Available online at www.sciencedirect.com
ScienceDirect
Phytomedicine 15 (2008) 284–291
Phytomedicine
www.elsevier.de/phymed

Piper longum Linn. Extract inhibits TNF-α-induced expression of cell adhesion molecules by inhibiting NF-κB activation and microsomal lipid peroxidation

Naresh Singh[a], Sarvesh Kumar[a], Prabhjot Singh[c], Hanumantharao G. Raj[c], Ashok K. Prasad[b], Virinder S. Parmar[b], Balaram Ghosh[a,*]

[a]*Molecular Immunogenetics Laboratory, Institute of Genomics and Integrative Biology, University of Delhi Campus (North), Mall Road, Delhi 110 007, India*
[b]*Bioorganic Laboratory, Department of Chemistry, University of Delhi, Delhi 110 007, India*
[c]*Department of Biochemistry, V.P. Chest Institute, University of Delhi, Delhi 110 007, India*

Received 23 December 2005; accepted 17 April 2007

Abstract

Recruitment of specific leukocyte subpopulations at the site of inflammation requires a series of cell adhesion molecule (CAM)-mediated interactions. The major CAMs, viz., intercellular adhesion molecule-1 (ICAM-1), VCAM-1 and E-selectin are expressed on endothelium in response to various cytokines or bacterial LPS. Here, we have evaluated the effect of *Piper longum* chloroform extract (PlCE) on TNF-α-induced expression of ICAM-1 on endothelial cells and on NADPH-catalyzed rat liver microsomal lipid peroxidation with a view to identify modulators for the expression of CAMs. We demonstrate that PlCE inhibits adhesion of neutrophils to endothelial monolayer. This inhibition is due to the ability of PlCE to significantly block the TNF-α-induced expression of CAMs, i.e. ICAM-1, VCAM-1 at 17.5 μg/ml concentration and E-selectin at 15 μg/ml concentration on human umbilical vein endothelial cells. The inhibition of ICAM-1, VCAM-1 and E-selectin by PlCE is mediated through inhibition of NF-κB in endothelial cells. To demonstrate the antioxidant activity of PlCE, we showed that PlCE inhibited the NADPH-catalyzed rat liver microsomal lipid peroxidation significantly. These results suggest a possible mechanism of anti-inflammatory as well as antioxidant activity of PlCE.

Keywords: Anti-inflammatory; Cell adhesion molecules; Human endothelial cells; Inflammation; *Piper longum*

Introduction

Leukocytes are major players in the inflammatory response because of their antimicrobial, secretory and phagocytic activities. They are recruited to the inflamed tissue by sequential adhesive interactions between leukocytes and the endothelium which are mediated by cell adhesion molecules (CAMs) on the surface of the interacting cells (Bochner et al., 1991). Various inflammatory mediators induce the expression of CAMs (Mantovani et al., 1992) on the endothelial cells. The increased expression of CAMs alters the adhesive property of the vasculature leading to indiscriminate infiltration of the leukocytes across the blood vessels

*Corresponding author. Tel.: +91 11 2766 2580; fax: +91 11 2766 7471.
E-mail address: bghosh@igib.res.in (B. Ghosh).

0944-7113/$ - see front matter © 2007 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2007.06.007

(Krieglstein and Granger, 2001). The transcriptional regulation of expression of CAMs is controlled by transcription factor kappa B (NF-κB), which plays pivotal role in the immune responses (Baldwin, 2001). The blockade of adhesion pathways are being actively explored as a potential strategy to therapeutically manage various inflammatory diseases including asthma and allergic diseases (Gorski, 1994; Weiser et al., 1997).

TNF-α-induced free radical generation like H_2O_2 activates inflammatory signalling pathway, including NF-κB in vascular cells (Garg and Agrawal, 2002; Takacs et al., 2001), and regulating the expression of CAMs on endothelial cells and hence play an important role in various inflammatory diseases (Rahman and MacNee, 1998). Many compounds that inhibit lipid peroxidation and influence the generation of ROS, in turn lead to decrease in the expression of the CAMs and subsequently decrease inflammation, hence are found to be useful therapeutic agents in various inflammatory diseases (Cuzzocrea et al., 2001). Treatment of endothelial cells with antioxidants is also shown to down-regulate the expression of intercellular adhesion molecule-1 (ICAM-1) on endothelial cells (Marui et al., 1993; Walther et al., 1999).

