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p-Methoxycinnamic acid, an active phenylpropanoid induces mitochondrial mediated apoptosis in HCT-116 human colon adenocarcinoma cell line

Sivagami Gunasekaran, Karthikkumar Venkatachalam, Nalini Namasivayam*

Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalai Nagar, Tamil Nadu 608 002, India

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ABSTRACT

Among the eight phytochemicals (dihydrocarveol, sinapic acid, vanillic acid, ethylgallate, myrtenol, transcarveol, p-methoxycinnamic acid, and isoferulic acid) we tested, p-methoxycinnamic acid (p-MCA) [10 μM] showed the most potent in vitro growth inhibition on human colon adenocarcinoma (HCT-116 cells). Antiproliferative activity of p-MCA at 24 h was associated with DNA damage, morphological changes and the results were comparable with doxorubicin. p-MCA induced phosphatidylserine translocation, increased the levels of reactive oxygen species (ROS), thiobarbituric acid reactive substances (TBARS), protein carbonyl content (PCC) and decreased enzymic antioxidant status (SOD, CAT, GPx) in HCT-116. p-MCA treatment increased the percentage of apoptotic cells, decreased the mitochondrial membrane potential and triggered cytochrome C release to cytosol. The induction of apoptosis by p-MCA was accompanied by an increase in caspase 3 and caspase 9 activities, increased expression of Bax and decreased expression of Bcl-2. Thus p-MCA induces mitochondria mediated intrinsic pathway of apoptosis in HCT-116 and has potential for treatment and prevention of colon cancer.

1. Introduction

Colorectal cancer is the second most common cancer and ranks second among cancer related deaths worldwide (Walker et al., 2014). Chemoprevention is a major strategy in cancer prevention, because therapies have not proved effective to date in controlling the high incidence or low survival rate of human colon carcinoma (Wei-Li et al., 2009). Cells from a variety of human malignancies showed decreased ability to undergo apoptosis in response to certain stimuli. An attractive method for cancer chemoprevention or chemotherapy is dietary or pharmaceutical agents that induce death of tumor cells in an effort to overcome proliferation. Apoptosis is a gene-directed form of cell death with well characterized morphological and biochemical features (Min-Hsiung et al., 2007). Apoptotic events are characterized by a series of distinct morphological changes that include blebbing, loss of cell membrane asymmetry and attachment, cell shrinkage, chromatin condensation, and chromosomal DNA fragmentation.

Mitochondrial dysfunction, a hallmark of programmed cell death (apoptosis), produces reactive oxygen species (ROS) that influence numerous cell processes (Boelsterli, 2002). Mitochondria are known to be a major source of intracellular ROS generation and are particularly vulnerable to oxidative stress (Tripathi et al., 2010). ROS may also act as intracellular messengers that are induced by a diverse range of stimuli and that trigger apoptosis. Although ROS have important roles in cell signaling, extended high levels of ROS can cause severe damage to DNA, RNA, and proteins, which eventually lead to cell death via either apoptotic or necrotic mechanisms (Fiers et al., 1999). Moreover during oxidative stress-induced cell death, ROS can target mitochondrial membrane potential (Choi et al., 2008). Apoptotic cell death activates caspases, the major executioners of these processes, initiated either through death receptor (extrinsic) or mitochondrial (intrinsic) pathways (Boatright and Salvesen, 2003; Okada and Mak, 2004; Jin and el-Deiry, 2005). The mitochondrion-mediated pathway begins with the disruption of the mitochondrial membrane potential (MMP) and release of apoptogenic proteins such as cytochrome C into the cytosol. Once in the cytosol, cytochrome C can activate caspase-9, which in turn cleaves and activates the executioner caspase-3 (Yang et al., 1997; Kroemer and Reed, 2000; Mohamad et al., 2005). The caspase–cascade signaling system is regulated by a number of different molecules, such as proteins from the Bcl-2 and the inhibitor of apoptosis protein (IAP) families. The intrinsic pathway is regulated by the Bcl-2 family of proteins which has both anti-apoptotic

* Corresponding author.
E-mail address: nalininam@yahoo.com (N. Namasivayam).

