C-9

Anti-inflammatory activity from brown seaweed *Ishige okamurae*

Gyawali YP, Khan MNA, Choi JS, Kang SE, Yoon SJ, Park SM and Hong YK

Department of biotechnology, Pukyong National University, Busan 608-737

Introduction

PLA_2 is an enzyme produced from the *phlA* gene of *Vibrio mimicus* which cleaved fatty acids at *sn*-2 position of phosphatidycholine, however, it didn't show lysophospholipase, sphingomyelinase and phospholipase activities (Lee et al, 2002). This enzyme play a major role in both phospholipids turnover and the regulatory mechanisms controlling inflammatory responses, since the release of arachidonic acid by PLA_2 leads to the synthesis of the bioactive eicosanoids (prostaglandins, thromboxanes and leukotrienes) (Flower& Blackwell, 1976). In this study, the PLA_2 enzyme *in vitro* assay and *in vivo* porbol myristate acetate (PMA) induced mouse ear oedema and erythema assay were carried out from purified compound (MCS 14-2) obtained from *I. okamurae*. PMA induced mouse ear oedema and erythema reducing and this enzyme inhibitor compound might be useful as a therapeutic drug for treatment of inflammatory disease.

Materials and methods

Purification of the extract: Seaweed thalli were collected from Namhe and Cheju Island , Korea during May 2002 to May 2004. MeOH-water (4:1) extract and polarity classes fractions were prepared according to method of Harborne (1998). The most inhibitory chloroform fraction was then filtered through Sephadex LH-20 column (2×120 cm) using methanol as eluant and collected 50 mL each at a flow rate of 0.5 mL/min. The active Sephadex fractions (13-14) then subjected to HPLC (3.9mm x 300mm, Silica column) using n-hexane and iso-propanol(0-6%) as eluants.

Enzyme assay Assay procedure was followed using the method described by Cho and Kezdy (1991) with modification. Acetone was used as a vehicle control.

Anti-inflammatory activity BALB/c mice(8-10 weeks of age) were used to ear edema and erythema assay. Porbol myristate acetate (Sigma, St. Louis, USA) was topically applied at 0.2ug in 10ul acetone with different concentrations of equal volume of purified compound (25ug, 50ug, 75ug, 100ug in 10uL acetone) to each inner side of mouse ear. Ear oedema and erythema was measured using a spring loaded micrometer (Mitutoyo Corp., Tokyo, Japan). Erythema was measured by taking digital photographs and compared the magenta value using Adobe photoshop 7.0.

Result and discussion

1. IC_{50} and MIC of purified compound were 1.86ug/ml and 3.96 ug/ml respectively. IC_{50} and MIC of rutin were 4.8uM and 98uM respectively.

2. For ear oedema IC_{50} and MIC of purified compound were 3.57g/ml and 5.21 mg/ml, rutin IC_{50} and MIC were 17mM and 114.84mM.

3. Erythema IC_{50} and MIC of purified compound were 4.55mg/ml and 9.1mg/ml. Similarly for rutin, erythema IC_{50} and MIC were 12.34mM and 49.05mM.

4. KI value of purified compound against bacterial PLA_2 was 3.89 ug/ml.

Reference

Cho W and Kezdy FJ (1991) Chromogenic substrates and assay of Phospholipase A_2. Meth. Enzymol. **197**: 75-79

Flower RJ and GJ Blackwell (1976) The importance of phospholipase A_2 in prostaglandin biosynthesis. Biochem. Pharmacol. **25**: 285-291.

Harborne J.B. 1998. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman & Hall, London, pp. 302.

Lee JH, Ahn SH, Kim SH, Choi YH, Park KJ, and Kong IS (2002) Characterization of *Vibrio mimicus* phospholipase A (Phl A) and cytotoxicity on fish cell, Bioch. and Bioph. Res. Comn. **298**: 269-276.