Time dependent changes of arterial distensibility induced by cholesterol and balloon injury in rabbits: an in vivo intravascular ultrasound study

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Abstract

The aim of this study was to validate in vivo measurement of intravascular ultrasound (IVUS) for the analysis of structural and functional vessel wall alterations in a chronic animal model. Furthermore, the relation between functional and structural alteration of the vessel wall should be investigated. Fifteen cholesterol-fed rabbits (1%) and 15 control rabbits underwent balloon injury of the abdominal aorta. Immediately before and after balloon traumatization as well as 2 and 6 weeks later IVUS depiction of 10 aortal vessel segments was performed (n = 1100 measurements). In vivo IVUS measurements and morphometric analysis of the neointimal area of same aortal segments showed a high correlation (n = 148, r = 0.844, p < 0.001). Plaque area determined by morphometry revealed larger areas than the evaluation by IVUS (0.162 ± 0.138 vs. 0.130 ± 0.126 mm², p < 0.001). Before balloon traumatization, pulsatility of the aortal vessel segments was less in cholesterol-fed rabbits (0.067 vs. 0.090, p < 0.01) and neointimal index higher (0.003 vs. 0). Investigation using IVUS 2 and 6 weeks after balloon traumatization demonstrated a continuous loss of arterial distensibility and an increase of neointimal index, being more pronounced in the cholesterol-fed group. As demonstrated by IVUS the loss of distensibility preceded the atherosclerotic alterations. Our investigation suggests using IVUS in this animal model is a reliable setting for long-term investigation of characteristics of the vessel wall. We could demonstrate that altered function of the vessel wall precedes the structural atherosclerotic vessel wall alterations.

Introduction

Balloon catheter injury of arteries and cholesterol-rich diet of rabbits has become a standard model for studying atherogenesis and restenosis after percutaneous transluminal angioplasty [1–5]. Both procedures result in the formation of atherosclerotic plaques, mostly described by histological and morphometric analysis. This approach, however does not allow to describe functional aspects, like pulsatility, of same atherosclerotic vessel wall segments over a defined period of time. A possible relationship between atherosclerotic plaque formation and related changes of functional aspects of the vessel remains unclear, because so far no existing animal model allows in vivo examinations of same vessel segments over a period of time and is able to demonstrate functional and morphological aspects simultaneously. Intravascular ultrasound (IVUS) has the unique ability to provide cross-sectional images of the vessel wall in vivo [6–9]. The purpose of this study was to validate an
animal model with in vivo measurements over a fixed period of same aortic vessel segments. Furthermore a relationship between the endothelial function and atherosclerotic vessel wall alterations should be described.

Material and methods

Animals

Thirty male New Zealand White rabbits (weight 3.9 ± 0.4 kg) were used for the experimental studies. Two groups of 15 rabbits in each group were examined. Group 1 received regular chow food, group 2 were fed a 1%-cholesterol-rich diet, beginning 4 weeks prior to the initial experiment. The cholesterol-rich diet was continued until sacrifice for another 2 weeks in five animals. The remaining 10 animals out of this group received cholesterol-rich diet for another 4 weeks after the balloon traumatization, they were euthanized 6 weeks after the baseline study. The rabbits of group 1 were just as the animals of group 2 euthanized 2 weeks (n = 5) or 6 weeks (n = 10) after the baseline study.

Intravascular ultrasound system

For this study a 3.5F 30 MHz mechanical IVUS catheter (Sonicath® CV, Mansfield™, USA) was used. This device consisted of a polyethylene shaft with distance markings on the surface, which allows reproducible positioning within the aorta. In our experiments the catheter was used without a guide wire, avoiding the well-known artifacts being caused by the guide wire. The catheter assembly houses a flexible drive cable connected to a driving unit which is used to rotate the mirror at the catheter tip 900 times/min. The ultrasound beam reflected by the rotating mirror creates a 360° real-time image perpendicular to the longitudinal axis of the catheter. Axial resolution of the system was 150 μm, lateral resolution was 250 μm. The catheter system was attached to the Sonos 1000® imaging device (Hewlett Packard, USA). All cross-sectional images were recorded on VHS-video tape for later analysis.

Study protocol

The animals were anesthetized with 40 mg/kg ketamine and 3 mg/kg xylacaine i.m. The surgical access was achieved by arteriae sectio as described by Skinner et al. [10]. The 30 MHz IVUS-catheter was introduced and positioned at the aortic bifurcation, where the first measurement was performed. For the following measurements the catheter was placed 1 cm proximal to the former position. The procedure was repeated until 10 measurements were obtained for each animal. Local anatomic markers were used as additional control for the identification of the corresponding histologic sections. After depiction of cross-sectional areas had been performed, the IVUS-catheter was removed and an aortal balloon traumatization procedure was performed. This procedure was previously described in detail [10]. Briefly, a Fogarty 4F balloon catheter was introduced and positioned in the proximal abdominal aorta. The inflated balloon was withdrawn three times to the distal abdominal aorta. Afterwards, the IVUS-catheter was introduced again and the corresponding positions of the aorta were monitored as described above. Two weeks later, IVUS measurements of the corresponding segments of the aorta were performed. Now, the right femoral artery served as access. After this second investigation five animals of each group were sacrificed and aortal specimens gained for histology. The remaining 10 animals of each group underwent a third IVUS investigation 6 weeks after balloon injury. Then, access was gained via the left femoral artery proximal to the former occluded vessel. After this measurements the animals were sacrificed and aortas were gained for histologic evaluation.

Histology

The animals received general anesthesia as described above. The rabbits were sacrificed via exsanguination after median sternotomy. After incision of the pulmonal artery the left ventricular apex was cannulated and perfusion fixation was performed with infusion of 250 ml of 2.5% glutaraldehyde solution at a mean arterial pressure