Phytomedicine 23 (2016) 558–565

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.com/locate/phymed

Cytotoxic and pro-oxidative effects of *Imperata cylindrica* aerial part ethyl acetate extract in colorectal cancer *in vitro*

Amy Ho Yan Kwok, Yan Wang, Wing Shing Ho*

School of Life Sciences, The Chinese University of Hong Kong, Hong Kong

ARTICLE INFO

Article history:
Received 18 June 2015
Revised 26 January 2016
Accepted 14 February 2016

Keywords:
Imperata cylindrica
Colorectal cancer
Prostanoids
Cytotoxicity
Oxidative stress

ABSTRACT

Background: Colorectal cancer (CRC) is the third most common cancer. Its global incidence and mortality have been on the rise. Recent strategy of therapies has involved the use of non-steroid anti-inflammatory drugs and cyclooxygenase-selective inhibitors. Aerial parts of *Imperata cylindrical L. Raeusch* (IMP) have been used as an anti-inflammatory agent in traditional Chinese medicine.

Hypothesis: Asarachidonate acid cascadeis often involved in inflammation-related malignancy and IMP is an anti-inflammatory agent, hence it is hypothesized that IMP aerial part ethyl acetate extract exerts cytotoxic effects on colorectal cancer cells *in vitro*.

Study design: The HT-29 adenocarcinoma cell line was used to elucidate its pro-apoptotic activities. Flow cytometry and fluorescent microscopy were performed to assess cell cycle arrest and the accumulation of reactive oxygen species (ROS). The mRNA and hormone levels of arachidonate acid pathways were studied via quantitative reverse transcription PCR (qRT-PCR) and ELISA.

Results: The 50% growth inhibitory effect (GI_{50}) of the IMP extract on HT-29 was measured with a value of 14.5 μg/ml. Immuno-blot and caspase-3/7 activity assay showed the pro-apoptotic effect of IMP on the activation of caspase cascade. G2/M arrest was observed via flow cytometry. The ROS activity was modulated by the IMP extraction a concentration-dependent manner in HT-29 cells. The IMP extract increased PGE_2 and $PGF_{2\alpha}$ levels qRT-PCR revealed that transcripts of rate-limiting PGE_2- and $PGF_{2\alpha}$-biosynthetic enzymes – COX-1, mPGES1 and AKR1C3 were notably up-regulated. Among the prostanoid receptors, EP_1 and FP transcripts were up-regulated while EP_4 transcripts decreased. The findings suggest that the proliferative effect of PGE_2, which is generally believed to associate with heightened DNA synthesis and cross-talk with MAPK pathways, is likely triggered by the pro-apoptotic or -oxidative effects exerted by IMP extract in HT-29 cells. Concurring with this notion, indomethacin (COX-1/2-inhibitor) was demonstrated to potentiate the cytotoxic effect of IMP extract ($GI_{50} \leqq 10.8$ μg/ml). The results show that the cytotoxic effect of IMP extract predominates over the influence of proliferative prostanoids released by challenged colorectal cancer cells, and may present a potential source for development of novel anti-cancer drugs.

Introduction

Colorectal cancer is ranked the third and fourth amongst neoplasm in term of prevalence and death rate (Ferlay et al., 2013). Its tumorigenesis is generally believed to be associated with or, in most cases, preceded by chronic inflammation and adenoma polyp formation along the distal colon (Philip et al., 2004), hence endoscopic colon examination is the common procedure for detection of these early signs (Rutter et al., 2004), as it helps with both prevention via surgical removal or diagnosis via collection for further histological examination of abnormal growth or tissues.

