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Fish oil reduces the proliferation of cells cultured from human breast and colorectal tumours: An *in vitro* study

Sandro J.R. Bonatto [a,*], *Adriana Aya Yamaguchi* [a], *Isabela Coelho* [b], *Everson Araújo Nunes* [b], *Fabíola Iagher* [b], *Jorge Itsuo Fukushima* [c], *Luciano V. Saboia* [c], *Luiz Claudio Fernandes* [b]

[a] *Instituto de Pesquisa Pelé Pequeno Príncipe, Curitiba, PR 80250-200, Brazil*

[b] *Department of Physiology, Biological Sciences Building, Federal University of Paraná, Curitiba, PR 81540-990, Brazil*

[c] *Angelina Caron Hospital, Campina Grande do Sul, PR 83430-000, Brazil*

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ABSTRACT

Diet plays a key role in the aetiology of cancer. Some studies have shown that diets rich in n-3 polyunsaturated fatty acids (n-3 PUFA) reduce tumour growth. This study aimed to investigate the effects of fish oil, rich in n-3 PUFA, in cell proliferation and death of cells from breast and colorectal tumours. In the presence of 1:5 (v/v) dilution of fish oil, 1.8-fold reduction in cell proliferation ($p < 0.05$) was observed. In addition, the presence of fish oil at a 1:5 proportion led to 1.6-fold increase in the rate of apoptosis, 6.7-fold increase in hydroperoxides, and 5-fold reduction in necrosis ($p < 0.0001$).

1. Introduction

Although advances in treatment have improved survival and reduced mortality rates, cancer remains the leading cause of death among men and women under 80 years of age worldwide (Jemal et al., 2009; Klein, 2009). Cancer originates from mutant DNA sequences that reroute the crucial pathways controlling tissue homeostasis, cell survival, and cell death (Visser, Eichten, & Coussens, 2006). Because carcinogenesis is dependent on the sequential accumulation of multiple rare genetic or epigenetic events, tumours have a clonal nature. However, strong evidence indicates that the extent of cells' continuing evolution and deviant behaviour is partly determined by interactions with their surrounding environment, even in the case of fully malignant cells (Greaves & Maley, 2012; Nguyen, Vanner, Dirks, & Eaves, 2012). It has been increasingly recognised that cancer is not a uniform disease, but is a multitude of diseases that, in certain cases, have surprisingly disparate characteristics (Nguyen et al., 2012). While tissue homeostasis is maintained by collaborative interactions between diverse cell types, cancer development is enhanced when mutant cells harness these same collaborative capabilities to favour their own survival (Stratton, 2011).

* *Corresponding author.* Instituto de Pesquisa Pelé Pequeno Príncipe, Curitiba, PR 80250-200, Brazil. Tel.: +55 4133221446; fax: +55 41 3322 1446.
E-mail address: sandrobonatto@gmail.com (S.J.R. Bonatto).
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Changes in the profile of dietary fatty acids can modify the fatty acid composition of many cell types, including those that are susceptible to cancerous transformation (Bonatto et al., 2012; Calder, 2007, 2008). Many food items, beverages, and dietary constituents have been reported to have cancer-preventive or anticancer activities (Yang, Wang, Lu, & Picinich, 2009). There has been a great interest in the effects of n-3 polyunsaturated fatty acids (PUFA) on cancer cells. The importance of PUFA in the carcinogenic process is confirmed by the observations that tumour cells show several changes in lipid composition that confer a proliferative advantage, and that fatty acid supplementation can affect the growth and differentiation of tumour cells (Pizato et al., 2006).

In the epidemiological literature, correlational studies and studies of migrants have suggested that n-3 PUFA may protect against cancer (Mandal, Ghosh-Choudhury, Yoneda, Choudhury, & Ghosh-Choudhury, 2010; Trombetta et al., 2007). However, it is difficult to identify the role of fat and fatty acids in carcinogenesis, mainly due to the absence of intermediate effect markers. Although there are plausible biological explanations for an effect of fat and fatty acids on carcinogenesis, the evidence is somewhat weak for most cancer (Berquin, Edwards, & Chen, 2008). Therefore, additional studies are needed to prove or refute the proposed roles of n-3 PUFA in carcinogenesis (Benetou et al, 2008; MacLennan et al., 2013). Here, we have investigated the effect of fish oil (FO), which is rich in n-3 PUFA, in reducing cell proliferation on human tumour cells. Our hypothesis was that fatty acids from fish oil could reduce tumour cells growth.

