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Original Article

Piper sarmentosum attenuates TNF-α-induced VCAM-1 and ICAM-1 expression in human umbilical vein endothelial cells

Siti M. Ismail, M.Bt., Uma M. Sundar, B.Sc., Chua K. Hui, Ph.D, Amilia Aminuddin, Ph.D and Azizah Ugusman, Ph.D *

Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latiff, Cheras, Kuala Lumpur, Malaysia

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الملخص

أهداف البحث: يلعب الالتهاب دورا رئيسا في التسبب في تصلب الشرايين. يعتبر بيبرسارمنتوسوم عشب له أنشطة كمضاد للأكسدة ومكافحة تصلب الشرايين. تهدف هذه الدراسة إلى تقييم الخصائص المضادة للالتهابات من المستخلص المائي لبيبر سارمنتوسوم في الخلايا البطانية للوريد السري البشري.

طرق البحث: تم تقسيم الخلايا البطانية للوريد السري البشري إلى ست مجموعات؛ التحكم، والعلاج بواسطة ١٠ نانوجرام/مل لعامل نخر الورم –ألفا، والعلاج المشترك ١٠ نانوجرام/مل لعامل نخر الورم –ألفا بأربعة تراكيز مختلفة من المستخلص المائي لبيبر سارمنتوسوم (١٠٠, ١٥٠, ٢٥٠ و٣٠٠ نانوجرام/مل) لمدة ٢٤ ساعة. بعد ذلك، تم استخراج البروتين من المركب-١ لالتصاق الخلايا الوعائية، والمركب-١ للالتصاق بين الخلايا، وتم قياس ي ٩٣٧ لالتصاق الخلايا الأحادية والعامل النووي-كابا ب والبروتين ٦٥ في الخلايا البطانية للوريد السري البشري.

النتائج: حفز العلاج بعامل نخر الورم-ألفا الخلايا البطانية للوريد السري البشري مع المستخلص المائي لبيبر سارمنتوسوم في تركيزات مختلفة خفض استخراج البروتين من المركب-١ لالتصاق الخلايا الوعائية والمركب-١ للالتصاق بين الخلايا بطريقة تعتمد على –الجرعة. علاوة على ذلك، منع المستخلص المائي لبيبر سارمنتوسوم أيضا عامل نخر الورم –ألفا ي ٩٣٧ تحفيز التصاق الخلايا الأحادية إلى الخلايا البطانية للوريد السري البشري. بالإضافة إلى ذلك، خفض المستخلص المائي لبيبر سارمنتوسوم عامل نخر الورم-ألفا من تحفيز العامل النووي –كابا ب والبروتين ٦٥ معتمدا على الجرعة.

الاستنتاجات: أظهرت النتائج أن المستخلص المائي لبيبر سارمنتوسوم حظر عامل نخر الورم- ألفا الناجم عن المركب-١ لالتصاق الخلايا الوعائية والمركب-١ للالتصاق بين الخلايا من خلال آلية تنطوي على العامل النووي –كابا ب.

الكلمات المفتاحية: الخلايا البطانية للوريد السري البشري؛ الالتهاب؛ المركب-١ الالتصاق بين الخلايا؛ بيبر سارمنتوسوم؛ المركب-١ التصاق الخلايا الوعائية

Abstract

Objectives: Inflammation plays a key role in the pathogenesis of atherosclerosis. *Piper sarmentosum* is an herb with antioxidant and anti-atherosclerotic activities. The aim of this study was to evaluate the anti-inflammatory properties of an aqueous extract of *P. sarmentosum* (AEPS) in human umbilical vein endothelial cells (HUVECs).

Methods: HUVECs were divided into six groups: control, treatment with 10 ng/ml TNF-α, and co-treatment of 10 ng/ml TNF-α with four different concentrations of AEPS (100, 150, 250, and 300 μg/ml) for 24 h. Subsequently, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) protein expression, U937 monocyte cells adhesion, and nuclear factor-kappaB (NF-κB) p65 expression in HUVECs were measured.

Results: Treatment of TNF-α-stimulated HUVECs with AEPS at different concentrations resulted in decreased VCAM-1 and ICAM-1 protein expression in a dose-dependent manner. Furthermore, AEPS also inhibited TNF-α-stimulated U937 monocyte cells adhesion to HUVECs. In addition, AEPS reduced TNF-α-induced NF-κB p65 expression in a dose-dependent manner.

* Corresponding address: Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latiff, 56000 Cheras, Kuala Lumpur, Malaysia.
E-mail: dr.azizah@ppukm.ukm.edu.my (A. Ugusman)

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Conclusions: The results indicated that AEPS suppressed TNF-α-induced VCAM-1 and ICAM-1 expression NF-κB signaling.

