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In vivo modulation of LPS induced leukotrienes generation and oxidative stress by sesame lignans

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Abstract

The role of inflammation and oxidative stress is critical during onset of metabolic disorders and this has been sufficiently established in literature. In the present study, we evaluated the effects of sesamol and sesamin, two important bioactive molecules present in sesame oil, on the generation of inflammatory and oxidative stress factors in LPS injected rats. Sesamol and sesamin lowered LPS induced expression of $cPLA_2$ (61 and 56%), 5-LOX (44 and 51%), BLT-1 (32 and 35%) and LTC_4 synthase (49 and 50%), respectively, in liver homogenate. The diminished serum LTB_4 (53 and 64%) and LTC_4 (67 and 44%) levels in sesamol and sesamin administered groups, respectively, were found to be concurrent with the observed decrease in the expression of $cPLA_2$ and 5-LOX. The serum levels of TNF-α (29 and 19%), MCP-1 (44 and 57%) and IL-1β (43 and 42%) were found to be reduced in sesamol and sesamin group, respectively, as given in parentheses, compared to LPS group. Sesamol and sesamin offered protection against LPS induced lipid peroxidation in both serum and liver. Sesamol, but not sesamin, significantly restored the loss of catalase and glutathione reductase activity due to LPS ($P<.05$). However, both sesamol and sesamin reverted SOD activities by 92 and 98%, respectively. Thus, oral supplementation of sesamol and sesamin beneficially modulated the inflammatory and oxidative stress markers, as observed in the present study, in LPS injected rats. Our report further advocates the potential use of sesamol and sesamin as an adjunct therapy wherein, inflammatory and oxidative stress is of major concern.

Keywords: LPS; Inflammation; Cytokines; Leukotrienes; Sesamol; Sesamin

1. Introduction

Inflammation and oxidative stress are the major cause of metabolic complications including CVD [1], rheumatoid arthritis [2], diabetic retinopathy [3] and obesity [4]. Though, inflammation and generation of small amounts of reactive oxygen species (ROS) are the normal physiological response to infection, tissue damage and a prerequisite for healing process, their production is sternly regulated by feedback mechanisms to avoid adverse effects [5]. Diminished activity of antioxidant enzymes results in elevated generation of free radicals that leads to oxidative stress in cells and culminates in cellular damage. When the circulating levels of ROS spiral out of control causing disruption in redox signalling, it may also elevate the generation of proinflammatory factors including chemoattractant leukotrienes and cytokines [6].

5-Lipoxygenase (5-LOX), one of the main enzymes in the inflammatory pathway, is involved in the generation of inflammatory mediators. 5-LOX is a non-heme iron-containing enzyme that catalyses the stereospecific incorporation of molecular oxygen into polyunsaturated fatty acids with 1, 4-*cis, cis* pentadiene moieties [7]. In the presence of five lipoxygenase activating protein (FLAP), 5-LOX metabolizes arachidonic acid to hydroperoxyeicosatetraenoic acid (HPETE) and subsequently to leukotriene A_4 (LTA_4). LTA_4 is further converted to leukotriene B_4 (LTB_4) and leukotriene C_4 (LTC_4) by the action of LTA_4 hydrolase and LTC_4 synthase, respectively. LTC_4 is further converted to leukotriene D_4 (LTD_4) and leukotriene E_4 (LTE_4) by the action of transpeptidase and dipeptidase (Fig. 1.). LTB_4 and LTC_4 are potent proinflammatory mediators that act through G protein coupled receptors, namely BLT-1 and CysLT1, respectively. LTB_4 stimulates superoxide anion production, aggregation of leukocytes at the site of injury, promotes ion influx and enhances lysosomal enzyme release whereas LTC_4 and LTD_4 are known to regulate vascular permeability [8]. Expanding research findings on the involvement of leukotrienes in various inflammatory complications and therapeutic potential of anti-leukotriene drugs have paved the way for newer line of treatments. In recent years, bioactive molecules are gaining importance due to their immense health promoting activity with limited or no side effects [9].

