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Sesamol regulates plasminogen activator gene expression in cultured endothelial cells: a potential effect on the fibrinolytic system

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Abstract

Sesamol is a component in the nutritional makeup of sesame that was identified as an antioxidant. In recent years, the importance of the plasminogen activator (PA) and its adjustment factor, plasminogen activator inhibitor-1 (PAI-1), in the prevention of atherosclerosis has gradually received recognition. The objective of this in vitro study was to demonstrate the effects of sesamol on PA and PAI-1. We also compared the effects of sesamol with two well-known antioxidants, vitamins C and E, by using human umbilical vein endothelial cells as an experimental model and by treating them with the above-mentioned three nutrients with doses up to 100 μmol/L. After 24 h, cells and cultural medium were collected for analysis. The concentrations of tissue PA (tPA), urokinase PA (uPA) and PAI-1 were measured by an enzymatic immunity method. Northern blot method was used to analyze the expression of mRNA of these three types of proteins. The results showed that sesamol increased the production of uPA and tPA significantly and also up-regulated the mRNA expressions of these proteins. On the other hand, vitamins C and E could induce tPA but not uPA. As for PAI-1, none of the nutrients induced any evident response. These findings suggest that the overall vascular fibrinolytic capacity may be enhanced by using sesamol to regulate PA gene expression.
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Keywords: Sesamol; Plasminogen activator; Plasminogen activator inhibitor-1; Gene expression; Endothelial cells

1. Introduction

Sesame seeds, from *Sesamum indicum*, are one of the most important edible oil seeds. Sesame seeds and sesame oil have been known as traditional health foods and have been used in ancient Chinese medicine for a long time. However, the scientific evidence of their miraculous functions, especially in the prevention of aging—as the food has often been prescribed for in traditional medicine, has not been well established. Sesamol is a potent phenolic antioxidant found mainly in roasted sesame or in processed sesame oil [1]. The biological effects of sesamol on health have been determined as follows: sesamol exhibited powerful inhibitory effects on lipid peroxidation [2], carried out the synergistic suppression of carcinogenesis and combined with other antioxidants [3]. An in vitro study indicated that sesamol inhibited the mutagenicity of mutagens in various tester stains of *Salmonella typhimurium* [4]. Sesamol could also attenuate the production of nitric oxide and hydrogen peroxide and reduce monoamine oxidase (MAO) activity in glial astrocyte cells [5]. Since a distinct relationship exists between MAO activity and the development of neurodegenerative diseases associated with aging such as Alzheimer's disease and stroke, sesamol might play a role in the prevention of these types of diseases. Fibrinolysis defects play a pivotal role in cardiovascular diseases such as atherosclerosis and atherothrombosis. Furthermore, it is considered as a risk factor for severe cardiovascular diseases such as myocardium infarction and stroke [6–8]. The blood fibrinolytic system is composed of an active enzyme, plasmin, which degrades fibrin into soluble fibrin degradation products. The plasmin is converted from its inactive proenzyme, plasminogen, by a

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plasminogen activator (PA) [9,10]. Two major forms of PAs, tissue type [tissue PA (tPA)] and urokinase type [urokinase PA (uPA)], have been identified. Tissue PA-mediated plasminogen activation occurs upon the dissolution of fibrin in the circulation whereas the main role of uPA appears to be in the induction of pericellular proteolysis. The fibrinolytic system is regulatively inhibited by PAI-1, a high level of which is associated with myocardial function and coronary artery disease [11,12]. Therefore, it is crucial to understand the interaction between these fibrinolytic regulated proteins and to study the correlation of their activity with occurrences of cardiovascular diseases. The aim of this in vitro study was to demonstrate the effects of sesamol on the gene and protein expression of PAs and PAI-1 in human umbilical vein endothelial cells (HUVECs). While sesamol and vitamins C and E antioxidantal properties have been well documented, their effects on PA and PAI have not been verified by recent studies. As such, the objective of this report is twofold: to demonstrate the effects that these three antioxidants have on PA and PAI and to further explore sesamol's medicinal values.

