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Basic nutritional investigation

Nelumbo nucifera leaf extract inhibits neointimal hyperplasia through modulation of smooth muscle cell proliferation and migration

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ABSTRACT

Objective: Endovascular injury induced by balloon withdrawal leads to the increased activation of matrix metalloproteinases (MMPs) in the vascular wall allowing the proliferated smooth muscle cells (SMCs) to digest the surrounding extracellular matrix and migrate from the media into the intima leading to the intimal thickening. The objective of this study was to examine the effect of *Nelumbo nucifera* leaf extract (NL) on intimal thickening of rat carotid artery injured by balloon catheter and on the proliferation and migration of cultured vascular smooth muscle cells (VSMCs) induced by tumor necrosis factor-α.

Methods: NL was administered orally using gastric sonde at three different doses, 100 mg kg^{-1} (NL100), 400 mg kg^{-1} (NL400), and 800 mg kg^{-1} (NL800) for 4 wk from the day of balloon injury in the rats. VSMC proliferation and migration were assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and Boyden chamber methods, whereas enzymatic action of matrix metalloproteinase-2 and -9 (MMP-2 and MMP-9) was carried out by gelatin zymography, and MMP-9 protein expression, extracellular signal-regulated kinase 1/2, and c-Jun N-terminal kinase phosphorylations were assessed by Western blot analyses.

Results: NL reduced the intimal thickening by suppressing VSMC's proliferation through inhibition of extracellular signal-regulated kinase 1/2 phosphorylation and their migration by reducing the expression of MMP-2 and -9 through inhibition of JNK1/2 phosphorylation.

Conclusion: Thus, the results suggest that NL can be considered of therapeutic value in the prevention of atherosclerosis because restenosis after percutaneous transluminal coronary angioplasty can be considered a model of "accelerated atherosclerosis."

Introduction

Nelumbo nucifera Gaertn. (Nymphaeaceae), a large aquatic herb widely found in India, China, Japan, and Korea, not only possesses ornamental and dietary value but also has been used as a medicinal herb in eastern Asia. Almost every part of *N. nucifera* including leaves, flowers, seeds, and rhizomes have been reported to possess different therapeutic effects [1]. The leaves have been mentioned to show antiobesity activity by increasing lipolysis in the adipose tissue of mice [1,2]. *N. nucifera* leaf extract has a greater content of phenolic acids and flavonoids, which include gallic acid, rutin, quercetin, catechin, epicatechin, and epigallocatechin gallate [3]. Moreover, the water extract of the *N. nucifera* leaf has been reported to contain 14.8% of total phenolic acids and 56.1% total flavonoids, respectively [4]. The high content and composition of polyphenolic constituents seem to play roles for the wide range of biological activities.

Percutaneous transluminal coronary angioplasty (PTCA) is an important therapeutic option in the treatment of patients with coronary artery disease. However, a problem with this method remains the restenosis of the artery occurring within the first 6 months after the procedure [5]. Although systemic pharmacologic approaches to reduce restenosis have not been successful in clinical use, local treatment with drug-eluting stents demonstrated significant reduction in restenosis rate and the subsequent need for revascularization [6]. Nevertheless, drug-eluting stents do not resolve all the problems arising from percutaneous coronary intervention and may be associated with an increased risk for late stent thrombosis [7].

The mechanism of restenosis can be described as the complex involvement of several growth factors and cytokines including

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tumor necrosis factor-α (TNF-α) and platelet-derived growth factor (PDGF) that induce proliferation and migration of vascular smooth muscle cells (VSMCs) and degradation of extracellular matrix (ECM) by matrix metalloproteinases (MMPs) [8]. TNF-α, although mostly secreted by activated macrophages in atherosclerotic lesions, is also produced by VSMCs in the neointima after balloon injury [9]. Similarly, PDGF, a potent growth factor produced by vascular endothelial cells, platelets, macrophages, or VSMCs in the injured vascular walls, can also induce migration of VSMCs, as PDGF is the strongest reported chemoattractant for VSMCs [10]. During the early stages of arterial wall injury or atherosclerosis, VSMCs may undergo transition from a contractile to a synthetic phenotype and begin proliferating in response to various growth factors including TNF-α and PDGF, causing intimal thickening of the arterial walls [11]. VSMCs, which accumulate within the intima, are derived from the proliferation of the intimal VSMCs and their migration from the media [12]. Migration of VSMCs may require the degradation or remodeling of ECM surrounding the cells [13]. For ECM degradation or remodeling, a number of in vivo and in vitro studies have implemented MMPs, specifically MMP-2 (72 kDa) and MMP-9 (92 kDa), proliferation and migration of VSMCs into the intima [14]. The main function of MMPs, known as matrixins, is a degradation of the ECM and contributes to pathologic conditions including rheumatoid arthritis, coronary artery disease, and cancer [15]. On endothelial denudation, there is release of MMPs that disrupt the ECM, thereby facilitating VSMCs to migrate from the medial layer of arterial wall into the intimal space and then intimal VSMCs undergo proliferation, progressively resulting in neointima formation [16]. In the present study, we endeavored to delineate the edifying effect of NL in proliferation, migration, and expression of MMPs in VSMCs, which are the key steps in the progression of intimal thickening.

