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Fish oil prevents colon cancer by modulation of structure and function of mitochondria

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ABSTRACT

Cancer cells are more susceptible to metabolic perturbations due to impaired electron transport chain (ETC) that promote uncontrolled proliferation. Mitochondria play a pivotal role in bioenergetics and apoptosis, hence are considered as a promising target in tumor cell eradication. Therefore, the present study is designed to elucidate chemopreventive action of fish oil (FO) in combination with corn oil (CO) on mitochondria in colorectal cancer (CRC). Male Wistar rats were divided into groups depending on dietary regimen—Control group, FO + CO(1:1) and FO + CO(2.5:1). These groups were further subdivided depending on whether these received a weekly intraperitoneal injection of ethylenediamine tetra-acetic acid (EDTA) or *N,N*-dimethylhydrazine dihydrochloride (DMH) for a period of 4 weeks. The animals sacrificed 48 h and 16 weeks after EDTA/DMH treatment constituted initiation and post-initiation phase respectively. The structural and functional alterations in mitochondria were evaluated using transmission electron microscopy (TEM) and by assaying electron transport chain (ETC) enzymes. Mitochondrial lipid composition and cholesterol levels were also assessed. DMH treatment led to mitochondrial degeneration, disrupted cristae and a significant decrease in ETC complexes suggestive of metabolic reprogramming. Moreover, an increase in cholesterol and cardiolipin (CL) levels in post-initiation phase led to evasion of apoptosis. FO in both the ratios resulted in stabilization and increase in number of mitochondria, however, FO + CO(2.5:1) + DMH group also exhibited mitophagy and crystolysis alongwith altered dynamics in ETC which facilitated apoptosis. It also decreased cholesterol and CL levels to increase apoptosis. Fish oil targets mitochondria in a dose dependent manner that augments apoptosis and hence attenuates carcinogenesis.

1. Background

Colorectal cancer (CRC) is a major public health problem worldwide and the second most common malignancy in the Western world [1]. CRC is a multistep process that involves an accumulation of mutations in tumor suppressor genes and oncogenes [2] which disturbs the cell homeostasis between proliferation and apoptosis. The incidence of CRC has been linked with lifestyle factors such as lack of exercise, smoking and dietary factors [3]. Epidemiologic, clinical and animal based studies indicate that the dietary fat may modulate development of colon cancer particularly the amount and types of essential polyunsaturated fatty acids (PUFAs) [4–6]. Recent reports suggest that a high dietary intake of n-6 PUFAs with a low intake of n-3 PUFAs lead to an increase in risk for breast, colon and prostate cancer [7,8]. In fact, the ratio of n-6 to n-3 PUFAs is considered to be an important determining factor of health. The previous study conducted in our laboratory has observed that 2.5:1 ratio of fish oil (FO) and corn oil (CO) has better chemopreventive efficacy against colorectal carcinogenesis through multiple mechanisms [9–11].

Mitochondria play a central role in carcinogenesis by regulating energy homeostasis as well as apoptosis. The enhanced resistance to apoptosis and metabolic reprogramming are the two prominent features of cancer cell Thus, targeting mitochondrial energy production and apoptotic pathways can be an attractive lead in therapeutic strategies for cancer abrogation [12]. It has already been documented that the structure and function of mitochondria in cancer cells are highly distinct from the normal cells. In the present study, we have determined the effect of differential ratios

Abbreviations: CRC, colorectal cancer; PUFAs, polyunsaturated fatty acids; ETC, electron transport chain; FO, fish oil; CO, corn oil; ROS, reactive oxygen species; CL, cardiolipin; DMH, *N,N*-dimethylhydrazine dihydrochloride; ATP, adenosine triphosphate; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; EDTA, ethylenediamine tetra-acetic acid; TEM, transmission electron microscope; HBSS, Hank's balanced salt solution; DTT, dithiothreitol.

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of FO and CO on mitochondrial structure and function to validate the effectiveness of the treatment.

The lipid composition of the mitochondrial membrane control its fluidity, membrane potential and thereby cell death [13]. The two important lipids of mitochondrial membrane are cholesterol and cardiolipin (CL). Cholesterol is an important component of biological membranes which not only determines its physical properties but also plays an important role in regulation of multiple signaling pathways [14]. CL, on the other hand, keeps cytochrome *c* anchored to the mitochondrial inner membrane and thereby, regulates its release from mitochondria [13]. The peroxidation of CL due to increase oxidative stress would lead to detachment of cytochrome *c* from the mitochondrial membrane and its release in cytosol to induce apoptosis. Emerging data suggests that the balance of mitochondrial cholesterol to (peroxidised) CL regulates mitochondrial membrane properties and permeabilisation and thus acts as a rheostat in cell death [14]. As n-3 PUFAs present in FO may get incorporated into membrane phospholipids, it is imperative to determine its effect on mitochondria in detail.