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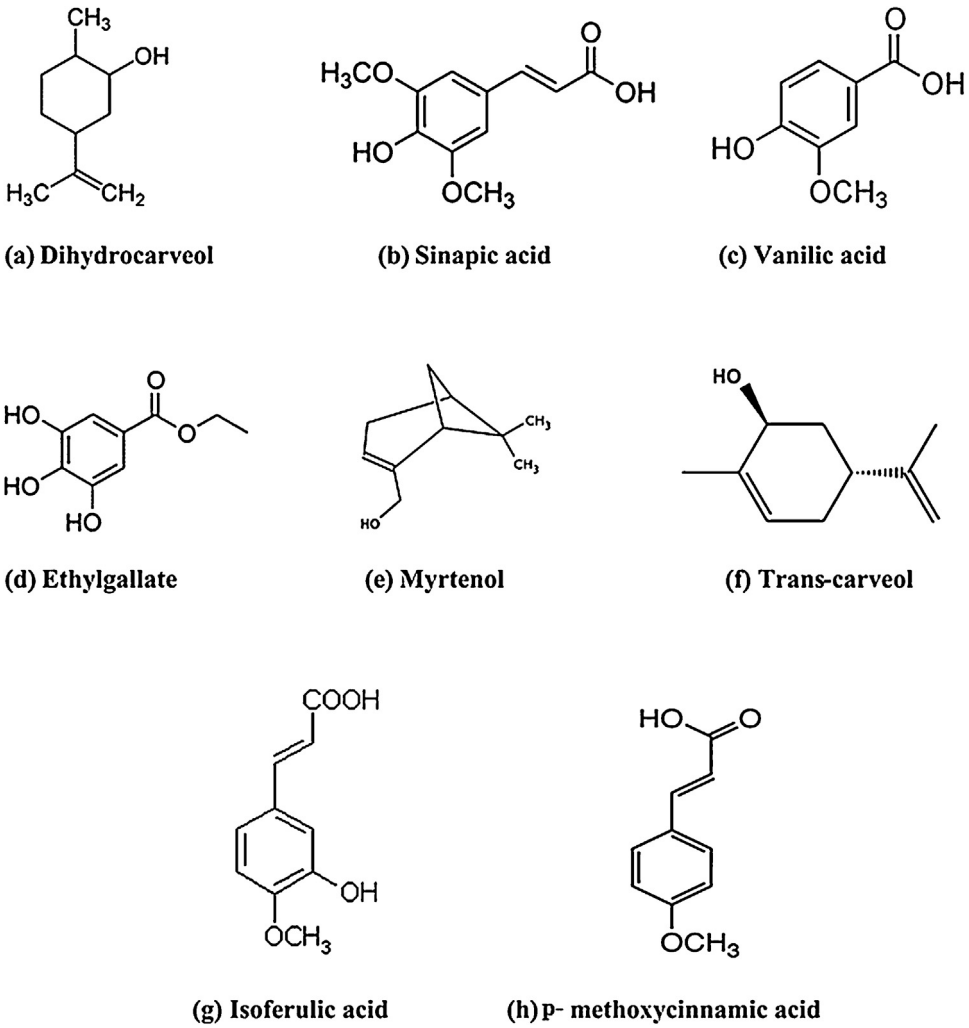

Fig. 1. Chemical structure of phytochemicals screened.

(Bcl-2 and Bcl-xL) and pro-apoptotic (Bax, Bak, and Bid) members. These act on the mitochondrion to prevent or facilitate the release of apoptogenic factors (Nechushtan et al., 2001; Mohamad et al., 2005; Dlugosz et al., 2006; Park et al., 2010).

Phytochemicals from dietary sources have been demonstrated to be potential cancer chemopreventive agents due to their ability to trigger critical pathways to induce cytotoxicity, specifically in cancer cells. Taking into account the use of sinapic acid, vanillic acid, p-methoxycinnamic acid, isoferulic acid, ethylgallate, myrtenol, transcarveol, and dihydrocarveol (Fig. 1) in Ayurvedic medicine for colon cancer treatment, their widespread pharmacological properties and lack of experimental studies on their antiproliferative property, our current investigation was undertaken to screen these phytochemicals for their antiproliferative activity and to elucidate the exact mechanism of action of the most potent phytochemical p-MCA against human colon adenocarcinoma cell line, HCT-116.

2. Material and methods

2.1. Chemicals

p-MCA, doxorubicin (DOX) acridine orange (AO), ethidium bromide (EB), rhodamine123 and Hoechst 33258 were obtained from Sigma–Aldrich Chemicals Co., USA. 3-(4,5-Dimethyl thiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT), trypsin EDTA, phenazinemethosulphate (PMS), nitrobluetetrazolium (NBT), 5,5-dithiobis(2-nitro benzoic acid) (DNTB), reduced glutathione (GSH), low melting agarose, and normal melting agarose were obtained from Himedia Lab Limited, Mumbai, India. All other chemicals were of analytical grade and obtained from SD Fine Chemicals Limited.

2.2. Cell line and maintenance

Three human colon cancer cell lines (HT-29, COLO320 DM, HCT-116) and a normal colonic epithelial cell line (NCM460) were procured from the National Centre for Cell Science (NCCS), Pune, India, and grown as a monolayer in RPMI (Rosewell Park Memorial Institute) 1640 medium with 10% FBS (fetal bovine serum) and 2% antibiotics. Stock cultures were sub-cultured every 7th day after harvesting the cells with trypsin EDTA (ethylene diamine tetra acetate) and then seeding them in tissue culture flask to maintain in an exponential phase.

2.3. Cytotoxicity assay

Growth inhibition by eight phytochemicals was determined on HCT-116, COLO320DM, HT-29 and NCM 460 using MTT assay (Mosmann, 1983). Cells were incubated with 0 or 200 μM of the eight phytochemicals individually for 24 h. After the preliminary screening, p-MCA was chosen for further investigations as it showed the maximum growth inhibition on the used cancer cell