Early Changes of Gene Expression Profiles in the Rat Model of Arterial Injury

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ABSTRACT

Purpose: Restenosis caused by intimal hyperplasia (IH) remains a significant drawback for vascular interventions. It is crucial to understand the molecular mechanisms that control activation of smooth muscle cells (SMCs) after the injury in order to develop strategies to prevent IH. The purpose of the present study was to investigate the early alterations in arterial-wall gene expression after balloon injury in the rat carotid artery with focus on the induction of an inflammatory response.

Materials and Methods: Twenty-four male Sprague–Dawley rats were subjected to injury of the left common carotid artery by using a 2-F Fogarty catheter. The arteries were harvested 5, 10, and 20 hours after injury. Uninjured arteries from an additional eight rats were used as controls. RNA was isolated and used for genome-wide microarray expression analysis, followed by validation of selected genes with quantitative real-time polymerase chain reaction (qRT-PCR). Immunohistochemistry was performed on the cross-sectioned vessels.

Results: Analysis of gene expression by microarrays showed that the most differentially expressed genes were primarily associated with inflammation, cell proliferation, migration, and adhesion. As confirmed by qRT-PCR, microarray data showed a significant ($P < .005$) upregulation of cytokines and chemokines (IL-6, CCL2, CXCL1, AIMP1, and CD44) just 5 hours after injury. Immunohistochemistry demonstrated that CCL2 and the adhesion receptor CD44 were expressed by SMCs in the early response to injury and in the absence of leukocyte infiltration.

Conclusions: Arterial injury is followed by an early induction of inflammatory genes in the vessel wall that appears to be confined to SMCs.

ABBREVIATIONS

CCA = common carotid artery, IH = intimal hyperplasia, qRT-PCR = quantitative real-time polymerase chain reaction, SMC = smooth muscle cell, SNEA = subnetwork enrichment analysis

Despite substantial progress in vascular biology and pharmacology, restenosis remains a drawback for vascular reconstructions, both percutaneous and open. Vascular interventions on diseased arteries trigger vessel wall repair processes that involve activation of the smooth muscle cells (SMCs) and inflammatory responses (1).

In order to develop strategies to prevent intimal hyperplasia (IH) after vascular interventions, it is crucial to understand the molecular mechanisms that control SMC activation and function in vessel wall healing. Previous studies in animal models have shown that mechanical stress, loss of endothelium-associated antiproliferative and antiinflammatory functions, as well as mitogens from platelets and inflammatory cells may trigger SMC activation (2,3). The rat model of arterial injury has been used in studies of IH for decades. In this model, SMC proliferation starts after 24 hours in the medial layer (4,5), and migration to the luminal surface follows a few days later. For the next 1–2 weeks, a neointima will be

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The work was supported by grants from the Ministry of Education and Science of the Russian Federation (project P1308), RFBR (project 14-04-01833), Swedish Heart–Lung Foundation, Swedish Research Council, and Cardiovascular Program funded by the Stockholm Council and Karolinska Institutet. None of the authors have identified a conflict of interest.

An **Appendix**, **Figures E1 and E2**, and **Tables E1–E3** are available online at *www.jvir.org*. A list of gene and protein abbreviations used is provided in the **Appendix**.

J Vasc Interv Radiol 2014; 25:789–796

http://dx.doi.org/10.1016/j.jvir.2013.11.031

formed through SMC proliferation and production of extracellular matrix components (6). In contrast to human restenosis, the rat carotid injury model involves remarkably mild inflammation and leukocyte infiltration (1). Nevertheless, activated SMCs, as well as scattered leukocytes, have been shown to express proinflammatory mediators (7,8). Most of these studies have focused on the later stages of IH formation. The importance of the earliest molecular events for the development of IH is demonstrated by the fact that short-term periprocedural treatment with paclitaxel via drug-eluting angioplasty balloons significantly decreases restenosis rates (9).

In a few publications (7,10,11), several individual factors and early molecular responses that trigger the later stages of IH have been discovered. However, it is difficult to estimate the relative importance of each signaling molecule from these studies. In contrast, microarray-based transcriptome profiling can provide more useful information, as it allows analysis of coordinated changes in genome-wide expression. In animal models, global gene expression analysis has previously been performed 4, 7, and 14 days after balloon injury (12). Interestingly, there are no reports on global changes in gene expression at earlier stages (< 24 h) in response to injury.

