The role of the kallikrein-kinin system, matrix metalloproteinases, and tissue inhibitors of metalloproteinases in the early restenosis of covered stents in the femoropopliteal arterial segment

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ABSTRACT

Objective: The purpose of this study was to investigate the roles of the kallikrein-kinin system and matrix metalloproteinases (MMPs) in the development of arterial restenosis attributable to intimal hyperplasia in the femoropopliteal arteries.

Methods: This report describes a single-center prospective study of 27 patients with peripheral artery disease who required percutaneous transluminal angioplasty and stenting of the femoropopliteal segment using covered stent grafts. The blood concentrations of total and kininogen fractions were evaluated using immunoenzymatic methods. Plasma kallikrein was evaluated by the colorimetric method. Tissue kallikrein was evaluated by the spectrophotometric method. The activity of kininase II was measured by fluorometric analysis. Quantification of MMPs was performed by zymography, and tissue inhibitors of metalloproteinases were measured by enzyme-linked immunosorbent assay.

Results: Four (15%) of the treated patients developed restenosis at the 6-month follow-up evaluation. These patients had significantly lower levels of high-molecular-weight kininogens (24 hours; $P < .05$) and low-molecular-weight kininogens (before, $P < .05$; 24 hours, $P < .01$; 6 months, $P < .05$) and lower levels of tissue inhibitor of metalloproteinases-2 (6 months; $P < .05$) than the patients without restenosis. The activity levels of plasma and tissue kallikrein, kininase II, and MMPs did not differ significantly between the patients with and without restenosis.

Conclusions: This study demonstrates an involvement of the kallikrein-kinin system in in-stent restenosis, although we could not confirm the participation of metalloproteinases in the restenosis process. (J Vasc Surg 2016;■:1-9.)

Covered stents are composed of fabric or synthetic material supported by a metal mesh or stent platform. Although initially conceived to exclude peripheral arterial aneurysms and treat vessel perforations,[1] the use of covered stents has expanded to act as a barrier to neointimal formation by excluding the vessel wall from the lumen. One such device is the Viabahn endoprosthesis (W. L. Gore & Associates, Flagstaff, Ariz), a tube of expanded polytetrafluoroethylene with a self-expanding helical nitinol stent mounted to the outside surface. Microporous polytetrafluoroethylene coating of stent surfaces has been found to inhibit the development of in-stent restenosis in the femoropopliteal (FP) segment, and clinical data suggest that polytetrafluoroethylene-covered stents can lead to better patency rates than angioplasty using bare metal stents for this very challenging segment.[2,3]

Stenting of the FP segment has been associated with a more intense inflammatory response than the same procedure for the iliac and carotid arteries.[4] Study results have consistently indicated that inflammatory mechanisms play a key role in the proliferative process associated with neointimal and restenosis after stent placement.[5-7] Inflammatory markers have been studied to better understand the process and potentially identify future therapeutic interventions.

The kallikrein-kinin system (KKS) is an endogenous metabolic cascade leading to the release of vasoactive kinins. This complex system includes the precursors of kinins, known as kininogens, and the kallikrein serine proteases. Kinins exhibit both proinflammatory and

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cardioprotective activity and are involved in a variety of physiological and pathologic processes, including blood pressure control, the coagulation cascade, and inflammation.[8] Kinins are rapidly hydrolyzed by a group of peptidases known as kininases. Kininase II, also known as angiotensin-converting enzyme (ACE), is the most studied kininase and functions to degrade the biologically active kinins, notably bradykinin (BK). Kininase II also acts to convert angiotensin (Ang) I to Ang II. Through various mechanisms, Ang II plays a role in endothelial dysfunction.[9]