Keywords: Human umbilical vein endothelial cells; Inflammation; Intercellular adhesion molecule-1; *Piper sarmentosum*; Vascular cell adhesion molecule-1

Introduction

Atherosclerosis is a progressive vascular disease that is characterized by endothelial dysfunction, elevated and modified low-density lipoprotein (LDL), and the proliferation of smooth muscle cells and fibrous tissue in the arterial wall.[1] Endothelial dysfunction will trigger inflammatory reactions, leading to development of atherosclerotic plaques.[2] During the early stages of atherosclerosis development, injury to the endothelium will trigger the endothelial cells to express cell adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin on its surface.[3] The adhesion molecules enable the circulating monocytes to adhere to the endothelium followed by their infiltration and differentiation into macrophages.[4]

Pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), increased the expression of chemotactic factors, other cytokines, and cell adhesion molecules, all of which contribute to the inflammatory process.[5,6] TNF-α also stimulates nuclear factor-kappaB (NF-κB) which is an important transcription factor involved in endothelial dysfunction, expression of adhesion molecules, and inflammatory responses.[7] TNF-α is one of the pleiotropic cytokines involved in most cases of inflammation and other immune response inductions.[8,9] Inducing Rat-2 fibroblasts with TNF-α led to the generation of reactive oxygen species.[8] TNF-α was also able to upregulate the expression of adhesion molecules including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) as well as induce the adhesion of monocytes in cultured endothelial cells.[10,11]

Piper sarmentosum (PS) is an herbaceous plant that belongs to the Piperaceae family and is widely distributed in the tropical and subtropical regions of the world.[12] Recent studies have reported that an aqueous extract of *P. sarmentosum* (AEPS) exhibited multiple cardiovascular protective effects such as antioxidant,[13] anti-hypertensive,[14] and anti-inflammatory effects.[15] Besides, AEPS is effective in reducing atherosclerotic lesions in hypercholesterolemic animals.[16]

Although the precise mechanisms by which AEPS reduces atherosclerosis have not been completely elucidated, it has been hypothesized that the anti-atherosclerotic activity of AEPS relies on its antioxidant potential and its ability to promote endothelial nitric oxide production to prevent endothelial dysfunction.[7,17] However, the effect of AEPS on the expression of adhesion molecules as well as monocyte adhesion to endothelial cells in response to inflammation has not been thoroughly studied yet. Therefore, this study investigated whether AEPS can modulate TNF-α-induced expression of VCAM-1 and ICAM-1 as well as the subsequent monocyte adhesion to human umbilical vein endothelial cells (HUVECs). Furthermore, the activation of NF-κB was studied, as it is the key molecule that regulates the expression of adhesion molecules.

Materials and Methods

Materials

Basal culture medium 200 (M200) (Cascade Biologics, Portland, Oregon, USA), low serum growth supplement (LSGS) (Cascade Biologics), phosphate buffered saline (PBS), TNF-α (Sigma−Aldrich Co, St. Louis, Missouri, USA), Human Soluble VCAM-1 and ICAM-1 ELISA kits (Chemicon® International Inc, Temecula, California, USA), Nuclear extraction kit (Chemicon® International Inc), NF-κB p65 UPSTATE® EZ-TFA Universal Transcription Factor Assay (Millipore Inc, Bedford, Massachusetts, USA), radioimmunoprecipitation assay (RIPA) buffer, (Sigma−Aldrich Co), and U937 monocytes (ATCC® CRL-1593.2™) (American Type Culture Collection, Manassas, Virginia, USA) were used for the experiments.

Preparation of the AEPS

Fresh leaves of PS were obtained from the Ethnobotanical Garden, Forest Research Institute Malaysia (FRIM) (voucher specimen: FRI 45870). The AEPS was prepared according to previous methods.[17] The fresh leaves were sun-dried and grinded into the powder form. The powder was mixed with water at 80 °C for 3 h (10%, w/v). The AEPS was then freeze-dried and kept at 4 °C.

HUVEC isolation and culture

This study was approved by the Ethical Research Committee of Universiti Kebangsaan Malaysia Medical Centre (approval number: FF-138-2007). Human umbilical cords were obtained with informed consent from healthy subjects in the labor room of Hospital Kuala Lumpur. HUVECs were isolated from human umbilical cords via a collagenase perfusion technique as described previously.[17] Briefly, the cells were isolated from umbilical cords using 0.1% collagenase and cultured in M200 supplemented with LSGS at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. M200 is a basal medium regularly used with LSGS to support the growth of human large vessel endothelial cells.[18]

HUVEC treatment protocol

HUVECs at passage 3 were cultured in 6-well plates at the density of 1×10^5 cells per well. At 80% confluency, the cultured HUVEC were treated with AEPS at four different concentrations (100, 150, 250, and 300 μg/ml) concomitantly with TNF-α (10 ng/ml) for 24 h. The treatment protocol was adopted from a previous study.[19] These four concentrations of AEPS were chosen as they were able to reduce oxidative