Regulation of lysyl oxidase in vascular cells: lysyl oxidase as a new player in cardiovascular diseases

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Lysyl oxidase (LOX) plays a crucial role in the maintenance of extracellular matrix stability and could participate in vascular remodelling associated with cardiovascular diseases. Evidence from in vitro and in vivo studies shows that LOX downregulation is associated with the endothelial dysfunction characteristic of earlier stages of the atherosclerotic process. Conversely, upregulation of this enzyme in vascular cells could induce neointimal thickening in atherosclerosis and restenosis. In fact, LOX is chemotactic for vascular smooth muscle cells and monocytes, is modulated by proliferative stimulus in these cells, and could control other cellular processes such as gene expression and cell transformation. Furthermore, it is conceivable that LOX downregulation could underlie plaque instability and contribute to the destructive remodelling that takes place during aneurysm development. Overall, LOX could play a key role in vascular homeostasis and, hence, it emerges as a new player in cardiovascular diseases. This review addresses the experimental evidence related to the role of LOX in vascular disorders and the potential benefits of controlling its expression and function.

1. Introduction

Lysyl oxidase (LOX) is a copper-dependent amine oxidase that initiates the covalent cross-linking of collagen and elastin. LOX catalyses an oxidative de-amination of lysine and hydroxylysine residues to peptidyl \( \alpha \)-aminoacidic-\( \delta \)-semialdehydes. These highly reactive semialdehydes can spontaneously condense to form intra- and intermolecular covalent cross-linkages that assure extracellular matrix (ECM) stability (Figure 1). LOX activity is essential to maintain the tensile and elastic features of connective tissues of skeletal, pulmonary, and cardiovascular systems, among others.1,2

LOX is synthesized as a pre-proLOX that undergoes a series of post-translational modifications to yield a 50 kDa pro-enzyme. This pro-enzyme is secreted into the extracellular environment where it is proteolytically processed by bone morphogenetic protein-1 (BMP-1) and other pro-collagen C-proteinases to release the mature and active 32 kDa form and its pro-peptide (Figure 2).3 Four closely related enzymes, LOX-like1 (LOXL1), LOXL2, LOXL3, and LOXL4, have been identified. LOX and LOXL isoenzymes have a conserved C-terminal region corresponding to the catalytic domain that includes the lysine tyrosylquinone (LTQ) cofactor, the copper-binding site, and the cytokine receptor-like domain.1 These isoenzymes exhibit different in vivo expression patterns, thus although their specific functions remain undefined, they probably would play different biological roles.4

Novel biological functions of LOX have been reported in the last few years. These include the control of epithelial-to-mesenchymal transition, cell migration, adhesion, transformation, and gene regulation.2,5 Interestingly, some of these functions are fulfilled by its pro-peptide independently of LOX catalytic activity (Figure 2).6 Furthermore, catalytically active forms of LOX have been identified in the cytosolic and nuclear compartments,7 and although intracellular substrates are currently unknown, these data suggest the existence of undefined roles of LOX in cellular homeostasis.

A different pattern of LOX expression/activity has been associated with distinct pathological processes. These include fibrotic diseases such as hepatic, lung, or kidney fibrosis,8-11 Alzheimer’s disease and other neurodegenerative processes,12,13 and tumour progression and metastasis.14,15 Moreover, in the last few years, several studies including our own research results emphasize the potential role of LOX in cardiovascular function.16-19
2. Lysyl oxidase in cardiovascular diseases

In the vascular wall, LOX is expressed in fibroblasts, endothelial cells, and vascular smooth muscle cells (VSMC). The prominent role of this enzyme in the development and function of the cardiovascular system has been established through gene knockout strategies. LOX deficiency leads to the death of most animals at the end of the gestational period or within the first hours of life. High incidence of aortic aneurysms, aortic tortuosity, and signs of aortic rupture were observed in LOX \(^{2}/^{2}\) mice. Microscopic analysis revealed an extended fragmentation of elastic fibres, disruption of VSMC contact, discontinuities of both internal elastic lamina and lamellae, detachment of endothelial cells from the basal lamina, and alterations on endothelial cell morphology.

In normal adult animals, experimental evidence suggests that LOX deregulation could be involved in different stages of the atherosclerotic process from endothelial dysfunction to plaque progression and rupture. Likewise, other cardiovascular diseases characterized by an intense destruction of ECM, such as aneurysms and coronary dissections, have been related to a disturbance of LOX expression.  