In the present work, we used microarray gene expression profiling to analyze the early molecular events and local inflammatory reactions within the vessel wall in the rat carotid injury model.

MATERIALS AND METHODS

Study Design

As shown on the flowchart depicting the study design (**Fig 1**), a total of 32 rats were used in the study. The animals were euthanized with isoflurane 5, 10, or 20 hours after vascular injury, and the injured left common carotid arteries (CCAs) were harvested ($n = 8$ animals in each group). Arteries were rinsed with phosphate-buffered saline solution to remove blood. Uninjured left CCAs from an additional eight animals were used as controls. Total RNA was isolated from whole arteries ($n = 4$ per group). Arteries from four animals in each group were divided into a proximal segment used for RNA isolation and a distal segment used for histologic examination. Three of four samples with the best RNA quality were used for gene expression analysis by microarray hybridization and quantitative real-time polymerase chain reaction (qRT-PCR).

Rat Carotid Artery Balloon Injury

Carotid artery balloon injury was performed on male Sprague–Dawley rats (weight, 350–400 g) (13,14). The left carotid artery was exposed under isoflurane inhalation anesthesia, an arteriotomy was performed in the external carotid artery, and a 2-F Fogarty embolectomy catheter was inserted in to the left CCA. The balloon at the tip of the catheter was passed down to the aorta, inflated, and withdrawn with simultaneous rotation through the whole length of the artery while inflated. The procedure was repeated three times to remove the endothelial layer. The experiments were performed according to protocols approved by the regional animal ethic committee (Stockholm, Sweden), and institutional guidelines and protocols for animal care were followed.

Microarray Hybridization and Transcriptome Analysis

RNA labeling, microarray hybridization, scanning, and raw data analysis were done by using a GeneChip Rat Genome 230 2.0 Array (Affymetrix, Santa Clara, California). A gene was considered differentially expressed if it had twice or more change in absolute intensity

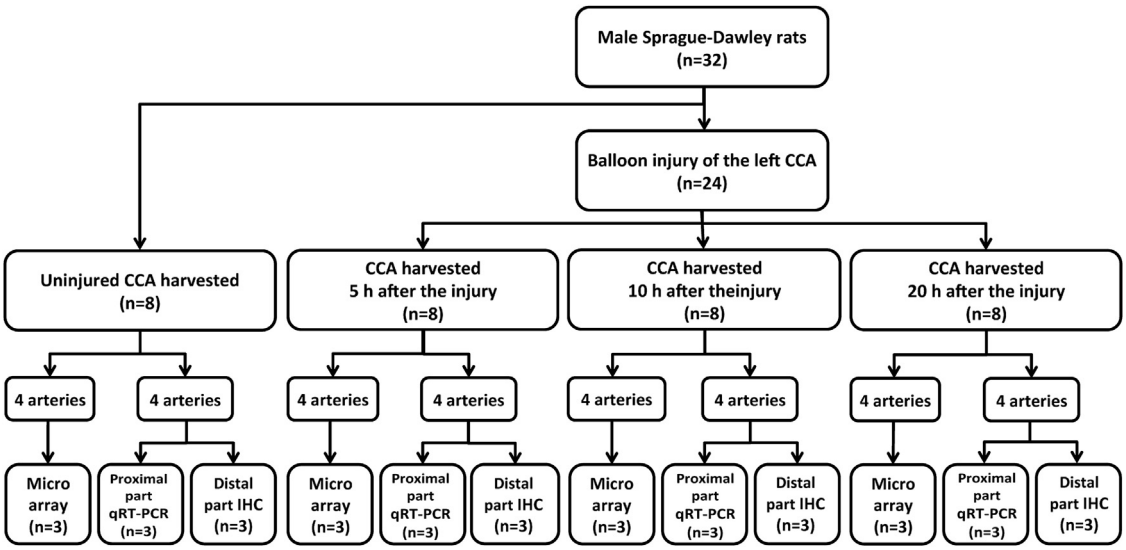

Figure 1. Flowchart of the experimental design.