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SHORT COMMUNICATION

Knipholone, a selective inhibitor of leukotriene metabolism

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Abstract

Inhibition of leukotriene formation is one of the approaches to the treatment of asthma and other inflammatory diseases. We have investigated knipholone, isolated from the roots of *Kniphofia foliosa*, Hochst (Asphodelaceae), for inhibition of leukotriene biosynthesis in an ex vivo bioassay using activated human neutrophile granulocytes. Moreover, activities on 12-lipoxygenase from human platelets and cycloxygenase (COX)-1 and -2 from sheep cotyledons and seminal vesicles, respectively, have been evaluated. Knipholone was found to be a selective inhibitor of leukotriene metabolism in a human blood assay with an IC_{50} value of 4.2 μM. However, at a concentration of 10 μg/ml, the compound showed weak inhibition of 12(S)-HETE production in human platelets and at a concentration of 50 μM it produced no inhibition of COX-1 and -2. In our attempt to explain the mechanism of inhibition, we examined the antioxidant activity of knipholone using various in vitro assay systems including free radical scavenging, non-enzymatic lipid peroxidation, and metal chelation. Knipholone was found to be a weak dose-independent free radical scavenger and lipid peroxidation inhibitor, but not a metal chelator. Therefore, the leukotriene biosynthesis inhibitory effect of knipholone was evident by its ability either to inhibit the 5-lipoxygenase activating protein (FLAP) or as a competitive (non-redox) inhibitor of the enzyme. Cytotoxicity results also provided evidence that knipholone exhibits less toxicity for a mammalian host cell.

Keywords: *Kniphofia foliosa*; Knipholone; Leukotrienes; 5-Lipoxygenase; 12-Lipoxygenase; Cycloxygenases

Introduction

5-Lipoxygenase (5-LOX) is the key enzyme involved in the first two biosynthesis steps of leukotrienes from arachidonic acid: the stereospecific oxygenation leading to formation of 5(S)-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (5-HPETE), and further dehydration to leukotriene A_4 (Samuelsson, 1983). Leukotrienes play a major role in the inflammatory response to injury and they have been implicated in the pathogenesis of several chronic inflammatory diseases, most notably asthma, psoriasis, rheumatoid arthritis and inflammatory bowel diseases. So far, two therapeutic strategies have been developed to inhibit the enzyme, 5-LOX, involved in leukotrienes biosynthesis: direct 5-LOX inhibitors and indirect inhibitors which interfere with 5-lipoxygenase activating protein (FLAP). The direct 5-LOX inhibitors interact with the enzyme via a redox,

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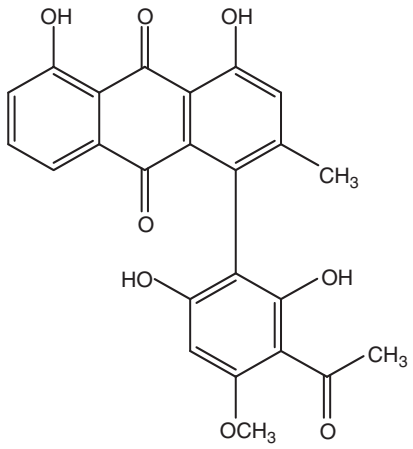

Fig. 1. The structure of knipholone.

a nonredox or an iron chelating mechanism (McMillan and Walker, 1992), while FLAP inhibitors block the association of the enzyme with the cellular membrane (Dixon et al., 1990).

Knipholone, a binary compound composed of the anthraquinone chrysophanol and an acetylphloroglucinol (Fig. 1), was originally isolated from the roots of *Kniphofia foliosa*, Hochst (Asphodelaceae) together with chrysophanol (Dagne and Steglich, 1984) and evaluated for its antiprotozoal properties (Bringmann et al., 1999, 2003). In the present study, we evaluated the leukotriene inhibitory activity and mechanism of inhibition by knipholone isolated from the roots of *K. foliosa*. The perennial herb *K. foliosa* grows wild in the central and southern highlands of Ethiopia. The roots have long been used in traditional medicine for the treatment of abdominal cramps and wound healing (Abate, 1989).