2. Materials and methods

2.1. *Tissue sampling*

Tumours were obtained from surgical resections performed at Angelina Caron Hospital (Curitiba, PR, Brazil). These procedures were approved by the ethics committee of the hospital under Protocol 48/09. Biopsy specimens were obtained from tumour tissue and transported in an RPMI-1640 (Gibco, New York, NY, USA) supplemented with 0.1% antibiotics (penicillin 10,000 U/L and streptomycin 10 mg/L) (Gibco, New York, NY, USA). The tumour tissue was minced into small pieces with a surgical scalpel and filtered through a 100 μm nylon mesh. Cells were washed and cultured in tissue culture flasks with complete medium (RPMI-1640 supplemented with 10% foetal bovine serum [FBS], 4 mM L-glutamine, and antibiotics) for 1 h at 37 °C to ensure macrophage adhesion. Subsequently, the cells were centrifuged at 153 *g* for 5 min and cell viability was assessed with a trypan blue exclusion test, the outcome of which was at least 98% for all samples.

2.2. *Fish oil and albumin complex*

Fish oil was kindly donated by the Herbarium Foundation (Curitiba, PR, Brazil). It was added to 2% bovine serum albumin (BSA) and blended into RPMI-1640 medium at a 1:10 (v/v) dilution. This procedure was performed under sterile conditions, and the flasks were maintained in movement, 200 rpm, for 24 h. Subsequently, the flasks were stored at 4 °C.

2.3. *Cell culture*

2.3.1. *Proliferation*

Tumour cells were diluted to 2×10^5 cells/well in a 96-well flat-bottomed tissue culture plate. The cells were cultured in complete medium RPMI-1640 at 37 °C in a 5% CO_2 humidified atmosphere. Twenty microlitres of [2-^{14}C]-thymidine were added to each well (0.01 μCi/well) and incubated for 24 h. The cells were maintained in the presence or absence of fish oil plus BSA. Subsequently, cells were harvested onto glass fibre disks (Cox Scientific, Kettering, England) and washed in a Skatron Cell Harvester (Skatron Instruments AS, Lierbyen, Norway). Radioactive thymidine incorporation into DNA was determined using liquid scintillation counting in a Beckman LS 6000IC scintillation counter.

2.3.2. *Hydroperoxides and flow cytometry*

Tumour cells were cultured in 6-well flat-bottomed microtiter plates maintained at 37 °C in a humidified 5% CO_2 atmosphere for 24 h. The tumour cell density was 2×10^6 cells/well in complete medium with or without fish oil plus BSA.

2.4. *Determination of tumour cell hydroperoxides*

To determine markers of lipid peroxidation, hydroperoxides were measured in sonicated tumour cells using a ferrous ion oxidation-xylenol orange assay (Fox2) in conjunction with a specific ROOH reductant, triphenylphosphine (TPP), following the method of Nourooz-Zadeh, Tajaddini-Samardi, and Wolff (1994). This method is based on the rapid peroxide-mediated oxidation of Fe^{2+} to Fe^{3+} under acidic conditions. In the presence of xylenol orange, the latter forms a Fe^{3+}-xylenol orange complex, which can be measured spectrophotometrically at 560 nm. Hydroperoxide content in the samples was determined as a function of the mean difference between the absorbance of samples with and without the elimination of ROOH by TPP. Calibration was performed with standard peroxides, such as hydrogen peroxide.

2.5. *Apoptosis and necrosis analyses*

Tumour cell apoptosis events were evaluated using annexin V-FITC (Pharmingen, San Diego, CA, USA). Cells were stained with annexin V-FITC for 15 min. The fluorescence was analysed using flow cytometry; the cells positive for annexin V-FITC were considered to be in an early stage of apoptosis. Necrosis was evaluated using 7AAD (Pharmingen).

2.6. *Protein determination*

The protein concentrations of tumours cells were measured following the method of Bradford (1976), using bovine serum albumin as is standard.

2.7. *Fatty acid composition of tumour cells*

Total lipids were extracted from tumour cells using chloroform-methanol (2:1 v/v). The crude lipid extracts were suspended in methanol, and pH was adjusted to ≥ 12 with 5 mol/L NaOH.