2.1 Lysyl oxidase and endothelial function

LOX is strongly expressed in the endothelium of healthy coronary arteries from humans and animal models (unpublished data), and recent studies suggest that LOX downregulation is associated with endothelial dysfunction. Indeed, studies performed by our group revealed that atherogenic concentrations of LDL reduce LOX expression and activity (Figure 3A and B). LDL triggers LOX downregulation through a transcriptional mechanism; however, the regulatory factors involved in this effect are not known. Nowadays, little information is available concerning active cis-acting elements present in the LOX promoter and in intron 1, which seems to control cell-type-specific responses. Both LDL and \(\beta\)-aminopropionitril (BAPN), an inhibitor of LOX activity, increase the exchange of macromolecules across an endothelial monolayer; thus, it could be considered that the alteration of ECM structure due to LOX downregulation impairs endothelial barrier function (Figure 3C).

Inhibition of LOX activity has been related with hyperhomocysteinaemia, an atherosclerotic risk factor that alters the elastic properties of the vascular wall and is a well-known inducer of endothelial dysfunction. Studies performed using purified LOX \(in vitro\) showed that homocysteine thiolactone (HCTL) and other analogues, but not homocysteine (HC) itself, irreversibly inhibit LOX activity. Recently, we reported that pathophysiological concentrations of HC inhibit LOX activity in vascular endothelial cells. Since, HC can only generate modest amounts of...
HCTL in cell culture, we ruled out that HCTL could account for this HC-mediated inhibition of endothelial LOX. Indeed, we observed that the HC thiol-group and the oxidative stress triggered by this amino acid are involved in LOX inhibition (Figure 4A). Moreover, HC causes a late reduction on both LOX mRNA and transcriptional activity.

Pro-inflammatory cytokines that impair endothelial function, such as TNFα, reduce LOX expression and activity in endothelial cells and in the vascular wall. In rats, TNFα administration decreases vascular LOX mRNA levels about two-fold. This effect seems to be mediated by the activation of TNF receptor-2 and is independent of the pro-apoptotic effect elicited by this cytokine. Furthermore, a decrease in LOX transcriptional activity seems to underlie these effects, similar to the LDL-dependent LOX downregulation. Thus, these results support the hypothesis that LOX downregulation could underlie the alteration of endothelial function elicited by atherosclerotic risk factors.

### 2.2 Lysyl oxidase regulation in vascular smooth muscle cells

LOX has been associated with VSMC migration and proliferation, and its activity could be essential in the insolubilization of ECM components, mainly because LOX is the isoform responsible for the 80% LOX activity in aortic SMC.

The coordinate regulation of ECM synthesis and their modifying enzymes in response to growth factors and cytokines, such as transforming growth factor β (TGFβ) and platelet-derived growth factor (PDGF), is critical in vascular remodelling associated with atherosclerosis and restenosis. TGFβ and PDGF are growth factors involved in the pathogenesis of restenosis and atherosclerosis that have been considered as therapeutic targets. In this regard, TGFβ increases LOX expression and activity in VSMC and lung fibroblasts in cell culture. Furthermore, TGFβ concomitantly upregulates collagen type I and III synthesis in VSMC and enhances the production of proteoglycans and fibronectin in the rat aorta injury model. Interestingly, fibronectin is involved in LOX proteolytic activation, a mechanism that could participate in the induction of LOX by TGFβ. PDGF, a key mitogen that promotes neointimal growth after coronary angioplasty, also increases LOX expression in VSMC.

Similarly, granulocyte macrophage colony-stimulating factor (GM-CSF), a cytokine implicated in vascular remodelling, increases both LOX and BMP-1 expression levels in VSMC. In fact, GM-CSF-deficient mice show decreased levels of tropoelastin and LOX and BMP-1 expressions, with a concomitant deficiency in the cross-linkage of elastic fibres.

Interestingly, enzymatically active forms of LOX have been detected in the nucleus of VSMC. The biological activity of nuclear LOX in VSMC remains to be unravelled, but affecting chromatin organization it could modulate the expression of ECM components such as elastin and collagen III, the most abundant collagen in atherosclerotic plaques.

Besides ECM maturation, LOX could contribute to neointimal growth through its demonstrated chemotactic activity for VSMC and monocytes (Figure 5). However, conflicting data have been reported regarding the relationship between...
Stimulation of cell proliferation increases LOX expression in quiescent rat adult VSMC, and a computational method identified LOX as a responsive gene to proliferative stimulus in these cells. However, in neonatal VSMC, an inverse correlation between the proliferation rate of these cells and LOX expression has been described. Surprisingly, LOX pro-peptide has been reported to directly inhibit VSMC proliferation and DNA synthesis in cell culture studies. However, since high amounts of pro-peptide are needed to observe this response, its physiological relevance remains doubtful and it is inconsistent with results obtained in animal models of restenosis (discussed subsequently).

