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Research paper

Cinnamon extract exhibits potent anti-proliferative activity by modulating angiogenesis and cyclooxygenase in myeloma cells

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ABSTRACT

Cinnamon is one of the most widely used herbal medicines with diverse bioactive effects. However, there is no report in regard to the potential anti-proliferative effects of cinnamon on myeloma cells. In this study, the authors investigated the anti-proliferative potential of cinnamon bark powder extract (CBPE) on myeloma cells specifically focusing on its anti-angiogenic and anti-inflammatory properties. The authors observed inhibition of myeloma cell proliferation by CBPE along with anti-inflammatory and anti-angiogenic effects in a human myeloma cell line RPMI 8226. After 24 h of treatment, CBPE caused approximately 50% inhibition of cell growth in RPMI 8226 cells at a concentration of 72 μg/mL (IC50), compared with the untreated controls. CBPE treatment strongly inhibited expression of angiogenic factors and cyclooxygenase at mRNA as well as at protein level in a time-dependent manner. Treatment with the CBPE resulted in cell cycle arrest in the G_0/G_1 phase in a time-dependent manner. CBPE besides altering growth kinetics of cells, induces DNA fragmentation resulting in apoptosis. In the treated group, DNA fragmentation increased in a time dependent manner with maximum intensity of labeled nucleotides in cells treated for 72 h as compared to 24 h and 48 h. In conclusion, the anticancer effects of cinnamon extract appear to be mediated by multiple mechanisms. These include inhibition of angiogenesis, inflammation and induction of apoptosis in myeloma cells. CBPE could be considered as a promising candidate for restricting the growth of myeloma cells. Hence, CBPE could lead to the development of an effective anti-cancer agent or herbal medicine for the treatment of MM.

1. Introduction

Tumour cells are generally resistant to apoptosis; hence selective killing of tumor cells by targeting the pathway of apoptosis is seen as an attractive and effective way for the development of anti-proliferative agents. Angiogenic factors and inflammatory markers are constitutively active in many kinds of cancers and play critical roles in tumour development and their further progression by promoting an angiogiogenic and inflammatory microenvironment leading to metastasis and cell survival (Joshi et al., 2011; Khan et al., 2013). It is a positive approach to use chemopreventive agents (natural or synthetic) to inhibit, delay, block, or reverse the initiation and developmental events associated with carcinogenesis (Abdullah et al., 2010). Currently, numerous scientific studies support herbal medicine as a potent anti-cancer drug (Molassiotis et al., 2005; Kwon et al., 2009). Plants, vegetables, herbs and spices used in folk and traditional medicine have been accepted as one of the main sources of chemopreventive drugs (Deorukhkar et al., 2007). Thus there is a need of the hour of the herb having anti-cancer activity along with anti-inflammatory and anti-angiogenic properties as previous reports established the role of inflammation and angiogenesis in multiple myeloma (MM) (Joshi et al., 2011; Khan et al., 2013). However, the development of any herbal medicine as an anti-proliferative agent needs substantial research in order for it to meet strict criteria such as those on standardization, quality control, safety, toxicity, and clinical trials (Buchanan et al., 2005). More importantly, the elucidation of the mechanism of action of herbal medicines will turn complementary and alternative medicine (CAM) into 'evidence based medicine' (Meijerman et al., 2006).

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In traditional Chinese medicine, cinnamon is indicated as an analgesic and antipyretic against colds, fever, headache, myalgia, arthralgia, and amenorrhea (Zhang et al., 2014). Many nutrients including manganese, dietary fiber, iron, and calcium, occur in cinnamon (Khan et al., 2014). Its extracts contain several active components such as essential oils (cinnamic aldehyde and cinnamyl aldehyde), tannin, mucilage and carbohydrates (Wijesekera, 1978; Tanaka, 2008). The volatile oils obtained from the bark, leaf, and root bark vary significantly in chemical composition. Each oil has a different primary constituent: cinnamaldehyde (in the bark oil), eugenol (in the leaf oil), and camphor (in the root-bark oil). Recent studies based on techniques such as gas-liquid chromatography and infrared spectrometry has revealed that the three oils possess the same array of monoterpene hydrocarbons in different proportions (Wijesekera, 1978). They have various biological functions including anti-oxidant, antimicrobial, anti-inflammatory (Asolkar et al., 1992), anti-diabetic effects (Kim et al., 2006), and anti-tumour activity (Schoene et al., 2005). Cinnamon exhibits potent anti-inflammatory properties by inhibiting the production of nitric oxide (NO), COX-2 and prostaglandin (PG) E2 in macrophage cell lines (Tung et al., 2008). Cinnamaldehyde (CNA) modulates inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) activation via the redox-related NF-kB inducing/inhibitor kinase (NIK/IKK) and mitogen-activated protein kinase (MAPK) pathways through the reduction of oxidative stress and it has also been shown to inhibit proliferation of several human cancer cell lines including breast, leukemia, ovarian, and lung tumor cells (Lee et al., 1999).