The relationships between inflammatory and immune responses and adverse clinical events after stenting, principally in peripheral arteries, have yet to be defined. The matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that are widely known for their ability to degrade various protein components of the extracellular matrix and promote its remodeling.[10] Unbalanced MMP activity promotes atherosclerotic processes, including cell migration, proliferation of vascular smooth muscle, and infiltration of vessel walls by inflammatory cells.[11,12] The inhibition of MMP activity has been proposed as a pharmacologic approach for the treatment of cardiovascular disease.[13,14] MMP-2 and MMP-9 have both been reported to play specific and important roles in various cardiovascular diseases.[15] The tissue inhibitors of metalloproteinases (TIMPs) are small proteins (~23 kDa) that interact with the zinc binding site within the catalytic domain of the active MMP at molar equivalence. Four TIMPs have been described: TIMP-1, TIMP-2, TIMP-3, and TIMP-4. Overall, the TIMPs are not specific for any particular MMP; however, TIMP-2 tends to interact with MMP-2, and TIMP-1 tends to interact with MMP-9.[16]

There are few specific studies of inflammation system following percutaneous transluminal angioplasty (PTA) with stent placement (PTA/stenting) to treat peripheral arterial disease specially using covered stents. The aim of this study was to investigate the systemic inflammatory response and the relationship between possible inflammatory markers and in-stent early restenosis in patients within 6 months of undergoing femoral PTA with covered stent implantation through a study of the serum kallikrein-kinin and metalloproteinase systems.

METHODS

We performed a single-center prospective study at the Department of Surgery and Anatomy, Division of Vascular and Endovascular Surgery, Ribeirão Preto Medical School, University of São Paulo in Brazil between July 2012 and September 2014. The institutional ethics committee approved the study, and each patient provided signed informed consent before participation.

Inclusion criteria. Selected patients were those with severe claudication, pain at rest, and/or ischemic ulceration not exceeding the digits of the foot (Rutherford stages 3, 4, and 5) who exhibited angiographic evidence of FP occlusion disease in the absence of any significant proximal disease. Patients were only included if they satisfied the TransAtlantic Inter-Society Consensus criteria (TASC) II for endovascular intervention (A, B, and C lesions).

Exclusion criteria. Patients were excluded if they had acute or chronic inflammatory disease, severe ischemic ulcers or extensive gangrene (Rutherford stage 6), TASC D lesions, if they primarily underwent local thrombolysis, or if they developed other arterial major complications (acute thrombotic events in other arterial segments not related to the site of stent implantation) in up to 6 months after stent implantation.

Laboratory examinations. Peripheral venous blood samples were obtained immediately before stent implantation and 24 hours and 6 months after the procedure. Blood was delivered into plastic tubes with sodium citrate for analysis of kallikrein and kininogens and into tubes with sodium heparin for analysis of MMPs, TIMPs, and ACE.

High- and low-molecular-weight kininogens. Total kininogen, low-molecular-weight kininogens (LMWK), and high-molecular-weight kininogens (HMWK) concentrations were determined by immunoenzymatic methods. LMWK concentrations were determined for plasma samples previously incubated (30 minutes) with kaolin (1.5 mg/mL). Before the determination of total and LMWK concentrations, respective untreated and kaolin-treated plasma samples were submitted to acid denaturation and trypsin hydrolysis as previously described.[17] The concentration of released BK was measured using an enzyme-linked immunosorbent assay that employed an antibody against BK (Markit M; Dainippon Pharmaceutical, Osaka, Japan).[17] The concentration of HMWK was calculated by subtracting the plasma concentration of LMWK from the total kininogen plasma concentration. The results are expressed as mEq BK/mL plasma.

Plasma kallikrein. Plasma pre-kallikrein was activated to kallikrein and evaluated for amidase activity on the selective chromogenic substrate H-D-Pro-Phe-Arg-p-nitroanilide (S-2302; Chromogenix, Mondal, Sweden). The paranitroaniline formed in this reaction was detected spectrophotometrically at 405 nm. The results are expressed as units (U) of kallikrein/mL plasma (U/mL) using a purified human PKal standard (Chromogenix).[18] To verify that the reactivity was due to PKal, additional reactions were performed in the presence or absence of soybean trypsin inhibitor (Sigma, St Louis, Mo).

Tissue kallikrein. To assay plasma tissue kallikrein activity, the plasma amidase activity of kallikrein was measured using the chromogenic substrate H-D-Val-Leu-Arg-paranitroanilide (S-2266; Chromogenix) in the presence or absence of aprotinin (Sigma), a tissue inhibitor of kallikrein, as previously described.[19] The