Sesame oil is known for both its nutritive value and its remarkable stability to oxidation. Sesame oil is shown to inhibit oxidative stress and inflammation [10]. The health benefits of oil are attributed to its lignans-

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sesamin, sesamolin, sesaminol and sesamol. They are reported to have anti-ageing, antidiabetic and anti-cancerous properties. Studies have shown that both sesamin and sesamol have delta-5 desaturase inhibiting activity [11]. Sesamin is stated to suppress chemokine production in LPS-activated human monocytes *via* MAPK, PPAR-α, and NF-κB pathways [12]. Reports suggest that sesamol down-regulates the production of IL-1β and TNF-α in LPS induced lung injury in rats [13]. Sesame lignans are known to rescue tissues from the detrimental effect of oxidative stress in arthritic models [14,15]. However, the effect of sesamol and sesamin on 5-lipoxygenase pathway is not yet understood completely under induced inflammatory conditions. In the present work, we hypothesize that sesame lignans modulate inflammatory markers *via* down regulation of 5-LOX pathway. This study would pave the way in promoting sesamol and sesamin as nutritional therapeutic molecules that may find applications in addressing inflammation complications.

2. Materials and methods

2.1. Chemicals

Sesamol, LPS, thiobarbituric acid, trichloroacetic acid, ascorbic acid, xanthine oxidase, glutathione reductase, glutathione oxidized, glutathione reduced, adenosine diphosphate, sodium nitrate, sulphanilamide, naphthly ethylene diamine, dinitrophenyl hydrazine, guanidine and bovine serum albumin were procured from Sigma-Aldrich Co, St. Louis, MO, USA. Arachidonic acid was purchased from New-Chek Prep (Elysian, USA). Sesame seeds and groundnut oil were purchased from the local market in Mysuru, India. Sesamin was isolated from sesame seed oil. HPLC grade solvents were procured from Merck Chemicals, India. Cytokine kits were purchased from Peprotech, USA. LTB_4 and LTC_4 ELISA kits were purchased from Cayman Chemicals, USA. C-reactive protein assay kit was procured from Aggape Diagnostics, Kerala, India. Antibodies were procured from Santa Cruz Biotechnology, USA. All other reagents used were of analytical grade.

2.2. Sesamin isolation and purification

Sesamin was isolated and purified as described by Kumar et al., 2009 [16] with minor modifications. Sesame seeds were crushed using food blender and oil extraction was carried using hexane in a Soxhlet apparatus for 8 h. Saponification of oil was done using 5% alcoholic KOH for 2 h and unsaponifiable fraction containing the lignans were extracted using petroleum ether (60–80°C). Lignans were recovered in methanol after evaporating the petroleum ether. Purified sesamin was obtained by subjecting methanol fraction to preparative HPLC analysis on a C_{18} column (250 × 4.6 mm, 10 μm) and elution was carried using methanol and water (70:30) at a flow rate of 8 ml/min for 70 min. Sample (1 ml) was injected and peak fractions, detected at 290 nm, were collected. Purified sesamin was characterized by LC–MS (Q-Tof Ultima Globa, Waters Associates, UK), equipped with atmospheric pressure chemical ionization, in positive mode. Evaluation of mass spectra was carried using LC–MS software equipped in the instrument.

2.3. Animal experiment design

Male Wistar rats weighing 150±5 g were maintained on *ad libitum* semi-synthetic diet (Sai Durga Feeds, Bengaluru, India). Standard conditions of temperature, humidity and light were provided to animals. The animal experiments were carried with approval from the Institutional Animal Ethic Committee (IAEC No. 391/15) and measures were taken to minimize pain or discomfort to the animals during the experimental period. Animals were divided into 6 groups of 6 animals per group. Group I – groundnut oil, control (C), Group II – LPS injected (LPS), Group III – sesamol+LPS (S+LPS), Group IV – sesamin+LPS (Sm+LPS), Group V – sesamol+sesamin+LPS (S+Sm+LPS) and Group VI – piroxicam+LPS (Px+LPS). Commercially purchased sesamol as well as sesamin isolated and purified from sesame oil, as described earlier, were used in the trials. The test molecules, solubilized in groundnut oil, were given to experimental animals by oral gavage for 15 days at concentration of 10 mg/kg body weight. For control and LPS group, only groundnut oil was given. On the 16th day, inflammation was induced by intraperitoneal injection of LPS (2.5 mg/kg body weight) to all groups except control. After 24 h, animals were sacrificed, blood and liver tissue was collected for further biochemical analysis. Liver homogenate and serum was stored at −80°C until further use.

