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ORIGINAL ARTICLE

Polyunsaturated Fatty Acids Differentially Modulate Cell Proliferation and Endocannabinoid System in Two Human Cancer Lines

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Background and Aims. Evidence suggests that quantity and quality of dietary polyunsaturated fatty acids (PUFAs) play a role in the development of cancer. However, the mechanisms involved in this interaction(s) are not clear. Endocannabinoids are lipid metabolites known to have growth modulatory actions. We studied the effect of supplementation with PUFAs ω-6 and ω-3 (essential fatty acids, EFAs), saturated and monounsaturated fatty acids (non-EFAs) on the growth of tumor cells and modifications in their endocannabinoid content.

Methods. Cell cultures of human glioblastoma (T98G) and breast cancer (MCF7) were supplemented with 50 or 100 mmol EFAs and non-EFAs for 72 h. Cell proliferation was then determined by MTT, anandamide (AEA) levels by HPLC, total fatty acids profiles by GLC, CB1 receptor expression by WB and FAAH activity by spectrophotometric method.

Results. Fatty acids profile reflected the incorporation of the lipids supplemented in each assay. Arachidonic acid (EFA ω-6) supplementation increased AEA levels and inhibited the growth of T98G, whereas palmitic acid (non-EFA) enhanced their proliferation. In breast cancer (MCF7) cells, eicosapentaenoic acid (EFA ω-3) reduced and oleic acid (non-EFA) enhanced their proliferation. CB1 expression was higher in T98G and no differences were observed in FAAH activity.

Conclusions. The growth of tumor cells can be differentially modulated by fatty acids and, at least in part, can be attributed to their ability to act on the components of the endocannabinoid system.

Key Words: Cell proliferation, Endocannabinoid system, Anandamide, Human glioblastoma cells, Human breast cancer cells, Essential fatty acids.

Introduction

Clinical and experimental trials indicated some correlation between quantity and quality of dietary fat intake and the incidence and growth of cancer (1,2). Epidemiologic studies reported a positive correlation with saturated dietary fat intake with an increase in cancer risk for breast, colon, bladder and prostate (1–5). On the other hand, increased consumption of ω-3 fatty acids (FA) was found to be related to a low incidence of cancer (1) and the same was observed for high dietary intake of essential fatty acids (EFA) (3,4). Data from studies performed in animal tumor models and experimental treatments of humans show that ω-6 PUFAs can inhibit cell growth and/or promote cell death in central nervous system (CNS) tumors (5–8). The inhibitory effects of PUFAs may be linked to their ability to serve as precursors to several bioactive lipids that have anti-tumor activities. Some of these bioactive lipids derived

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mainly from ω-6 PUFAs are eicosanoids and endocannabinoids (EC) (9).

The relative proportions and quality of PUFAs in cell membranes as well as cell type are the primary factors that determine the type and quantity of eicosanoids and EC that will be formed in a given situation. Intriguingly, the hydrolytic release of ω-3 and ω-6 PUFAs from phospholipids appears to occur indiscriminately (6). Since the main PUFA substrate for EC in cell membrane is AA (20:4 ω6), most of the ligands are endogenous AA-derived lipids. These ECs are released in the brain and many other tissues and have been implicated in a wide array of physiological and pathological processes including cancer (10−15). The two major endogenous cannabinoid ligands are arachidonoylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG).

The biological effects of EC are due to their binding and activation of the cannabinoid receptors (CBR), called CB1 and CB2, a family of transmembrane G-protein-coupled receptors, densely distributed throughout the autonomic and central nervous system (CNS), immune system and other human tissue (11). Binding of CB1 or CB2 receptors to endogenous ligands can cause excitation or inhibition of certain cellular activities, depending on the enzyme cascades linked to the receptors (12,13). It is likely that EC arising from other PUFAs (other than AA) or even monounsaturated FAs may have different activities, although this has not yet been fully explored.

EC are not stored as in the case of conventional water-soluble neurotransmitters, but are rather rapidly synthesized from PUFAs of the cell membrane (9). Once extracellularly released by a putative EC transporter, they are quickly degraded by fatty acid amide hydrolase (FAAH), which cleaves AEA and 2-AG into AA and ethanolamide and into AA and glycerol, respectively (16).Thus, EC are akin to eicosanoids and are very short-lived substances, a property very useful for regulatory mechanisms in almost all body tissues (9,12).

In vitro and *in vivo* stimulation of CBR by EC ligand influence intracellular events that play a significant role in the proliferation and apoptosis of a wide variety of cancer cells, thereby leading to antitumor effects (10,17−19). Studies in mammals (rat, mouse and pigs) showed that dietary PUFAs influence tissue and cellular concentration of EC (20−23). These data indicate that dietary PUFAs can alter the levels and types of EC to a significant extent mainly due to the availability and release of the precursor AA or other PUFAs from the membrane phospholipids (12). Evidence shows that high availability of different kinds of FA could have pro- or anti-tumor effects and these effects could at least be partially mediated by EC.

Human tumor cell lines with different origins have been reported that express CBR (19) as glioblastoma T98G, an astrocytic-derived line and human breast cancer MCF7, an epidermal ductal strain. The level of expression of receptors, agonists and related enzymes in these different cell lines may lead to diverse physiological responses after activation of the ECS.

Our previous epidemiological and experimental studies revealed the modulatory effects of FA, particularly PUFAs, on cancer process (2,3,24−27). In an extension of these studies, we evaluated supplementation of EFAs (precursors of EC metabolites) and non-EFAs to two human tumors cell lines with different origins, physiology, and metabolic responses. Data obtained confirm that the kind of FA used differentially affects cell growth and viability *in vitro*. This effect could be due, at least in part, to ECS activation in T98G cells, whereas in the MCF7 line the observed response was independent of endocannabinoids.

Materials and Experimental Procedures

The cell lines were purchased from American Type Culture Collections (ATCC, Rockville, MD). Free and methyl esters of fatty acids were obtained from Nu Check (USA). Cell culture medium was obtained from Gibco (USA). Decanoyl p-nitroaniline, URB597, Rimonabant (SR141716), MTT kit and Anandamide standards were purchased from Cayman Chemicals (USA). Antibodies and Western blot molecular weight standards were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and Sigma-Aldrich (St. Louis, MO). Solvents and other chemicals of analytical grade were obtained from Anedra (Argentina) and Sigma-Aldrich.

Cell Culture

Human glioblastoma T98G (ATCC CRL-1690) and human breast cancer MCF7 (ATCCHTB-22) were cultured in 75 cm^2 flasks or 96-well plates (for MTT essays) in DMEM medium with 10% fetal bovine serum (FBS), 100 IU/mL penicillin G and 40 μg/mL gentamycin sulfate and incubated at 37°C in a 5% CO_2 atmosphere. Twenty four hours after seeding, cells were supplemented with AA, EPA, oleic acid (OA) and palmitic acid (PA). All FA were dissolved in ethanol at 50 or 100 mmol concentration (final ethanol concentration was $<0.1\%$). The FAAH specific inhibitor URB597 (5 mmol) and CB1 agonist Rimobanant® (50 mmol) were added in DMSO (final concentration $<$ 0.05%). Controls were treated with ethanol and DMSO at the same concentration as experimental treatments. Cells were cultured for 72 h with and without fatty acids and were harvested for various analyses (26). FA concentrations and culture time used were selected after exploratory dose-time assays (data not shown).

Fatty Acid Determination

The fatty acid profile of the cells under different experimental conditions was determined. Cell lipids were extracted