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ORIGINAL ARTICLE

Vascular lysyl oxidase over-expression alters extracellular matrix structure and induces oxidative stress☆,☆☆

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KEYWORDS
Lysyl oxidase;
Collagen;
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Abstract
Introduction: Lysyl oxidase (LOX) participates in the assembly of collagen and elastin fibres. The impact of vascular LOX over-expression on extracellular matrix (ECM) structure and its contribution to oxidative stress has been analysed.
Methods: Studies were conducted on mice over-expressing LOX (Tg), specifically in smooth muscle cells (VSMC). Gene expression was assessed by real-time PCR analysis. Sirius Red staining, H_2O_2 production and NADPH oxidase activity were analysed in different vascular beds. The size and number of fenestra of the internal elastic lamina were determined by confocal microscopy.
Results: LOX activity was up-regulated in VSMC of transgenic mice compared with cells from control animals. At the same time, transgenic cells deposited more organised elastin fibres and their supernatants induced a stronger collagen assembly in *in vitro* assays. Vascular collagen cross-linking was also higher in Tg mice, which showed a decrease in the size of fenestrae and an enhanced expression of fibulin-5. Interestingly, higher H_2O_2 production and NADPH oxidase activity was detected in the vascular wall from transgenic mice. The H_2O_2 scavenger catalase attenuated the stronger deposition of mature elastin fibres induced by LOX transgenesis.

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Conclusions: LOX over-expression in VSMC was associated with a change in the structure of collagen and elastin fibres. LOX could constitute a novel source of oxidative stress that might participate in elastin changes and contribute to vascular remodelling.

PALABRAS CLAVE
Lisil oxidasa;
Colágeno;
Elastina;
Estrés oxidativo

La sobreexpresión vascular de la lisil oxidasa altera la estructura de la matriz extracelular e induce estrés oxidativo

Resumen
Introducción: La lisil oxidasa (LOX) contribuye al ensamblaje de las fibras de colágeno y elastina de la matriz extracelular (MEC). Hemos determinado las consecuencias de la sobreexpresión vascular de LOX sobre la estructura de la MEC y su contribución al estrés oxidativo.
Métodos: Los estudios se desarrollaron en ratones que sobreexpresan la LOX (Tg) específicamente en células musculares lisas vasculares (CMLV). Se realizaron análisis por PCR a tiempo real, tinción de rojo sirio, producción de H_2O_2 y actividad NADPH oxidasa. Se caracterizaron las fenestras de la lámina elástica interna mediante microscopia confocal.
Resultados: Las CMLV de ratones transgénicos presentaron niveles de actividad LOX superiores a los de animales control. En consonancia, las células transgénicas depositaron más fibras de elastina organizada y sus sobrenadantes indujeron un mayor ensamblaje de colágeno en ensayos *in vitro*. El nivel de colágeno maduro fue superior en la pared vascular de ratones Tg, que presentaban una menor área de las fenestras y un aumento de la expresión de la fibulina-5. La producción vascular de H_2O_2 y la actividad NADPH oxidasa fueron superiores en los ratones transgénicos. La incubación de CMLV con catalasa atenuó el incremento en la deposición de fibras de elastina madura inducido por la transgénesis de LOX.
Conclusiones: La sobreexpresión de la LOX en CMLV se asocia a una alteración de la estructura vascular del colágeno y la elastina. La LOX podría constituir una nueva fuente de estrés oxidativo que participaría en la alteración estructural de la MEC y podría contribuir al remodelado vascular.

Introduction

Lysyl oxidase (LOX) is an enzyme that catalyses one of the key steps in the synthesis and stabilisation of the extracellular matrix (ECM).[1,2] LOX is a copper-dependent amino oxidase that participates in the covalent cross-linking of collagen and elastin fibres in the ECM. Specifically, LOX catalyses the oxidative deamination of lysine and hydroxylysine residues, resulting in the formation of highly reactive peptidyl semialdehydes that condense together to form both intramolecular and intermolecular bonds; a reaction that generates H_2O_2 as a by-product.[1]

LOX activity determines the mechanical and structural properties of connective tissues and changes in that activity have been linked to many different pathological processes, including cancer and cardiovascular disease.[2,3] Recent studies suggest that LOX activity and increased cross-linking in associated collagen fibres may play a key role in vascular stiffness.[4] Alteration in the structure of elastin affects vascular mechanics in both large and small arteries[5–7] and at least partly contributes to increased vascular stiffness in hypertension,[8,9] although there is less evidence of the role of LOX in this process.

Surprisingly, in addition to its merely structural function, LOX has been implicated in the control of multiple cellular processes, including cell differentiation, migration, transformation and regulation of gene expression.[1,2] It is interesting to note that some of these biological functions, such as control of cell migration and vascular smooth muscle cell (VSMC) chemotaxis, have been linked to increased production of H_2O_2 as a result of LOX activity.[10,11] However, whether LOX may contribute to vascular oxidative stress and its possible impact on vascular structure have not been established. Our results obtained in a transgenic mouse model that overexpressed LOX specifically in VSMC show that LOX overexpression is associated with greater oxidative stress in the vascular wall, and that this then contributes to the alteration of elastin structure, which may have significant pathophysiological repercussions.

Material and methods

Animal model

The studies were performed on a transgenic mouse model that overexpressed LOX in VSMC (Tg) on a C57BL/6J genetic