2.4. Protein estimation

Protein was estimated according to method as described by Lowry et al., 1951 [17] using bovine serum albumin as reference standard.

2.5. Estimation of inflammatory markers in serum

Levels of cytokines (TNF α, IL-1β and MCP-1) and leukotrienes (LTB_4 and LTC_4) were estimated in serum using specific ELISA kits according to manufacturer's instructions. CRP levels in serum were measured as per the kit instructions by Aggape Diagnostics, Kerala, India.

2.6. Western immunoblot analysis

Expression of $cPLA_2$, 5-LOX, LTC_4 synthase and BLT-1 were analysed in liver homogenate using immunoblot technique. Liver homogenate (10%) was prepared using cell lysis buffer. Samples were subjected to SDS polyacrylamide gel (8–10%) separation according to Laemmli, 1970 [18]. The separated bands were transferred to PVDF membrane (pore size 0.2 μ, Pall Life Sciences, India) at 100 V for 2 h and thereafter blocked using skim milk (5%). Overnight primary antibody treatment was done at 4°C against $cPLA_2$, 5-LOX, LTC_4 synthase and BLT-1 independently. β-Tubulin was used as the loading control. The blots were washed with TBST to remove unbound primary antibody and incubated with respective HRP-conjugated secondary antibody for 1 h at room temperature (27°C). After washing with TBST, (to remove unbound secondary antibody), the blots were treated with enhanced chemiluminescence reagent for 1 min. The protein bands were visualized using Syngene Gel Doc system equipped with blue light transilluminators (G: BOX Chemi XT4)

2.7. Measurement of oxidative stress markers and antioxidant enzyme activity

Lipid peroxidation in serum and in liver was estimated using thiobarbituric acid reaction (TBA) according to Buege and Aust, 1978 [19] with slight modification. For estimation of malondialdehyde (MDA), liver was homogenized in 0.74% KCl buffer and an

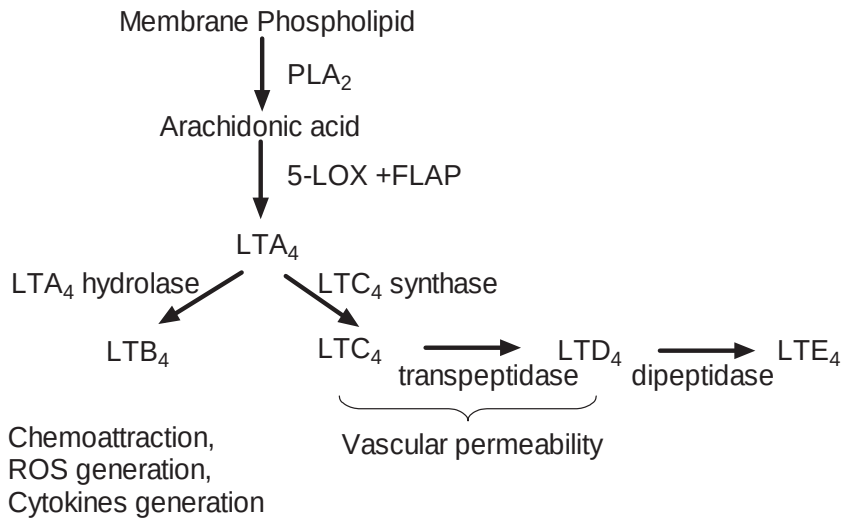

Fig. 1. Biosynthesis and physiological effects of leukotrienes.