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Sesamol induces mitochondrial apoptosis pathway in HCT116 human colon cancer cells via pro-oxidant effect

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ABSTRACT

Aims: Sesamol lignan is a phenolic compound found in sesame seeds. We investigated the effect of different concentrations of sesamol on oxidative stress in colorectal carcinoma cells (HCT116).
Main methods: Antioxidation *in vitro* was determined from elimination of the DPPH radical, ferric reducing-antioxidant power (FRAP), $O_2^{\bullet -}$, and peroxyl radical scavenging activity. Intracellular $O_2^{\bullet -}$, H_2O_2 and GSH levels were determined by DHE, DCFH-DA, and CMF-DA assay, respectively. Cell viability was detected by neutral red assay. Cell cycle proportion and mode of apoptotic HCT116 cells death was analyzed by flow cytometry. Apoptosis in sesamol-treated HCT116 cells was confirmed by morphological changes in the nuclei using DAPI staining and changes in mitochondrial membrane potential using the $DiOC_6(3)$ assay.
Key findings: Sesamol at both low (0.05 and 0.25 mM) and high (0.5, 2, 5, and 10 mM) concentrations concurrently reduced FRAP reagent and scavenged DPPH$^{\bullet}$, and $O_2^{\bullet -}$. Sesamol at low concentrations scavenged ROO$^{\bullet}$, but ROO$^{\bullet}$-scavenging was decreased at higher concentrations. Sesamol suppressed cell viability via disruption of cell cycle progression at high concentrations (0.5, 1, 2, and 5 mM), thereby causing S-phase arrest and inducing apoptosis—through the production of intracellular $O_2^{\bullet -}$, mitochondrial dysfunction, and DNA fragmentation.
Significance: High concentrations of sesamol induced the mitochondrial apoptosis pathway in human colon cancer HCT116 cells via a pro-oxidant effect.

1. Introduction

Sesamol (Fig. 1) has a wide range of biological actions, including: anti-mutagenesis [1]; antioxidant [2]; suppression of neuro-inflammation [3]; inhibition of atherosclerosis progression [4]; inhibition of NF-κB in platelet aggregation [5,6]; and, reduction of plasma cholesterol and triacylglycerol levels [7]. Its anti-inflammation character was detected as suppression of basal COX-2 transcriptional activity in mice colon cancer (DLD-1 cells) [8]. Black sesame seed extract was found to induce apoptosis in HT-29 colon cancer [9]. Sesamol exerted a chemopreventive effect on various anti-cancer cells by inducing apoptosis, including in: human leukemic cells (Molt-4 and K562) [10,11]; breast cancer cell lines (MCF-7 and T47D) [12]; hepatocellular carcinoma cells (HepG2) [13]; and, MA-10 mouse Leydig tumor cells [14]. Evidence of the non-toxicity of sesamol on cells was shown by its protection of rat thymocytes (normal cell) [10]. Sesamol protected lymphocytes from UVB and γ-radiation [15] and cardiac myoblasts in H9C2 cells from doxorubicin-exposure [16]. In lightening cosmeceutical usage, sesamol inhibited tyrosinase activity with no harmful on melanocytes in SK-MEL-2 cells [17].

Intracellular reactive oxygen species (ROS)—such as superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2)—are produced as byproducts of normal metabolism as well as via activation of ROS-producing enzymes in response to exogenous stimuli. The oxidant defense system modulates the fate of ROS in the cell by superoxide dismutase (SOD) catalyzed $O_2^{\bullet -}$ to produce H_2O_2 and is transformed by glutathione or catalase to H_2O [18]. ROS are associated with cellular proliferation and cell death, depending on the intensity and location of the oxidative burst, and functionality of the antioxidant system [19]. Intracellular accumulation of ROS is toxic [20], as it induces cell necrosis through passive cellular dysfunction in response to the stress. A role for $O_2^{\bullet -}$ in tumor cell sensitivity to cell death was induced by cell surface receptor CD95 (Apo-1/Fas) [21–23]. Apoptosis is an active process involving caspase proteases and apoptogenic factors released from mitochondria, regulated by (a) signals transmitted through cell surface

