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Entitled

ROLE OF NEROLIDOL, AN ALIPHATIC SESQUITERPENE, IN COLON INFLAMMATION

by

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Abstract

Inflammatory bowel diseases, which comprise Crohn's disease (CD) and ulcerative colitis (UC), are chronic and progressive immune-mediated inflammatory conditions of the gastrointestinal (GI) tract. Both genetic and environmental factors influence this condition. Conventional therapy to suppress aberrant immune seen in IBD with corticosteroid or with biological agents has a high relapse rate and limiting their use. Approximately 40% of IBD patients switch to alternative therapies that include dietary supplements rich in phytochemicals. Nerolidol (NED) is a naturally occurring sesquiterpene alcohol present in various plants with potent anti-inflammatory properties. Therefore, in the current study, we investigated the role of NED in preclinical models of colon inflammation. In our initial experiments, we investigated the anti-inflammatory properties of NED in lipopolysaccharide (LPS)-stimulated RAW 264.7 murine macrophage cells. NED inhibited the lipopolysaccharides induced TNF-α, IL-1β, and IL-6 release and its mRNA expression. In addition, NED also prevented the increase in the expression of proinflammatory mediators such as COX-2 and iNOS suggesting its potential anti-inflammatory properties. We further investigated NED as a putative anti-inflammatory compound in *in vivo* and *in vitro* models of colonic inflammation. *In vivo* colon inflammation model was established using C57BL/6J male black mice administered with 3% dextran sodium sulfate (DSS) in drinking water for 7 days κto induce colitis. Colitis groups received either vehicle or oral NED (50, 100, and 150 mg/kg body weight/per day by gavage). Colon length, Disease activity index (DAI), colonic histology, and various biochemical parameters were measured. *In vitro* model of inflammation was established by challenging HT-29 cells (human colorectal adenocarcinoma cell) with TNF-α (1ng/ml concentration). Two different concentrations of NED (25μM and 50 μM) were used for *in vitro* experiments. NED significantly decreased the DAI and reduced the inflammation-associated changes in colon length and macroscopic and microscopic architecture of the colon. Changes in tissue MPO concentrations, neutrophil and macrophage mRNA expression (CXCL-2 and CCL2), and proinflammatory cytokine content (IL-1β, IL-6, and TNF-α) both at the protein and mRNA level were significantly reduced by NED. The increase in the content of the proinflammatory mediators COX-2 and iNOS induced by DSS was also significantly inhibited by NED along with tissue nitrate levels. NED promoted Nrf-2 nuclear translocation dose-dependently. NED significantly increased antioxidant enzyme activity (SOD and CAT), HO-1 and SOD-3 mRNA levels. NED treatment in TNF-α-challenged HT-29 cells significantly decreased proinflammatory chemokines (CXCL-1, IL-8, CCL2, and COX-2) mRNA levels.

Mitogen-activated protein kinases (MAPKs) and nuclear factor kappa B (NF-κB) are important signaling pathways involved in inflammation. Therefore, we investigated the effect of NED on MAPK and NF-κB signaling mechanisms. Our results suggest that the phosphorylation of MAPK (p38, JNK, and $ERK^{1/2}$) and NF-κB were significantly increased in the DSS-induced colitis model, and LPS stimulated RAW macrophages. NED treatment significantly inhibited phosphorylation of MAPK and NF-κB signaling proteins both in the mouse model and in LPS-stimulated RAW macrophages. These results suggest that NED's anti-inflammatory action is mediated by inhibition of the NF-κB/MAPK signaling mechanism.

Intestinal epithelial barrier dysfunction leading to enhanced intestine permeability seen in IBD. Therefore, we investigated the effect of NED on intestine tight junction integrity in colon inflammation using the DSS-induced colitis model (*in vivo* model) and in LPS challenged Caco-2 monolayers (*in vitro* model). NED significantly decreased the concentration of FITC-dextran in the serum of mice compared to the DSS group suggesting decrease colon permeability. Our results also showed that LPS induced a decrease in the transepithelial electrical resistance (TEER) in Caco-2 monolayers was significantly prevented by the treatment with NED. It was observed that NED protected the intestinal barrier integrity by enhancing the expression of tight junction proteins such as claudin-1, -3, -7, and occludin in *in vitro* and *in vivo* models of colon inflammation. Collectively our results elucidate that NED supplementation attenuates colon inflammation through its potent antioxidant and anti-inflammatory activity both in *in vivo* and *in vitro* models of colonic inflammation. NED also enhanced intestine tight junction integrity to prevent barrier dysfunction observed in colon inflammation.

Keywords: Nerolidol, aliphatic sesquiterpene, LPS-stimulated RAW macrophages, DSS colitis, Nrf-2/Keap-1, MAPK, NF-κB signaling, Caco-2 cells, HT-29 cells, FITC-Dextran permeability, transepithelial electrical resistance (TEER), intestine epithelial tight junction, occludin, and claudins.