Several medicinal herbs have been shown to augment specific cellular and humoral immune responses (Duke, 1985). *Piper longum* is a component of Indian traditional medicine reported to be used as a remedy for treating gonorrhea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infection, chronic gut-related pain and arthritic conditions (Ghoshal et al., 1996; Choi and Hwang, 2003; Mata et al., 2004). Other reported beneficial effects of *P. longum* include analgesic and diuretic effects, relaxation of muscles tension and alleviation of anxiety (Vedhanayaki et al., 2003). Piper extracts and piperine possess inhibitory activities on prostaglandin and leukotrienes COX-1 inhibitory effect and thus exhibit anti-inflammatory activity (Stohr et al., 2001). Recently, biochemical activities of some important medicinal plants including *Piper* species and their metabolites have been described (Prasad et al., 2005; Kumar et al., 2005a). However, very little is done to elucidate the possible targets of its action.

In the present study, we have evaluated the inhibitory activity of *P. longum* chloroform extract (PlCE) on the adhesion of neutrophils to TNF-α-induced HUVECs. We have also demonstrated that this inhibition is due to the ability of PlCE in blocking the TNF-α-induced expression of ICAM-1, VCAM-1 and E-selectin on HUVECs. Furthermore, as the induction of these CAMs is taking place at the level of transcripts by transcription factor, NF-κB, we checked the status of NF-κB in PlCE-treated cells. We found that PlCE inhibited the NF-κB activation. This extract, therefore, could be useful for the identification of small molecule(s) towards the development of anti-inflammatory molecule(s).

Materials and methods

Materials

Anti-ICAM-1, anti-VCAM-1, anti-E-selectin antibodies were purchased from Pharmingen (USA). M-199, L-glutamine, penicillin, streptomycin, amphotericin, endothelial cell growth factor, trypsin, *O*-phenylenediamine dihyrochloride, anti-mouse IgG-HRP were purchased from Sigma Chemical Co. (USA). Fetal calf serum was purchased from Biological Industries (Israel). Sodium phosphate, sodium chloride, citric acid and other chemicals were purchased from Merck.

Procurement of plant material

Plant material (dry fruits) was collected from Khari Baoli, Delhi. The plant material was identified as fruits of *P. longum* Linn., which is commonly known as Pipli or Indian long pepper by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (CSIR), Delhi. Specimen sample has been deposited in the herbarium, voucher no. NISCAIR/ RHM/F-3/2005/ Conslt/619/99.

Preparation of *P. longum* extracts

P. longum fruit powder (100 g) was dissolved in 150 ml of 50% ethanol and incubated at room temperature (28–30 °C) for 16 h. The supernatant (140 ml) collected by centrifugation at 14,000 rpm was dried in vacuum (3.5 g), designated as ethnolic extract (F001). This was further fractionated using hexane (35 ml, b.p. 68–70 °C), soluble fraction dried under vacuum and designated as hexane extract (F002). The insoluble fraction was further dissolved in chloroform (40 ml, b.p. 65 °C), the supernatant was separated by using a separatory funnel. The lower fraction was dried under vacuum, and designated as PlCE (F003). Finally, all the extracts were dissolved in DMSO individually, and used for testing ICAM-1 inhibitory activities. Among various extracts prepared, PICE was found to be more inhibitory in TNF-α-induced ICAM-1 expression (data not shown), and was chosen for further study. The phytochemical profile of PICE was determined by HPLC.

HPLC system and chromatographic condition

The HPLC system consisted of an Agilent 1100 series isocratic pump and an Agilent 1100 series diode array (DAD) detector. A Hypersil-C18 column (4.6 mm × 150 mm) (Thermo, USA) was used. The mobile phase was methanol–H_3PO_4 (75:25, v/v), filtered through a 0.2 μm filter and degassed prior to use. The flow rate was