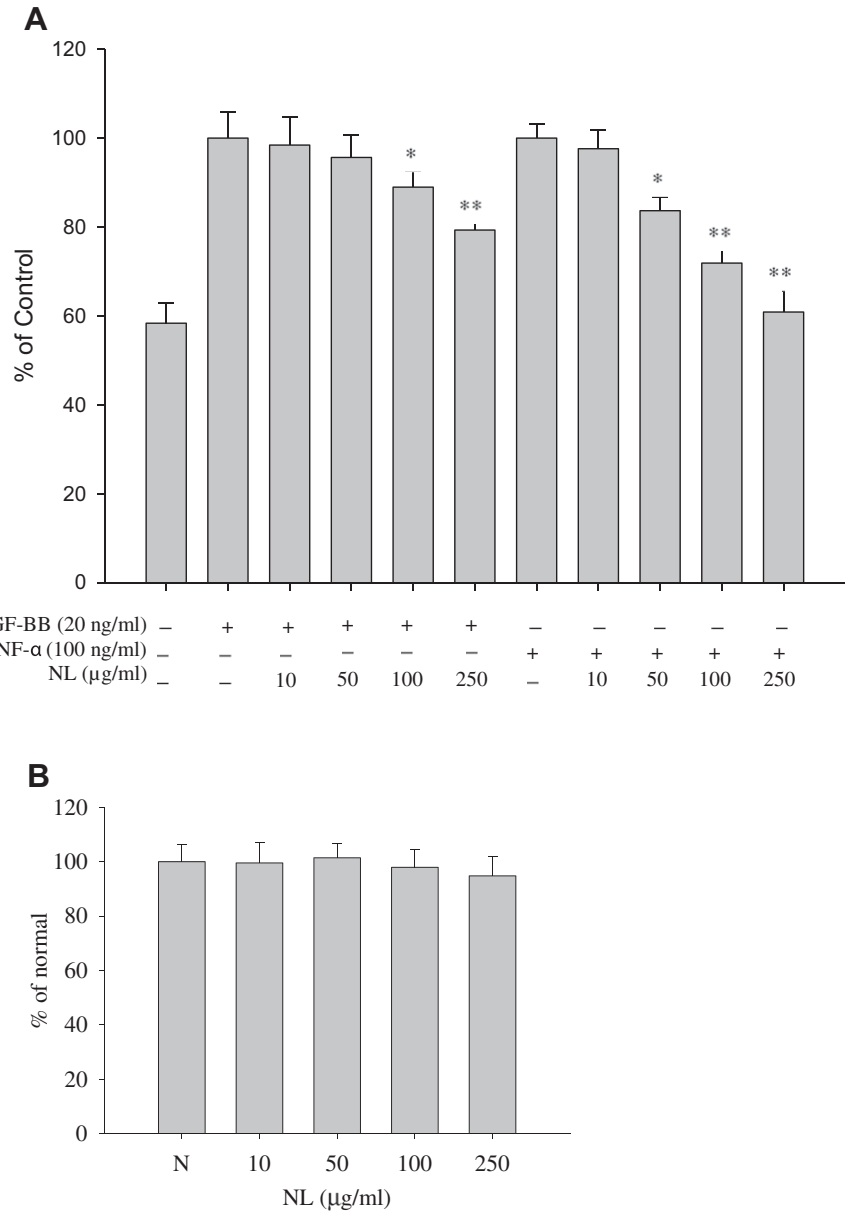

Fig. 1. Effect of NL on (A) PDGF and TNF-α–induced proliferation of VSMCs and (B) viability of VSMCs.