2.3 Lysyl oxidase in the onset, progression, and instability of atherosclerotic plaques

The absence of extensive immunohistochemical studies characterizing vascular LOX expression in humans has hampered the knowledge about the role of LOX in the development of atherosclerosis. However, multiple studies have revealed that disturbances of LOX expression could be related with endothelial dysfunction, lesion progression, or plaque rupture.

Our own research demonstrates that LOX is strongly downregulated in the earlier stages of the atherosclerotic process. Indeed, in agreement with the in vitro studies described in Section 2.1, we have observed that systemic hypercholesterolaemia downregulates aortic LOX expression in the porcine model of diet-induced atherosclerosis that develops fatty streaks in aorta after 100 days of diet intervention (Figure 4B). Conversely, in chickens fed a cholesterol-rich diet, vascular LOX activity was not modified. Differences in the experimental approach (animal model, diet composition, and/or length of each study) and the type of lesions analysed could explain these discrepancies. Indeed, the study conducted by Chvapil et al. used very young chickens (1–2 days old) and a short time of diet supplementation (6 weeks). Consistent with the above-mentioned data, a microarray study revealed that diabetes reduces vascular LOX expression in rats, together with a concomitant decrease in mRNA levels for elastin, different collagens, and other ECM components. The magnitude of LOX reduction (three-fold) was comparable with that reported in our in vivo studies analysing the effect of hypercholesterolaemia or TNF-α, supporting the hypothesis that LOX downregulation could be a common denominator in the earlier phases of plaque development.

In advanced stages of atherosclerosis, an increase in vascular LOX activity has been reported. In a study performed in rabbits, Kagan et al. administered a diet rich in cholesterol and peanut oil (2% cholesterol, 8% peanut oil) that can induce cholesterol levels over 1000 mg/dL within a month and highly fibrotic lesions. The authors observed an increase in vascular LOX exclusively in advanced lesions from the aortic arch. Thus, vascular LOX changes observed in the earlier atherosclerotic lesions could differ from those observed in advanced stages of disease.

Finally, it has been speculated that LOX inhibition could be associated with plaque instability and rupture. LOX contributes to the regulation of the biomechanical properties of ECM, and hence a defective cross-linking due to low-LOX activity could promote ECM disorganization. This situation could be aggravated by the fact that LOX inhibition favours the presence of soluble forms of collagen that are highly susceptible to metalloproteinase degradation. In accordance with this, interferon γ (IFNγ), a cytokine induced in ruptured atherosclerotic plaques and involved in inflammation, has been shown to downregulate LOX expression in vascular smooth muscle cells and monocytes, and actively participates in extracellular matrix (ECM) deposition. Discrepant results have been reported regarding the role of lysyl oxidase in vascular smooth muscle cells proliferation. These processes contribute to neointimal formation in restenosis and promote the development of a more stable atherosclerotic lesion. Inhibition of lysyl oxidase by inflammatory mediators could participate in extracellular matrix disorganization associated with vulnerable plaques.
in matrix remodelling, reduces LOX expression and activity in VSMC through both transcriptional and post-transcriptional mechanisms (Figure 5). The concomitant decrease on collagen I synthesis caused by IFN-γ could also participate in the alteration of ECM stability associated with this cytokine. Furthermore, novel biological functions of LOX related with plaque instability, such as the control of neovascularization, could not be ruled out due to the reported regulation of LOX by hypoxia. Finally, Schieffer et al. have shown that aortic LOX mRNA levels are reduced in ApoE<sup>−/−</sup>/IL-6<sup>−/−</sup> double-knockout mice, animals that develop extensive atherosclerotic lesions and exhibit an altered lipid profile characterized by high levels of LDL, VLDL, and total cholesterol. ApoE<sup>−/−</sup>/IL-6<sup>−/−</sup> mice show a reduction of collagen content in atherosclerotic plaques, a higher gelatinolytic activity of MMP-9, and a deficient vascular ECM assembly consistent with the reduction of LOX expression. However, specific studies directed to establish the role of LOX inhibition in plaque instability have not been performed and hence proper strategies will be necessary to clarify this issue.