Arachidonate acid (ARA) metabolites are important lipid mediators regulating inflammation, analgesia, angiogenesis and cell proliferation [Bogatcheva et al., 2005]. The common precursor ARA released by phospholipases (PLA2) then initiates either cyclooxygenase (COX) or lipoxygenase (LOX) pathways to yield the 2 classes of eicosanoids – prostanoids (PGs) or leukotrienes (LTs), respectively. COX and LOX catalyze the conversion of ARA into unstable intermediates PGH_2 and LTA_4, which then underwent rapid conversion into PGs (includes prostaglandin D2, E2, F2α, I2 (PGD_2; PGE_2; $PGF_{2\alpha}$; PGI_2), and thromboxanes (TXA_2)) and LTs (*e.g.* leukotriene C4 (LTC_4)) via their specific terminal synthases, respectively. Amongst these eicosanoids, PGE_2 has been most frequently implicated in cancer tumorigenesis, proliferation, metastasis and

Abbreviations: CRC, colorectal cancer; DOX, doxorubicin; IMP, Imperata cylindrica; Indo, indomethacin; MTT, 3- (4,5-cimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; LTs, leukotrienes; PGs, prostanoids; ROS, reactive oxygen species.

* Corresponding author. Tel.: +852 3943 6114; fax: +852 2603 7732.
E-mail address: ws203ho@cuhk.edu.hk (W.S. Ho).

http://dx.doi.org/10.1016/j.phymed.2016.02.015
0944-7113/

immune-surveillance in previous studies (Wang and DuBois, 2006). Significantly up-regulated level of PGE_2 is often observed in neoplastic tissues, including breast, colon and ovarian cancer cells (Wang and DuBois, 2006). And non-steroidal anti-inflammatory drugs (NSAIDs) targeting COXs have been widely used in cancer prevention and treatment (Thun et al., 2002), and in recent decades, COX-2-specific inhibitors and antagonists for PGE_2 receptors (EPs) have been studied as potential targets in chemotherapies or prevention.

Imperata cylindrica L. Rausch (IMP), commonly known as cogon grass or spear grass, is a perennial rhizomatous plant that can grow on soils with a vast range of nutrients, moisture and pH (Santoso et al., 1996). Despite ecological concerns over its extensive vegetation (Gordon, 1998), its rhizome has been widely used in traditional Chinese medicine as a diuretic and anti-inflammatory agent (Pharmacopeia Committee of P. R. C., 2010), of which responses are closely linked to eicosanoids. There is little information on the use of its aerial part. Previous study showed that its aerial part which was extracted with ethyl acetate demonstrated the potential in growth inhibition of cancer *in vitro* (Colotta et al 2009). Hence, the growth-inhibiting, pro-apoptotic and pro-oxidative effects of IMP extract in colorectal cancer cell line especially the eicosanoid biosynthesis and signaling were investigated.

Materials and methods

Cells, chemicals & reagents

HT-29 and WRL68 cell line were obtained from ATCC (Manassas, VA). Methanol (RCI, Labscan, Thailand), trifluoroacetic acid (Sigma Aldrich, St. Louis, MO) were of HPLC grade. All other reagents were of analytical reagent grade purchased from Sigma Aldrich. Apoptosis antibodies sampler kit and antibody against caspase-8 were purchased from Cell signaling (Danvers, MA) and Santa Cruz (Dallas, TX), respectively.

IMP extract preparation

Imperata cylindrical L. Raeusch(IMP) was obtained from a local vendor. 200 g of its aerial parts was boiled with 6 l of 70% ethanol for 1.5 h twice. The 70% ethanol extract was then condensed to 100 ml in a rotary evaporator (R-210, BUCHI, Switzerland) with 120 mbar at 60 °C. It was partitioned with ethyl acetate (4 × 100 ml) to yield the final ethyl acetate extract. The extract was dried in the rotary evaporator and frozen at −80 °C deep freezer overnight before lyophilization. IMP extract powder was kept at −20 °C for a long-term storage and reconstituted with DMSO freshly before any experiment.

HPLC analysis

Individual components in the IMP extract were determined using high-performance liquid chromatography (HPLC). 30 mg of sample was dissolved in 1 ml solution [acetonitrile (CAN): DMSO = 1: 1, v/v] by sonication for 5 min. It was then filtered through a 0.22 μM polypropylene filter and 20 μl of the filtrate was injected into the analytical HPLC column (ALLTIMA C18,5 μM, 250×10 mm i.d.) and quantified on a HP1100 series HPLC system equipped with a UV detector at 323 nm. The elution profile (Fig. 1) was programmed with the gradient mobile phase composed of solvent A (0.1% trifluoroacetic acid) and solvent B (methanol). The gradient for separation was programmed as follows: 0 min, 65% B, at a flow rate of 1.5 ml/min;5–10 min, 70% B, at a flow rate of 1.5 ml/min; 15 min, 80% B, at a flow rate of 1.0 ml/min; 25–35 min, 85% B, at a flow rate of 0.8 ml/min; 40–50 min, 100% B, at a flow rate of 2.0 ml/min, and then was held for additional 5 min.

