Available online at www.sciencedirect.com
ScienceDirect
Phytomedicine 14 (2007) 263–272
Phytomedicine
www.elsevier.de/phymed

Antiproliferative activity and induction of apoptosis in human colon cancer cells treated *in vitro* with constituents of a product derived from *Pistacia lentiscus* L. var. *chia*

K.V. Balan[a], J. Prince[a], Z. Han[a], K. Dimas[b], M. Cladaras[c], J.H. Wyche[a], N.M. Sitaras[d], P. Pantazis[a,b,*]

[a]*Department of Biology, University of Miami, Coral Gables, Miami, FL, USA*
[b]*Laboratory of Pharmacology–Pharmacotechnology, Foundation for Biomedical Research, Academy of Athens, Greece*
[c]*School of Biology, Aristotle University of Thessaloniki, Greece*
[d]*Department of Pharmacology, Medical School, University of Athens, Greece*

Abstract

In this report, we demonstrate that a 50% ethanol extract of the plant-derived product, Chios mastic gum (CMG), contains compounds which inhibit proliferation and induce death of HCT116 human colon cancer cells *in vitro*. CMG-treatment induces cell arrest at G_1, detachment of the cells from the substrate, activation of pro-caspases-8, -9 and -3, and causes several morphological changes typical of apoptosis in cell organelles. These events, furthermore, are time- and dose-dependent, but p53- and p21-independent. Apoptosis induction by CMG is not inhibited in HCT116 cell clones expressing high levels of the anti-apoptotic protein, Bcl-2, or dominant-negative FADD, thereby indicating that CMG induces cell death via a yet-to-be identified pathway, unrelated to the death receptor- and mitochondrion-dependent pathways. The findings presented here suggest that CMG (a) induces an anoikis form of cell death in HCT116 colon cancer cells that includes events associated with caspase-dependent pathways; and (b) might be developed into a chemotherapeutic agent for the treatment of human colon and other cancers.

Keywords: Chios mastic gum; Colon cancer cells; Apoptosis; *Pistacia lentiscus* L. var. *chia*

Introduction

Dietary intake of phytochemicals has been associated with decreased risk of cancer and significant survivability of cancer patients (Ho et al., 2002; Setzer and Setzer, 2003; Weiss and Landauer, 2003). Several plant products contained in foods have exhibited activity against human colon cancer. Buckwheat protein (Brivida et al., 2002), resveratrol analogs (Wolter et al., 2001, 2002), linoleic acid conjugates (Kemp et al., 2003), and green tea extracts (Mueller-Klieser et al., 2002) have exhibited anticancer activity *in vitro* by targeting various molecular and cellular mechanisms, and juice or freeze-dried powder from Brussels sprouts significantly enhanced levels of apoptosis and reduced mitosis in the colonic crypts in an animal model (Smith et al., 2003).

In most cases, anticancer drug treatment results in the activation of the enzymes, caspases, which act effectively in the execution of various forms of cell death. The

*Corresponding author. Department of Biology, University of Miami, Coral Gables, Miami, FL, USA. Tel.: +1 305 284 8135; fax: +1 305 284 8226.
E-mail address: pantazis@bioacademy.gr (P. Pantazis).

0944-7113/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2006.03.009

death receptor-dependent apoptotic pathway is triggered at the cell surface and requires activation of caspase-8, whereas the mitochondrion-dependent pathway is initiated with release of mitochondrial cytochrome *c* into the cytoplasm and requires activation of caspase-9. Subsequently, caspase-8 or -9 can activate caspase-3, which in turn targets and degrades specific and vital cellular proteins, ultimately resulting in nuclear DNA degradation and apoptotic death of the cells (for reviews on caspases, see Krammer and Debatin, 2004; Strasser et al., 2001). There is accumulating evidence of existence of caspase-independent mechanisms of cell death executed by other proteases, however, thus leading to variant forms that may display some or no characteristics of the "classical" apoptotic pathways (Jäättelä, 2004; Lockshin and Zakeri, 2004).