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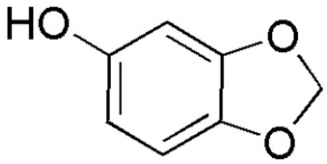

Fig. 1. Sesamol chemical structure.

receptors (i.e., CD95 and tumor necrosis factor receptors [24]), (b) growth factor deprivation [25], (c) exposure to ultraviolet/ionizing radiation [26], and/or (d) treatment with a wide range of chemical including chemotherapeutics [27].

ROS (such as $O_2^{\bullet -}$) can inhibit death signaling at concentrations not exceeding cellular antioxidant defense systems. Notwithstanding the sensitivity of cells to apoptotic stimuli, it also is significantly increased with decreasing intracellular levels of $O_2^{\bullet -}$ [28]. Production of H_2O_2 leads to activation of the effector components of the cell apoptotic death mode; by inducing reduction of the intracellular milieu and ceasing cytosolic pH-generating support [21]. Hence, the critical determinant between survival and apoptotic or necrotic cell death is pH, downstream of ROS production (i.e., $O_2^{\bullet -}$ and H_2O_2 levels).

The role of ROS on tumor cell survival was due to lower SOD activity in tumor cells [29], leading to a pro-oxidant state—as a result of accumulation of intracellular ROS, providing tumor cells a survival advantage over their normal counterparts. The enhancement of tumor cell survival occurs as $O_2^{\bullet -}$ or H_2O_2 function as mitogenic stimuli at low doses [30,31]. The proliferative effect of ROS is due to activation of the Erk1/2 and NF-κB pathways and upregulation of the mRNA levels of cyclins leading to cell cycle progression from the G1 to S phase [31]. Cell survival is favored with a slight increase in intracellular $O_2^{\bullet -}$ that helps maintain pH in the alkaline range. The change of intracellular redox status may either trigger or block apoptotic cell death, depending on the severity of the oxidative stress. Apoptosis induction from exposure to H_2O_2 (0.5 mM) in the M14 melanoma cell line was associated with a significant decrease in the intracellular concentration of $O_2^{\bullet -}$, which in turn was associated with an increase in the reduced GSH/oxidized GSSG ratio of GSH [21]. Increasing intracellular $O_2^{\bullet -}$ can, however, also restrict drug-induced tumor cell death via the CD95 system [28].

Since chemotherapeutic agents are mostly toxic to noncancerous cells, intensive study of natural compounds for chemotherapy—or as complementary or alternative treatments—have been conducted to identify and mitigate side effects. Increases in ROS in cancer cells play important roles in cancer initiation, promotion, progression, invasion, and metastasis. An antioxidant effect in carcinogenesis and tumor growth was reported. This was achieved by both preventing and deceasing intracellular ROS [32]. Pro-oxidant agents also increase the cellular concentrations of ROS to cytotoxic levels, which may induce the selective killing of cancer cells making them therapeutically useful; a single compound can achieve both albeit the combined effect is concentration-dependent [33]. Many compounds have a paradoxical effect on cells including curcumin [34], resveratrol [35], and melatonin [36]. The current study focused on the counterpart pro-oxidant properties of sesamol.

Colorectal cancer is the third most commonly diagnosed cancer globally, after lung and breast cancer. Poor diet has been associated with colorectal cancer, so a strategy for effective chemoprevention or chemotherapy includes non-toxic dietary substances or natural products. Epidemiological studies indicate that the incidence of some cancers in Asia (including colon cancer) is lower than other regions, perhaps because Asian food is high in plant-derived polyphenols [37]. Several studies on phenolic compounds—phyto-constituents commonly found in plant and fruits—reported some value for colon cancer therapy via the apoptosis induction mechanism [38–40]. Other dietary compounds such as sesamol—a monophenolic acid—may, therefore, play a role(s) in colon cancer therapy.

