multivessel coronary artery stenosis followed by PTCA

In this group, six sheep (body weight of 64.5±4.8 kg) were used. The preparation and initial 8-week protocol of sedation, echocardiography, hemodynamic measurements and microsphere injections were nearly similar to that in the eight week chronic stenosis group except that after surgery the echocardiography was only performed at 4 week intervals. At ten weeks after induction of coronary stenosis the animals were again anaesthetized and PTCA was performed at random of one of the two stenotic vessels. An *in vivo* coronary angiography was done before and after the PTCA procedure. A shot of microspheres was also given before and within 5 minutes after the PTCA. Echography was performed under sedation at 2 week intervals up to 20 weeks. At 20 weeks the sheep were again anaesthetized, hemodynamic parameters were measured and a last shot of microspheres was given. The heart was then removed and prepared