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Basic Research

The Extracellular Matrix Signature in Vein Graft Disease

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ABSTRACT

Background: Vein graft disease is a major and yet unsolved problem in cardiac revascularization surgery. Although accumulation of extracellular matrix is characteristic for vein graft disease, detailed analysis of the fibrotic material is lacking. Because alterations of collagen cross-links are typical for organ fibrosis, we performed a comprehensive analysis of collagen and elastin in vein graft disease.

Methods: Collagen, elastin, and their respective cross-links were analyzed using histology and amino acid analysis. The expression of collagen-modifying enzymes was analyzed using SYBR Green quantitative real-time polymerase chain reaction. Fibrillin expression was analyzed by immunohistochemistry and quantitative real-time polymerase chain reaction.

Results: Diseased vein grafts showed a marked increase of collagen and of intermediate collagen cross-links, which are markers for newly synthesized collagen. Furthermore, we identified in vein graft disease increased levels of mature hydroxylysine aldehyde-derived cross-links typical for skeletal tissues. This was accompanied by upregulation of

RÉSUMÉ

Introduction : La maladie du greffon veineux est un problème important, et encore non résolu, de la chirurgie de revascularisation du cœur. Bien que l'accumulation de matrice extracellulaire soit caractéristique de la maladie du greffon veineux, l'analyse détaillée des constituants fibreux est déficiente. Puisque les altérations des liaisons croisées du collagène sont typiques de la fibrose d'organes, nous avons réalisé une analyse exhaustive du collagène et de l'élastine lors de maladie du greffon veineux.

Méthodes : Nous avons analysé le collagène, l'élastine et leurs liaisons croisées respectives à l'aide des données histologiques et des acides aminés. Nous avons analysé l'expression des enzymes modificatrices du collagène à l'aide de la PCR (*polymerase chain reaction*) quantitative en temps réel à base du colorant SYBR Green. Nous avons analysé l'expression de la fibrilline par immunohistochimie et PCR quantitative en temps réel.

Résultats : Les greffons veineux atteints montraient une augmentation marquée du collagène et des liaisons croisées intermédiaires du

Vein graft disease is a major and yet unsolved problem in coronary artery bypass graft surgery, which resembles atherosclerosis of coronary arteries in an accelerated form. Vein grafts are the most commonly used grafts for cardiac revascularization surgery although vein graft failure occurs in approximately 50% of grafts at 10 years because of neointima formation and atherosclerosis. Even though one hallmark of vein graft disease is the accumulation of extracellular matrix due to an activation of vascular smooth muscle cells or adventitia fibroblasts, detailed biochemical data are missing.[1,2]

Collagen is characterized by post-translational modifications including the hydroxylation of prolyl and lysyl residues. Hydroxylysine (Hyl) residues located in the helical domain and the telopeptides of the collagen molecule determine the pattern of intermolecular cross-links formed between collagen molecule, which provide tensile strength to the tissue. Responsible for lysyl hydroxylation are the lysyl hydroxylases (LHs; LH1, LH2, and LH3). LH1 is capable of hydroxylating lysyl residues located within the triple helix, whereas only LH2 is a specific telopeptidyl hydroxylase.[3-5] LH3 has been shown to function mainly as a galactosylhydroxylysyl glucosyltransferase.[6] Recent reports provide evidence that an altered pattern of collagen cross-links is characteristic for fibrosis and wound-healing.[7,8]

Collagen cross-links constitute a group of compounds, which are end products of a complex process initiated by lysyl oxidase (LOX)-dependent oxidative deamination of lysine

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lysyl hydroxylase 2 and lysyl oxidase expression. Furthermore, vein graft disease showed a reduction of the elastin/collagen ratio, using elastin cross-links as a marker of elastin content, which was accompanied by an increase of fibrillin-1.

Conclusions: Vein graft disease was accompanied by marked alterations in the composition of the extracellular matrix. The altered collagen cross-link pattern and the reduced elastin/collagen ratio might synergistically increase the stiffness in diseased vein grafts. Furthermore, hydroxylysine aldehyde-derived cross-links can cause a decreased degradability of collagens by matrix-metalloproteinases. Our data suggest collagen cross-links as a therapeutic target in vein graft disease.

collagène, qui sont des marqueurs du collagène nouvellement synthétisé. Lors de maladie du greffon veineux, nous avons également observé une augmentation des taux de liaisons croisées matures dérivées de l'hydroxylysine aldéhyde typiques des tissus squelettiques, qui était accompagnée de la régulation à la hausse de l'expression de la lysyl hydroxylase 2 et de la lysyl-oxydase. De plus, la maladie du greffon veineux montrait une réduction du ratio élastine-collagène, utilisant les liaisons croisées de l'élastine comme marqueur de la teneur en élastine, qui était accompagnée d'une augmentation de la fibrilline 1.