Materials and methods

Analytical TLC was performed on Merck silica gel 60 F_{254} and RP-18 F_{254s} plates. Column chromatography (CC) was performed on Merck silica gel 60 (70–240 mesh) and size exclusion chromatography on Sephadex LH-20. Solid phase separation was conducted using Isolute C18 EC (10 g) columns. Semi-preparative HPLC was performed using LiChrospher® RP-18 (10 μm, 250 × 10 mm i.d.) column. NMR spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C on a Bruker AVANCE 500 spectrometer. Mass spectra were determined by LC–ESI–MS analysis on a Thermo Finnigan LCQ Deca XP Plus mass spectrometer connected to a Surveyer LC-system (Thermo Finnigan). Absorbances for antioxidant tests were determined with a WALLAC 1420 Multilable Counter, Perkin Elmer™ Life Sciences. UV–visible spectra were recorded using a SPECTROD 50 spectrophotometer (Zeiss). The absorbance for LTB_4 quantification was conducted using the photometric ELISA plate reader, Tecan RAIN BOW.

Trypan blue solution, eicosatetraenoic acid, type VII Folch bovine brain extract, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 1-butanol and glutathion were purchased from Sigma Chemicals. Ca ionophor A 23187, KH_2PO_4–K_2HPO_4 buffer pH 7.413 (25 °C) (PBS), Tween 80^{R}, trichloroacetic acid (TCA), thiobarbituric acid (TBA), butylated hydroxyltoluene (BHT), NaOH and epinephrin-hydrogentartrate were obtained from Fluka. Ethanol p.a., formic acid, citric acid solution, $CaCl_2 \cdot 2H_2O$ p.a., anhydrous D-glucose, $MgCl_2 \cdot 6H_2O$. KCl, TRIS p.a., HCl solution, NH_4Cl solution, NaCl, TÜRKS solution, $FeCl_3 \cdot 6H_2O$, $CuSO_4 \cdot 5H_2O$, $FeSO_4 \cdot 7H_2O$, dimethyl sulfoxide (DMSO) and Na_2EDTA were bought from Merck. Ascorbic acid and baicalein were obtained from Aldrich. Quercetin and Tris/HCl buffer (pH 8.0) were obtained from Roth. LTB_4 EIA Kit, purified PGHS-1 and -2, indomethacin and NS-398 were obtained from Cayman Chemical, Ann Arbor, MI, USA. Arachidonic acid and dextran were purchased from Amersham Pharmacia Biotech AB. 12(S)-HETE correlated-EIA kit was purchased from Assay Designs, Ann Arbor, MI, USA. PGE_2-EIA kit was obtained from R & D systems, Minneapolis, MN, USA. Zileuton was purchased from Sequoia Research Products Ltd., Oxford, UK.

Plant materials

The roots of *K. foliosa* were collected in April 2001 from Grassland very close to Dinsho, Bale, Ethiopia (alt. 2700–2850 m) and identified by Mr. Melaku Wondafrash, the National Herbarium, Department of Biology, Addis Ababa University. A voucher specimen (collection number 1482) has been deposited in the National Herbarium for future reference.

Extraction and isolation

Air-dried ground roots (500 g) of *K. foliosa* were extracted successively by petroleum ether, dichloromethane and methanol using a Soxhlet apparatus for 24 h. The dichloromethane extract was evaporated under reduced pressure to yield 5.5 g (1.1%) residue and subjected to CC on silica gel eluting with petroleum ether and a 10% stepwise gradient with dichloromethane and methanol to afford 20 fractions. Fractions 12–13 (1.2 g) from CH_2Cl_2/CH_3OH (9:1–8:2) were combined and subjected to solid phase separation using a CH_3OH/H_2O gradient elution followed by Sephadex LH-20, CH_2Cl_2/CH_3OH (1:1) to yield a reddish fraction (720 mg), which was purified further by semi-preparative RP-18 HPLC with CH_3CN/H_2O (6:4) isocratic system