2. Materials and methods

2.1. Cell culture

Human umbilical vein endothelial cells (HUVECs) were isolated from umbilical cord vein by collagenase treatment as described previously [13]. Human umbilical cords from normal deliveries or cesarean sections were collected. In a tissue culture hood, both ends of each cord (1 cm long) were excised with a sterile scalpel to expose a sterile surface. The umbilical vein was perfused with 1× PBS and was then perfused with 0.2% collagenase (Boehringer Mannheim) in PBS solution until the vein was distended. The two ends of the cord were clamped with sterile clamps and the cords were incubated for 30 min at 37°C. Then, the cords were gently massaged to facilitate endothelial cell detachment from the vessel wall. The solution was flushed out and the lumen of the vein was washed with M199 medium (containing 1% penicillin–streptomycin). The cell suspension was collected and centrifuged at 1000 rpm for 5 min at 4°C. The pellet was resuspended in medium M199 (containing 10% serum and 1% penicillin–streptomycin). The cells were plated on a 10-cm culture plate and were incubated in 37°C and 5% CO_2 overnight. After attaching overnight, the cells were washed with 1× PBS and grown in endothelial cell growth medium (BioWhittaker, #CC-3121) supplemented with 12% fetal bovine serum (Gibco). After they have fully grown, HUVECs were divided or stored in liquid nitrogen (medium containing 20% DMSO when stored). Before treatment, the cells (at a density of 5×10^5 cells/plate, 6-cm dish) were seeded in the medium containing 2% fetal bovine serum (Gibco, #10270-106) overnight. HUVECs between passages 3 and 7 were used for experimentation.

2.2. Cell treatments

Sesamol (Sigma, #S-8518) was predissolved in ethanol with concentrations of 1, 10 and 100 mmol/L and then diluted with culture medium to obtain three final concentrations of 1, 10 and 100 μmol/L. α-Tocopherol (Sigma, #T-3251) was predissolved in ethanol with 100 mmol/L and then diluted into the final concentration of 100 μmol/L with medium, whereas L(+)-ascorbic acid (Merck, #100127) was diluted directly in the culture medium with 100 μmol/L. The cultured medium and cells with various concentrations, solvent control (ethanol) and control treatments were isolated after 24 h.

2.3. Enzyme-linked immunosorbent assay

Imubind enzyme-linked immunosorbent assay kits, PAI-1 (American diagnostica no. 821), uPA (no. 894) and tPA (no. 860) were used for measuring the concentrations of the three proteins in cell extract and culture medium. These concentrations were adjusted by total protein of cell extracts. Total protein of cell extract was measured by Bradford protein assay (Bio-Rad, #69466A) [14].

2.4. Probes

Probes for Northern blot analysis were synthesized by reverse transcriptase polymerase chain reaction (PCR). The oligonucleotides PAI-1-5′ (gtctttggtgaagggtctgct) and PAI-1-3′ (ctcgtgaagtcagcctgaaa) were used to amplify the PAI-1 complementary DNA (cDNA) with 943 bp. The oligonucleotides uPA-F (gccaccatgagagccctgct) and uPA-R (cctgtagatggccgcaaacc) were used to amplify the uPA cDNA with 600 bp. The oligonucleotides tPA-5′ (ttccgccccaccacctgacg) and tPA-3′ (gaggagtcgggtgttcctgg) were used to amplify the tPA cDNA with 622 bp. The oligonucleotides glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-5′ (tggtatcgtggaaggactca) and GAPDH-3′ (agtgggtgtcgctgttgaag) were used to amplify the GAPDH cDNA with 370 bp. The PCR conditions for those probes were the same. The initial denaturation phase lasted 5 min at 94°C and was followed by a 35-cycle amplification phase consisting of 1 min at 94°C, 1 min at 60°C and 3 min at 72°C. Amplification was terminated after 7 min at 72°C. After PCR, the DNA fragments were purified with a QIA quick gel extraction kit (Qiagen). Twenty nanograms of DNA fragments was labeled with α-^{32}P-dCTP (Amersham) by a random prime labeling system (Rediprime, Amersham) for Northern blot assay.

2.5. Northern blot analysis

In order to detect the expression level of uPA, tPA and PAI-1 genes, Northern blot assay was used for detecting gene expression. Total RNA was isolated by the method that was described in the Tri-Reagent's protocol (Molecular Research Center). Ten to 20 μg of total RNA was separated on 1% agarose/formaldehyde gels as described previously [15] and was then transferred onto nylon membrane (Hybond-N+, Amersham). Hybridization was performed at

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