Viability assay

HT-29 or WRL68 cells were seeded at 8×10^3 cells per 96-well (~20% confluence) and incubated overnight. The medium was then replaced by either 0.05% DMSO [as solvent control (SC)] or various dosages of IMP extract, alone or in combination with various concentrations of indomethacin (Indo) or N-acetyl cysteine (NAC), and incubated for 24 h. The medium was removed at the end of incubation, and the cells were washed in phosphate-buffered saline (PBS) once before the addition of MTT (5 μg/ml)-supplemented incomplete RPMI1640 medium (1:100, v/v) (Gibco, Carlsbad, CA). The cells were incubated for 4 h, and then the medium was replaced by DMSO. MTT crystals were solubilized with gentle shaking and subjected to absorbance measurement at 540 nm (TECAN infinite M200, Switzerland). Each treatment or control was performed in 4 replicates. Mean absorbance readings from solvent control groups were defined as 100% viability and data from treatment groups were shown as % viability relative to the controls.

Cell cycle analysis

Cells were seeded at 1×10^6 cells per well on a 6-well plate. After overnight incubation, cells were treated with IMP extract for 48 h. The cells were washed with PBS once and trypsinized into a single cell suspension. The harvested cells were washed with PBS twice before fixation with 70% ethanol for 1 h. The cells were centrifuged at 500 x *g* for 5 min and washed twice with PBS before addition of propidium iodide (final conc: 40 μg/ml) with RNase A (8 μg/ml; Life Technologies). The cells were incubated in dark at 37 °C for 15 min. Flow cytometry was performed on FACSVerse (BD

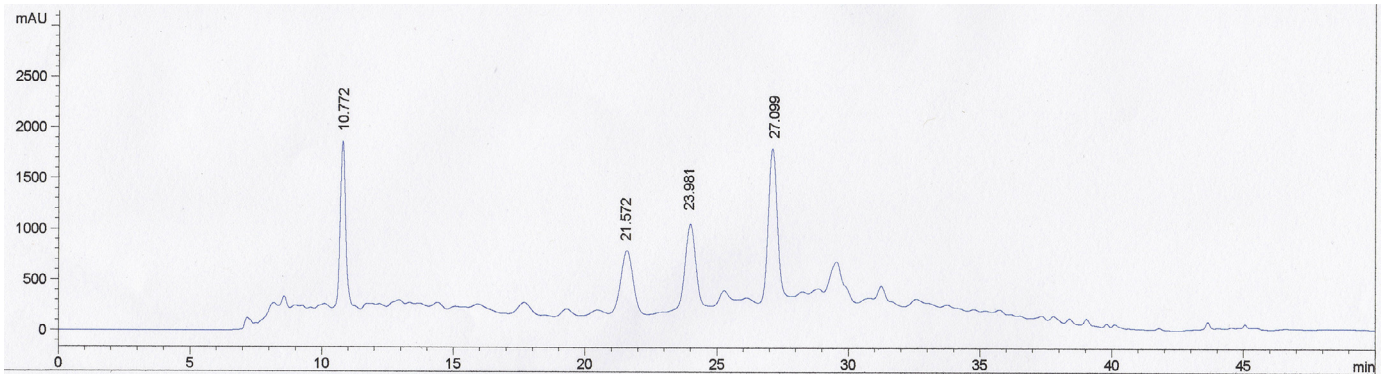

Fig. 1. HPLC profile of IMP aerial part ethyl acetate extract. The numbers denote the respective elution time (min) at which the various components are eluted from the HPLC column. These constituents have not been purified and remained unknown to our knowledge.