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Anti-proliferative effects of a new docosapentaenoic acid monoacylglyceride in colorectal carcinoma cells

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ABSTRACT

N−3 polyunsaturated fatty acids (*n*−3 PUFAs) have been shown to inhibit the induction and progression of many tumor types. However, the anticancer effect of *n*−3 PUFA monoglyceride on colorectal cancer has yet to be assessed. The aim of the present study was to determine the anti-tumorigenic effects of docosahexaenoic acid monoglyceride (MAG-DHA), eicosapentaenoic acid monoglyceride (MAG-EPA) and docosapentaenoic acid (22:5*n*−3) monoglyceride (MAG-DPA) in colorectal carcinoma cells. Our results demonstrate that MAG-DHA, MAG-EPA and MAG-DPA all decreased cell proliferation and induced apoptosis in HCT116 cells, with MAG-DPA having the higher anti-proliferative and pro-apoptotic effects *in vitro*. In a HCT116 xenograft mouse model, oral administration of MAG-DPA significantly inhibited tumor growth. Furthermore, MAG-DPA treatments decreased NFκB activation leading to a reduction in Bcl-2, CyclinD1, c-myc, COX-2, MMP9 and VEGF expression levels in tumor tissue sections. Altogether, these data provide new evidence regarding the mode of action of MAG-DPA in colorectal cancer cells.

1. Introduction

Colorectal cancer (CRC) is a major health problem in the more developed countries, with at least 1 million new cases per year diagnosed worldwide and is the second leading cause of death by cancer [1]. Despite the emergence of new targeted agents and the use of various therapeutic combinations, none of the treatment options available is curative in patients with advanced CRC [2].

N−3 polyunsaturated fatty acids (*n*−3 PUFAs) are essential fatty acids necessary for human health and are found primarily in coldwater fish in the form of eicosapentaenoic acid (EPA; 20:5*n*−3) and docosahexaenoic acid (DHA; 22:6*n*−3) [3]. Several epidemiologic and clinical studies have shown that *n*−3 PUFAs such as DHA and EPA have beneficial effects in the treatment of a wide variety of pathologies, ranging from autoimmune and inflammatory diseases to neurological disorders and, notably, several types of malignancies including breast, ovarian, prostate and CRC [4–8]. Moreover, diets rich in fish-oil are protective against chemically-induced colon cancer in animal models [9,10] while high fish consumption in humans appears to be chemopreventive [11]. Cyclooxygenase (Cox-2) inhibition has been suggested to mediate the anticarcinogenic effects of DHA and EPA on colorectal cancer [12]. *N*−3 PUFA competes with *n*−6 PUFA for desaturases which metabolize the former into longer fatty acid chains. Hence, increasing the dietary intake of *n*−3 PUFA decreases the desaturation of the *n*−6 PUFA linoleic acid and subsequent formation of arachidonic acid (AA) [13].

EPA and DHA also have the capacity to induce colonocyte apoptosis in rodent models of colorectal carcinogenic induction fed with fish oil as the source of fat [10,14] as well as in human colorectal adenocarcinoma cells [15,16]. However the exact mechanism by which *n*−3 PUFAs induce apoptosis in colorectal cancer is still largely unknown.

Furthermore, very little is known regarding the physiological effects of docosapentaenoic acid (DPA; 22:5*n*−3), an *n*−3 PUFA found in most fish and marine foods [17]. *N*−3 DPA is an elongated metabolite of EPA and is an intermediary product between EPA and DHA. Although current literature on *n*−3 DPA is scant, available data suggest that DPA displays beneficial health effects. For example, when compared with either EPA or DHA, DPA was found to be the most potent inhibitor of platelet aggregation in rabbit platelets by inhibiting cyclooxygenase-1 which is required for the synthesis of TXA2 [18]. In human subjects, DPA was also found to be as effective as EPA and DHA in inhibiting *ex vivo* platelet aggregation in female subjects [18]. Moreover, DPA has been shown to suppress the expression of lipogenic genes; these actions may be due, in part, to the ability of DPA to also induce the expression of peroxisome proliferator-activated receptor (PPARα) [19,20]. In addition, DPA is involved in the reduction

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of expression of inflammatory genes such as tumor necrosis factor (TNFα) in cell culture models [21]. However, to our knowledge, the effects of DPA on proliferation and survival of tumor cells have never been tested. A short term clinical study using highly purified DPA demonstrated that 7-day supplementation with DPA significantly increased the proportions of DPA in plasma phospholipids (PL) and triacylglycerol (TAG) fractions [22,23]. Moreover it was shown that DPA supplementation also significantly increased the proportions of EPA in TAG and cholesterol ester fractions as well as that of DHA in the TAG fraction [22,23]. Collectively, these studies suggest that DPA may provide an additional source of beneficial long chain $n-3$ PUFA.