The tumor suppressor protein, p53, functions as a key component of a cellular emergency response system to induce cell growth arrest or apoptosis (El-Deiry et al., 1993; Levine, 1997). Absence of p53 or p53 function markedly attenuates radiation- and drug-induced apoptosis in a variety of murine and human cell systems (reviewed in Eastman, 2004; Scherr, 2004). Further, p53-dependent apoptosis and chemosensitivity may require caspase activation in some cell systems (Schuler et al., 2000; Wu and Ding, 2002). Imbalance in favor of cell survival enables tumor progression and resistance to anticancer drugs. Thus, the anti-apoptotic protein, Bcl-2, can suppress p53-dependent apoptosis in HCT116 human colorectal cancer cells, and silencing of Bcl-2 induces massive p53-dependent apoptosis in absence of genotoxic drugs necessary to activate p53 (Jiang and Milner, 2003).

The plant *Pistacia lentiscus* L. var. *chia* grows particularly and almost exclusively in the South region of Chios Island, Greece, and produces a resin, known as Chios mastic gum (CMG). Only a few constituents have been identified from CMG (Papageorgiou et al., 1997); moreover, there is no report on anti-cancer activities of CMG. In this report, we demonstrate that a 50% ethanol CMG extract induces apoptotic death of human colon cancer cells *in vitro* and this process correlates with, but it is not dependent on caspase activation.

Materials and methods

Chemicals/reagents and preparation of gum extracts

Dry Chios mastic resin (gum) was provided by a producer of this product, Chios Island, Greece. Absolute ethanol was purchased from Florida Distillers (Lake Alfred, FL). Cell culture media, RPMI 1640, heat-inactivated bovine serum, trypsin, and antibiotics solutions were purchased from Mediatech, Inc. (Herdon, VA). Hard-dry CMG resin was pulverized to a fine powder with the aid of a kitchen mixer, and identical amounts of the powder were mixed with equal volumes of de-ionized water (Extract I), 25% ethanol in water (Extract II), 50% ethanol in water (Extract III) or 100% ethanol (Extract IV). The mixtures were continually agitated by vertical rotation for 24 h at room temperature, centrifuged to separate soluble (supernatant) from insoluble (pellet) material, and the soluble material was transferred into polypropylene tubes and stored at room temperature in the dark.

Cells and treatments

The HCT116 human colon cancer cell line expressing p53 (HCT116/p53$^{+/+}$) and a clone lacking p53-expression (HCT116/p53$^{-/-}$) were donated by Dr. Bert Vogelstein (Bunz et al., 1999). A clone of HCT116 cells over-expressing Bcl-2 (HCT116/Bcl-2) was developed in our laboratory. A clone of HCT116 cells transfected with FADD dominant-negative plasmid, and thus not expressing functional FADD (clone HCT116/DN.FADD) was also developed in our laboratory using a plasmid donated by Dr. Andreas Strasser (The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia). All cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated bovine serum and antibiotics. The cell cultures were maintained and propagated in a humidified 37 °C incubator with a 5% CO_2 atmosphere.

Flow cytometry, immunoblotting, and transmission electron microscopy

CMG-induced perturbations in the cell cycle and apoptosis were monitored with the aid of an EPICS XL flow cytometer (BeckmanCoulter, Miami, FL), equipped with the Multicycle AV program for cell cycle analysis (Phoenix Flow Systems, San Diego, CA). The cell samples were treated with RNAse and stained with propidium iodide (PI) immediately prior to analysis. We chose to use the PI methodology over the TUNEL and Annexin-V methodologies for flow cytometry analysis because the TUNEL and Annexin-V methods detect early stages of apoptosis, whereas the PI method detects sub-G_1 cells, that is, cells at late and irreversible apoptotic stages (Pozarowski and Darzynkiewicz, 2004; Span et al., 2002; Tao et al., 2004; Telford et al., 2004; Tuschl and Schwab, 2004; Wilkins et al., 2002). We have used this methodology in previously reported studies (Hu et al., 2003; Pantazis et al., 1993, 2000).

Whole cell extracts were prepared and subjected to immunoblot analysis as described (Chatterjee et al., 2001). The antibodies used in this report recognized human caspase-8 (Sigma–Aldrich, St. Louis, MO);