The current study focused on the effect of sesamol on oxidative stress although not ignoring its pro-oxidant activity. Anticancer action was evaluated with respect to concentrations in colorectal carcinoma cells (HCT116); the antioxidant effect of sesamol at low concentrations and the pro-oxidant effect at high concentrations in human colorectal carcinoma cells HCT116 were represented by intracellular $O_2^{\bullet -}$ generation. Here, we report the paradoxical effect of sesamol: apoptosis induction in human colorectal carcinoma based on its pro-oxidant effect.

2. Materials and methods

2.1. Chemical and reagents

Dulbecco's modified Eagle's medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium, and 0.25% trypsin-EDTA (1×) were from Gibco (Barcelona, Spain). Sesamol is the methylene ether of oxyhydroquinone. Its chemical name is 5-hydroxy-1,3-benzodioxole or 3,4-(methylenedioxy) phenol. Sesamol (PubChem CID: 68289) (98% purity) was from Spectrum (New Brunswick, NJ, USA). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-*s*-triazine (TPTZ), sodium carbonate, 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH), fluorescein, 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), and dihydroethidium (DHE), 3,3′-Dihexyloxacarbocyanine iodide ($DiOC_6$) were from Sigma-Aldrich (St. Louis, MO, USA). 5-Chloromethylfluorescein diacetate (CMF-DA) was from Molecular Probes (Eugene, OR, USA). The FITC-conjugated annexin V and PI Kit were from eBioscience (San Diego, CA, USA). PI was purchased from Sigma-Aldrich Ltd. (Gillingham, Dorset, UK). 4′-6-Diamidino-2-phenylindole (DAPI) was purchased from Sigma–Aldrich Chemie (Steinheim, Germany). Ferric chloride ($FeCl_3 \cdot 6H_2O$) and ferrous sulfate were from Hazardons (Taren Point, NSW, Australia). DMSO was from Lab-Scan, Analytical Science (Dublin, Ireland) while the other reagents were bought from standard commercial suppliers. Cisplatin was from Boryung Pharmaceutical (Kyunggi-do, Korea). Neutral red (NR) was from Sigma Chemical Co. (St. Louis, MO, USA). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was from Aldrich Chemistry (St. Louis, MO, USA).

2.2. Antioxidation in vitro

2.2.1. Reducing power based on FRAP assay

The FRAP assay evaluates the ferric reducing antioxidant power—the ability of an antioxidant to reduce the Fe^{3+}-TPTZ complex to Fe^{2+}-TPTZ [17,41]. Various 1 mL concentrations of each test compound (sesamol, standard ferrous sulfate, or standard antioxidant Trolox) were added to a conical tube. FRAP reagent (1 mL) was added to each tube with 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyls-triazine (TPTZ) solution and 20 mM $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio. Two hundred microliters of the reaction mixture were pipetted into each well of a 96-well plate and incubated for 10 min. The absorbance of the colored product (ferrous tripyridyltriazine complex) was measured at 593 nm against a blank with deionized water using a microplate reader (Sunrise, Tecan Group, Mannedorf, Switzerland). The standard curve was linear between 2.5 and 40 μM Trolox ($y = 0.0197x + 0.0389$; $R^2 = 0.9974$). Results are expressed in μM Trolox.

2.2.2. Radical scavenging activity based on DPPH assay

Radical scavenging activity was determined using DPPH as a free radical [42]. DPPH in absolute ethanol (150 μL, final concentration of 0.25 mM) was added to 50 μL of the test compounds (sesamol or Trolox) at different concentrations. Then the reaction mixture was maintained for 30 min in the dark at room temperature. Absorbance was measured at 515 nm against an ethanol blank. The free radical scavenging capacity of sesamol was expressed as an equivalent of Trolox. The experiment was done in triplicate. The results were calculated and expressed as a micromoles curve of Trolox equivalents (TE) using the calibration