Conclusions : La maladie du greffon veineux était accompagnée d'altérations marquées dans la composition de la matrice extracellulaire. L'altération des liaisons croisées du collagène et la réduction du ratio élastine-collagène augmenteraient de façon synergique la rigidité des greffons veineux atteints. De plus, les liaisons croisées dérivées de l'hydroxylysine aldéhyde peuvent causer une diminution de la dégradabilité des collagènes par les métalloprotéinases matricielles. Nos données suggèrent que les liaisons croisées du collagène sont des cibles thérapeutiques pour la maladie du greffon veineux.

(Lys) or hydroxylysine (Hyl) residues in the telopeptide region of collagen molecules that lead to aldehydes (Lys^{ald} or Hyl^{ald}). Subsequently, condensation of the aldehydes with juxtaposed Hyl, Lys, and histidine residues located in adjacent collagen chains results in difunctional intermediate compounds, a marker for newly synthesized collagen, and trifunctional mature end products. According to the nature of the amino acid residue located in the telopeptides, a Lys^{ald}- or Hyl^{ald}-pathway of cross-link formation can be distinguished (LCC or HLCC). LCCs are predominant in soft tissues including skin (mature LCC: histidinohydroxylysinonorleucine [HHL]). HLCCs are typical for stiff tissues like bone, cartilage, or fibrosis and affect collagen degradation by matrix metalloproteinases (MMPs; difunctional HLCC: dihydroxylysinonorlucine [DHLNL]; mature HLCCs: hydroxylysylpyridinoline [HP], and lysylpyridinoline [LP]; Table 1). The difunctional cross-link hydroxylysinonorleucine (HLNL) can be formed between telopeptidyl Lys^{ald} and helical Hyl or telopeptidyl Hyl^{ald} and helical Lys and, thus, can be grouped as LCC or HLCC, respectivley.[9,14,15]

A different group of cross-links is present in elastin, also depending on the oxidation of lysyl residues by LOX (isodesmosine, desmosine [DES]), which can be used as a quantitative determinant of elastin content.[16] Elastin and microfibrils are the main components of elastic fibres that provide elasticity to tissues and organs. In addition to their structural function, microfibrillar proteins (ie, fibrillins) are thought to modulate the activity of growth factors of the transforming growth factor β superfamily.[17,18] In the present study, we performed a comprehensive analysis of the extracellular matrix in vein graft disease, required for the design of new and effective therapeutic strategies.

Methods

Specimens

Specimens of diseased vein grafts (aortocoronary venous bypass [ACVB]) from 12 patients (aged 68.7 ± 6.5 years, 8 male and 4 female) and normal nondiseased human saphenous vein controls from 12 patients (age 66.4 ± 10.0 years, 8 male, 4 female) were used in this study. All patients (control and ACVB) had relapsed symptomatic coronary artery disease and were treated according to standard protocols with β-blockers, lipid-lowering drugs, and antiplatelet medication. The patients were matched for body mass index, smoking history, diabetes, hypertension, and hyperlipidemia. All specimens were taken from an intrastenotic segment of diseased vein grafts. Normal saphenous veins were obtained during primary coronary artery bypass procedures at the Department for Cardiac Surgery, University Hospital Lübeck. All biopsies were obtained after a written consent of the donors under protocols approved by the Ethical Committee of the University of Lübeck.

Table 1. Influence of collagen modifying enzymes on amino acid composition, cross-link pattern, and tissue parameters

Function	Enzyme	Read out: collagen cross-links or collagen amino acid composition
Telopeptidyl lysyl hydroxylation	LH2	HLCCs: DHLNL, HP, LP HLCC/LCC: (DHLNL + HP + LP)/HHL
Helical lysyl hydroxylation	LH1	HP (Hyl^{telo} × Hyl^{telo} × Hyl^{helix})/LP (Hyl^{telo} × Hyl^{telo} × Lys^{helix}); Hyl/Hyp
Oxidative deamination of telopeptidyl lysine or Hyl	LOX	Total number of aldehydes involved in collagen cross-links
Newly synthesized collagen[9]		Difunctional cross-links: DHLNL, HLNL
Increase of stiffness[10-12]		HP, DHLNL
Lower matrix degradation by MMP1[13]		HP

DHLNL, dihydroxylysinonorleucine; HHL, histidinohydroxylysinonorleucine; HLNL, hydroxylysinonorleucine; HLCC, hydroxylysine aldehyde-derived collagen cross-links; HP, hydroxylysylpyridinoline; Hyl, hydroxylysine; Hyl^{helix}, hydroxylysine, triple helix; Hyl^{telo}, hydroxylysine, telpeptide; Hyp, hydroxyproline; LCC, lysine aldehyde-derived collagen cross-links; LP, lysylpyridinoline; Lys^{helix}, lysine, triple helix; MMP1, matrix metalloproteinase 1.