$N-3$ PUFA formulations serve as the primary tool to obtain an efficient dose of $n-3$ PUFA for providing potentially beneficial effects on biological and cellular functions. To date, $n-3$ PUFA supplements are commercially available in various formulations and chemical binding form, such as free fatty acids, ethyl esters, triacylglycerides and phospholipids. Recently, Schuchardt et al. [24], provides an overview of the influence of chemical binding form (free fatty acids, ethyl esters, triacylglycerides and phospholipids) on bioavailability of omega-3 supplements. They concluded that triacylglycerides bound form can be used more effectively than ethyl esters form. However, no data have conclusively demonstrated the increased bioavailability of $n-3$ PUFA phospholipid supplements, especially found in krill oil, when compared with other formulations. Moreover, Dyerberg et al. [25] also concluded to the better bioavailability of triacylglycerides (especially re-esterified triacylglycerides) compared to ethyl esters and free fatty acids. The superiority of re-esterified triacylglycerides over natural triacylglycerides can be explained by the presence of diglycerides and monoglycerides in the re-esterified form. Cruz-Hernandez et al. [26] have shown that omega-3 monoglycerides did not need pancreatic lipase to be absorbed unlike omega-3 triacylglycerides who need to be hydrolyzed in free fatty acid and monoglycerides by pancreatic lipase before intestinal absorption. However, the effect of $n-3$ PUFA monoglycerides is much less characterized on biological and cellular functions. On the basis of these observations, our group has synthesized new $n-3$ PUFA monoacylglycerides derivatives in order to determine their increased bioavailability and mode of action *in vitro* and *in vivo*. The aim of the present study was to investigate and compare the effect of $n-3$ PUFA in free fatty acid or monoglycerides forms on human colorectal cancer cells and, in particular, the effect of MAG-DPA on tumor growth and NFκB activation pathway.

2. Materials and methods

2.1. Synthesis of $n-3$ PUFA monoglycerides

MAG-DPA, MAG-DHA and MAG-EPA were synthesized as previously described using highly purified corresponding ethyl ester as starting material. In the resulting molecule, DPA/DHA/EPA is attached at the s$n-1$ position of glycerol [27,28].

2.2. DPA metabolites

7,17-dhydroxy-DPA was obtained from Cayman Chemical and 17-hydroxy-DPA was synthesized according to Adcock [29].

2.3. Cell line and culture

Human HCT116 colorectal adenocarcinoma cells were obtained from the American Type Culture Collection (ATCC). HCT116 cells were maintained in McCoy′s 5A medium (Wisent, St-Bruno, QC, Canada) containing 10% FBS, 5 mM HEPES and 10 units/ml penicillin, 100 μg/ml streptomycin. Cells were grown in a 5% CO_2 incubator at 37 °C. Cells were used between passage 3 to 6 for all conditions and assays tested in this study. Cells were untreated or treated with MAG-ω3 at the indicated concentrations. All saturated and unsaturated monoglycerides, as well as free fatty acids tested was dissolved in 100% ethanol and diluted to a final concentration of 0.1% ethanol. Cell proliferation analyses were performed using the BrdU cell proliferation assay kit according to the manufacturer′s instructions (New England BioLabs, Pickering, ON, Canada). This kit detects the level of 5-bromo-2′-deoxyuridine (BrdU) incorporated into cellular DNA during cell proliferation using an anti-BrdU antibody. Apoptosis analyses were performed using (1) a terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) assay in accordance with the manufacturer′s instructions (EMD Millipore, Bellerica, MA, USA) and (2) a cleaved caspase-3 ELISA assay according to the manufacturer′s instructions (New England BioLabs, Pickering, ON, Canada) [30].

2.4. In vivo tumor xenograft experiments

Female nu/nu nude mice were obtained from Charles River Laboratories (Montreal, QC, Canada). All studies involving mice were approved by the institutional Animal Care Committee (Protocol: # 237-10). Human HCT116 xenografts were established in 4-week-old nude mice. Mice were subcutaneously inoculated with 0.2 ml of 1×10^6 HCT116 cells in McCoy′s 5A on the right flank. After formation of 100 mm^3 tumors, mice were randomly assigned into 2 groups, control (untreated) and MAG-DPA-treated ($n=6$ per group). MAG-DPA was administrated *per os* (618 mg/kg) [31] daily following cell inoculation. Tumor volumes (V) were calculated using the formula: $V=(a \times b^2)/2$, where "a" is the largest superficial diameter and "b" the smallest. CD31 staining was performed by immunohistochemistry on tumor tissue sections derived from control and MAG-DPA-treated mice. Western blot analyses using specific antibodies against phosphorylated forms of p65 NFκB, IκBα, COX2, CyclinD1, c-myc, Bcl2, VEGF, MMP9 and β-actin were performed on tumor homogenates derived from control and MAG-DPA-treated mice [30]. Apoptosis analyses were performed using a terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) assay previously described by Morin et al. [30] and according to the manufacturer′s instructions (EMD Millipore, Bellerica, MA, USA). Cleaved caspase-3 ELISA assay was performed on tumor homogenate derived from control and treated mice according to the manufacturer′s instructions (New England BioLabs, Pickering, ON, Canada).

2.5. Fatty acid composition of plasma and tumor tissues using high-performance liquid chromatography coupled to mass spectrometry

Plasma and tumor tissue fatty acid composition was measured using a high-performance liquid chromatography (HPLC)-mass spectrometry (MS) method [32] modified to enable determination of complete fatty acid composition. Briefly, 5 μl of KOH solution (9 N) were added to a 25 μl aliquot of plasma or homogenized tumor tissue samples and heated at 65 °C for 45 min. After cooling the samples to room temperature, 975 μl of extraction solution [150 mM ammonium formate buffer pH=3.0/acetonitrile (25:75 v/v)] were added and the samples were vortexed for 15 s and centrifuged ($12{,}000 \times g$) for 5 min. The resulting supernatant was transferred to a HPLC vial and 5 μl were injected onto an HPLC column. Analyses were performed using an Agilent 1200 series HPLC system at room temperature with a reversed-phase column (Zorbax, C8, 50 × 2.1 mm, 3.5 μm, Agilent). The binary gradient mobile phase [mobile phase A (5 mM ammonium acetate) and mobile phase B (acetonitrile with 0.01% formic acid)] were pumped at a flow rate of 1.0 ml/min using the following gradient: 0 min. (65% B), 1.5 min (65% B), 1.7 min (99% B), 2.9 min (99% B), 2.95 min (65% B) and 3.5 min (65% B). Run time was 3.5 min