Cinnamon bark has been typically used in the form of a water extract, whereby the volatile ingredients are seldom found thus it is likely that the established pharmacological activities of cinnamon bark depend on a mixture comprised of a variety of water-soluble components, thereby ensuring its safety as a traditional remedy. It has been found that cinnamon bark water extract (CWE) inhibits angiogenesis, NFκB and activator protein- 1 (AP1) and also modulates the function of CD8+ T cells (Kwon et al., 2009; Lu et al., 2009; Kwon et al., 2010). Vascular endothelial growth factor (VEGF) is viewed as an attractive therapeutic target for the development of novel anticancer agents (Ferrara and Kerbel, 2005) and a variety of approaches to inhibit VEGF activity are currently being assessed in preclinical and clinical trials. However serious side effects have been associated with currently available anti-VEGF agents, limiting their chronic use (Kamba and McDonald, 2007). Recently, polyphenols extracted from various plants, including soy, black raspberries, pomegranate, grape seed extract and green tea, have been found to be potent inhibitors of angiogenesis (Chen et al., 2006; Sartippour et al., 2008).

This is the first study to investigate the anti-proliferative potential of cinnamon bark powder extract (CBPE) from *Cinnamomum cassia* L. family Lauraceae on myeloma cells (RPMI 8226) specifically focusing on its anti-angiogenic and anti-inflammatory properties.

2. Materials and methods

2.1. Reagents and cultureware

Tissue culture plastic ware was purchased from Corning, NY, USA and Axygen Scientific Inc, CA, USA. RPMI-1640 Medium (ATCC® 30-2001™) was procured from American Type Culture Collection (ATCC) Manassas, VA, USA. Penicillin and streptomycin and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylthiazolium bromide (MTT) were obtained from Sigma-Aldrich St. Louis, MO, USA. Gibco® Sera—FBS (US origin) was used. Antibodies were purchased from Abcam®, Cambridge, England, United Kingdom. All other common reagents were procured from Merck Limited (Mumbai, India).

2.2. Cell line and culture

The myeloma cell line (RPMI 8226; ATCC® CCL-155™) was maintained in RPMI-1640 Medium (ATCC® 30-2001™) supplemented with 10% (v/v) FBS (Fetal Bovine Serum) and 1% Penicillin-Streptomycin at 5% CO_2 at 37 °C. ATCC characterized and authenticated the cell line utilizing Short Tandem Repeat (STR) profiling.

2.3. Cinnamon bark powder extract (CBPE)

CPBE is derived from the bark of the *C. cassia* Presl. tree. The water extract of Cinnamon bark was supplied in the form of a brownish powder by PureBulk Inc, Roseburg, USA. The powder was dissolved in culture media to make stock of 10 mg/mL concentration. It was then further diluted with working media for desired concentrations in all experiments (5–100 μg/mL).

2.4. Cell proliferation assay

To investigate the effect of CBPE on RPMI 8226 cell proliferation, cells were seeded in 96-well plates. Various concentrations (5–100 μg/mL) of CBPE were added and the cells were incubated (37 °C, 5% CO_2) for 24 h, at a density of 1×10^5 cells/mL. 20 μL of 5 mg/mL MTT was added and cells were kept in culture condition again for 4 h. 150 μL dimethyl sulfoxide (DMSO) was then added to dissolve the formazan precipitate before absorbance was measured at 570 nm using a microplate reader (Bio-Tek, USA). The cell viability of CBPE treated cells was then obtained by comparing to the control (untreated cells, without CBPE treatment) and the percentage inhibition was calculated (Freshney, 2005).

2.5. Cell cycle analysis

RPMI 8226 cells were cultured in 10% serum medium containing CBPE (72 μg/mL; IC50 value obtained from the MTT assay) for 24, 48 and 72 h. Cells were harvested, washed twice with cold phosphate-buffered saline (PBS) and fixed with precooled 70% ethanol and kept in ice for 1 h. Fifty μg/mL DNase-free RNase A was then added and left for 1 h at 37 °C before the addition of propidium iodide (PI), at a final concentration of 10 μg/mL for DNA staining in the dark at 4 °C until analysis (Moore et al., 1998). For each sample, more than 1×10^4 cells were analyzed using a FACS Canto (BD) Flow cytometer. The distribution of cell cycle stages was determined using BD FACSDiva software© Becton, Dickinson and Company.

2.6. TUNEL assay

RPMI 8226 cells were cultured in 10% serum medium containing CBPE (72 μg/mL) for 24, 48 and 72 h. Cells were harvested, washed thrice with PBS, fixed, permeabilised, labeled with TUNEL reaction mixture and analyzed by flow cytometry for DNA fragmentation as instructed by manufacturer's protocol (In Situ Cell Death Detection Kit, Fluorescein; Roche Applied Science, Mannheim, Germany; Gold et al., 1994).

2.7. Quantitative mRNA expression by real-time reverse transcription PCR

The mRNA levels of angiogenic factors [VEGF, hepatocyte growth factor (HGF), angiopoietin-1 (Ang-1), and angiopoietin-2 (Ang-2)] and cyclooxygenase (COX-1 and COX-2) were analyzed through relative quantitation using ABI 7500 real-time PCR (Applied Biosystems Inc.) before and after treatment with CBPE