Therefore, the goal of the present study was to delineate the structural and functional impact on mitochondria by dietary supplementation of fish oil in experimental colon carcinogenesis.

2. Materials and methods

2.1. Chemicals

N,*N*-Dimethylhydrazine dihydrochloride (DMH), adenosine triphosphate (ATP), glycyl glycine and cardiolipin were obtained from Sigma Chemical Company (St. Louis, USA). Fish oil under the brandname Maxepa was obtained from Merck Chemicals (Goa, India). It contains 180 mg/ml eicosapentaenoic acid (EPA) and 120 mg/ml docosahexaenoic acid (DHA) as per manufacturer's instructions. Corn oil was obtained from Sigma chemical company (St. Louis, USA). It contains 50% of n-6, 1.3% of n-3 and 26.4% of n-9 PUFAs. The mineral mixture (Agrimin) was obtained from Virbac Animal health India Pvt. Ltd. (Mumbai, India). All other chemicals used in the study were of analytical grade.

2.2. Experimental model

Male Wistar rats weighing 100–150 g were obtained from Central Animal House, Panjab University, Chandigarh. Animals were acclimatized for 7–10 days before inclusion in the study. The experimental protocols were approved by the Institutional Ethics Committee and conducted according to Indian National Science Academy Guidelines for the use and care of experimental animals.

2.3. Diet

The animals were randomly divided into the different groups and were maintained on experimental diet for four weeks. The diets were prepared on the basis of the American Institute of Nutrition standard reference diet AIN-76A (Committee on Laboratory Animal Diets, 1978). The composition of diet is documented in Table 1. The diets were adjusted so that animals in all the dietary groups would consume same amount of calories [6–8]. In the control group, soya bean oil was used to maintain the concentration of essential fatty acids. The animals maintained on FO + CO(1:1) and FO + CO(2.5:1) derives 35% of total calories from fat. The fat energy ratio for n-3 PUFAs and n-6 PUFAs was 5.28% and 14% in FO + CO(1:1) diet and the corresponding values in FO + CO (2.5:1) diet were 10% and 7.11%; and n-3/n-6 PUFA ratio in these diets was 1:2 and 1.26:1, respectively.

Experimental design: The animals (n = 112) were divided into the following groups depending on dietary regimen.

A. Control group: These animals received purified diet and a weekly intraperitoneal (i.p.) injection of 1 mM ethylenediamine tetra-acetic acid (EDTA pH 6.5, a vehicle of DMH) for a period of 4 weeks.
B. DMH group: Rats were given purified diet and administered a weekly i.p. injection of DMH (20 mg/kg body weight) for a period of 4 weeks.
C. FO + CO(1:1) group: The animals received modified diet supplemented with 1:1 ratios of fish oil and corn oil. The animals were further subdivided into FO + CO(1:1) + EDTA and FO + CO(1:1) + DMH groups depending on whether they received EDTA or DMH as per the protocol.
D. FO + CO(2.5:1) group: Rats received modified diet supplemented with 2.5:1 ratios of fish oil and corn oil. Rats were subdivided into FO + CO(2.5:1) + EDTA and FO + CO (2.5:1) + DMH groups based on the treatment given i.e. EDTA or DMH.

The initiation phase study comprised of animals sacrificed 48 h after EDTA/DMH injections and the animals kept further for 12 weeks after the treatment regimen constituted the post-initiation phase study. All the animals were sacrificed and samples were processed for further structural or functional studies.

2.4. Ultrastructural studies

For the transmission electron microscope (TEM) analyses, distal part of colon was fixed with 2.5% glutaradehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 h at 4 °C. They were washed with phosphate-buffered saline solution (PBS, pH 7.4) and fixed with 1% osmium tetraoxide for 2 h at 4 °C as the secondary fixative. After washing, they were embedded in CY 212 Araldite and cut with a Leica EM FCS (Vienna-Austria) ultramicrotome. One micrometer semi-thin sections were stained by toludine blue −Borax solution to select the region of interest in different treatment samples. Once the area of interest was selected, the ultrathin sections were made and double stained with uranyl acetate and lead acetate. These ultrathin sections were finally

Table 1
Composition of synthetic diet.

Diet content (%)	Group I Diet content (%) synthetic diet	Group II Diet content (%) modified synthetic diet	Group III Diet content (%) modified synthetic diet
Casein	20.00	23.50	23.50
Fat	5.00	5 + 7.5 corn oil + 7.5 fish oil	5 + 4.3 corn oil + 10.7 fish oil
Sucrose	65.00	44.72	44.72
Fiber	5.00	5.90	5.90
Mineral mix	3.80	4.46	4.46
Vitamins mix	1.00	1.18	1.18
Choline chloride	0.20	0.24	0.24