2.4 Lysyl oxidase in restenosis

The pathophysiology of restenosis is characterized by VSMC proliferation and migration and ECM deposition, processes in which LOX seems to be involved as described in Section 2.2. Better understanding of the molecular mechanisms involved in restenosis is crucial to develop suitable therapeutic approaches directed to avoid recurrent narrowing.

Studies in animal models have shown that vascular LOX expression is induced after balloon injury and precedes collagen accumulation. In fact, an exacerbated collagen and elastin cross-linking has been related to the constrictive remodelling and restenosis after balloon angioplasty. In this study, LOX staining was strongly induced in the neointima of rat injured vessels after 3 days and increased progressively through day 21, while maximal collagen expression occurred at day 28. In this model, the increase in LOX expression could be a requisite for ECM stabilization, but LOX induction also precedes VSMC proliferation and migration. Furthermore, in rabbits undergoing angioplasty, LOX inhibition reduces restenotic rates and causes a lower constrictive remodelling compared with control animals. The increase in LOX expression after catheter-based therapies could be associated with the well-established induction of LOX by both TGFβ and PDGF key factors in the restenotic process (Figure 5).

Overall, LOX is a pro-fibrotic enzyme, and LOX inhibition could limit restenosis in animal models, but paradoxically this strategy could be detrimental to plaque stability. In fact, although the pathophysiology of atherosclerosis and restenosis shares common elements, acute complications of atherosclerosis basically raise from the rupture of vulnerable plaques rather than from highly stenotic lesions. ECM disorganization is involved in plaque instability, a process in which LOX inhibition could be fundamental (Figure 5). Indeed, similar discrepancies have been reported regarding some drug-eluting stents and TGFβ inhibition strategies that are effective in reducing neointimal hyperplasia but can cause medial atrophy and fragmentation of internal elastic lamina, leading to a loss of vascular integrity. Thus, although pharmacological approaches directed to block vascular LOX expression could reduce vascular narrowing and limit restenotic cell growth, this kind of interventions should be localized and controlled to avoid the risk of increased plaque vulnerability in rupture-prone pre-existing neighbouring lesions.

2.5 Aneurysm development and aortic dissection

Strong evidence is available about the involvement of a reduction of LOX activity in the pathogenesis of abdominal aortic aneurysm (AAA), a vascular disease characterized by a destructive remodelling of the arterial wall that leads to an extensive degradation of ECM. The role of LOX in the development of AAA has been analysed in animal models. Elastase-induced AAA in rats takes place with a reduction of LOX expression. The concomitant inhibition of LOX with BAPN in these animals causes aortic dissections and thrombotic events. Indeed, it has been reported that cross-linking inhibition by BAPN reduces the diameter, strength, and stiffness of rat aorta and cause the death of these animals due to dissecting aneurysms. Similarly, a decrease of vascular LOX expression was observed in calcium chloride-induced AAA. In this model, vascular over-expression of LOX by local gene delivery normalizes aortic diameter and prevents AAA formation in vivo. LOX reduction has also been related with an impairment of vascular elasticity due to calcification, a phenomenon that confers a bad prognosis for small AAA.

Likewise, other vascular diseases characterized by an ECM disorganization, such as arterial dissection, have been related with LOX deficiency in a single case report study, although spontaneous coronary artery dissections should respond to a disturbance of multiple ECM macromolecules besides LOX.

Thus, taking into account the high prevalence and mortality of AAA and the lack of available pharmacological treatment (only open aneurysm repair or endovascular repair are suitable therapeutic approaches for this disease), LOX emerges as a potential new therapeutic target for the treatment of this disorder.

3. Conclusions and future perspectives

At present, experimental data from multiple studies indicate that LOX is differentially regulated depending on the phase of progression of the atherosclerotic process and that the disturbance of LOX expression could also be involved in ECM remodelling associated with aneurysm development. The role of LOX in endothelial homeostasis emerges as a new area that deserves further attention and investigation. Likewise, the hypothesis that LOX inhibition could promote plaque instability requires proper experimental strategies including suitable animal models to explore this tentative mechanism and to establish its clinical relevance. Furthermore, several intriguing questions will require an extensive analysis, among them: (i) the involvement of LOX in cardiac remodelling and (ii) the role of LOX in vascular angiogenesis. Interestingly, no data about specific biological functions of LOXL isoenzymes, some of them highly expressed in vascular cells, are currently available. Overall, the involvement of LOX in cardiovascular diseases opens up new prospects about LOX as an interesting therapeutic target in vascular disorders that are
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