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Original Contribution

Differential sensitization of cancer cells to doxorubicin by DHA: A role for lipoperoxidation

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Abstract

Polyunsaturated fatty acids have been reported to enhance the cytotoxic activity of several anticancer drugs. In the present study, we observed that doxorubicin chemosensitization of breast cancer cell lines by docosahexaenoic acid (DHA, a long-chain ω-3 polyunsaturated fatty acid) was cell-line selective, affecting MDA-MB-231 and MCF-7dox (a doxorubicin-resistant cell line) but not the parental MCF-7 cell line. DHA supplementation led to an increase in membrane phospholipid DHA level, but did not induce changes in intracellular [^{14}C]doxorubicin accumulation. In MDA-MB-231, doxorubicin efficacy enhancement by DHA was linked to an increase in malondialdehyde level, a final product of lipid peroxidation. DHA elicited by itself a 3.7-fold malondialdehyde level increase, additive to that induced by doxorubicin. Addition of doxorubicin to DHA further increased the glutathione level, indicative of the generation of an oxidative stress. In contrast to MDA-MB-231, doxorubicin did not increase the malondialdehyde level in MCF-7, although DHA induced lipid peroxidation. Therefore in MCF-7, lipid peroxidation induced by DHA itself was not sufficient to trigger an oxidative stress and to subsequently increase sensitivity to doxorubicin. These data indicate that the differential effect of DHA among cells on drug toxicity results from a differential oxidative response to doxorubicin. Chemosensitization through fatty acids appears as a new promising adjuvant therapeutic paradigm, since ω-3 fatty acids are physiological molecules found in food and are nontoxic in vivo.

Keywords: Breast cancer; Fatty acids ω-3; Anthracycline; Drug accumulation; Oxidative stress

Introduction

Several epidemiological studies have indicated that populations consuming high amounts of ω-3 (n-3) fatty acids display a lower breast cancer risk [1,2]. This effect seems to be related to specific n-3 polyunsaturated fatty acids and/or to the ratio of n-6 to n-3 fatty acids [3–6]. Long-chain n-3 fatty acids found in fatty cold-water fish, particularly eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), have been shown to inhibit the growth of human breast cells both in culture and in xenografts [7,8] and to increase the efficacy of anticancer drugs [9,10].

Several mechanisms have been proposed to account for the activity of n-3 polyunsaturated fatty acids (n-3 PUFAs) against cancer cells, including alteration in gene expression [11,12], modulation of cellular proliferation, apoptosis and differentiation [13], increase in drug transport across the cell membrane [14,15], generation of free oxygen radicals, and lipid peroxidation [16,17]. These biological mechanisms

Abbreviations: DHA, docosahexaenoic acid; DMSO, dimethyl sulfoxide; EPA, eicosapentaenoic acid; GSH, glutathione (reduced); GSSG, glutathione (oxidized); MDR, multidrug resistance; PBS, phosphate-buffered saline; P-gp, P-glycoprotein; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species.

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could explain that n-3 fatty acid-mediated inhibition of mammary carcinogenesis is not independent. For example, incorporation of n-3 polyunsaturated fatty acids into cell membrane phospholipids can alter membrane fluidity (and increase intracellular drug accumulation), modulate cell signaling [18], and enhance the production of free oxygen radicals [19] and lipid peroxidation [20].

Anthracyclines are among the most active anticancer agents, widely used in the treatment of solid tumors and leukemia. The cytotoxic action of doxorubicin has been mainly related to the inhibition of topoisomerase II and to the production of oxygen-reactive species [21,22]. Among polyunsaturated fatty acids, DHA, with its 6 double bonds, is very prone to oxidation. The increased membrane unsaturation index consequently would provide more abundant targets for oxygen-reactive species generated by doxorubicin metabolism. Products of lipid peroxidation such as hydroperoxides and aldehydes have been implicated in the cytotoxic process and the increase in drug efficacy. Thus, conditions favoring an increased lipid peroxidation in response to doxorubicin would lead to a higher activity of the drug. Among PUFAs studied, DHA was the most potent to enhance the cytotoxic effect of doxorubicin [9]. In vivo and in vitro studies on the influence of DHA supplementation have reported higher peroxidation and oxidative stress, as demonstrated by an increase of thiobarbituric acid-reactive substances, conjugated dienes, or malondialdehydes and a decrease of antioxidant vitamins [23–26]. Protection against oxidative damages is normally ensured by non-enzymatic (especially vitamins provided by diet) and enzymatic (catalases, superoxide dismutases, and glutathione peroxidases) defenses. Changes in the activity of these antioxidant enzymes have also been described during PUFA supplementation [27,28].

Chemotherapy has improved during the last decades, largely due to the introduction of effective drug combinations and treatment schemes. However resistance is frequently observed in tumors undergoing primary therapy or, more frequently, is a result of treatment with various antitumor drugs. This multidrug resistance, the so-called phenotype MDR, remains a major obstacle for successful chemotherapeutic cure [29]. Among drug resistance mechanisms, the most common are: (i) an increase in drug efflux, associated with overexpression of the mdr-1 gene product, a M_r 170,000 plasma membrane glycoprotein (P-gp) that functions as an energy (ATP)-dependent efflux pump for cytotoxic drugs, and (ii) an abnormal redox status developed secondarily after drug exposure, in which glutathione and antioxidant enzyme activities are elevated and protect the cell against free radical aggression [30]. Attention has been focused recently on the study of MDR reversing agents. Although hundreds of compounds have been found in vitro to be able to modulate the MDR phenotype, their clinical application was limited owing to high toxicity in vivo such as cardiotoxicity, nephrotoxicity, or immunosuppression [29].

To determine to what extent an oxidative stress could account for the sensitization of cancer cells to doxorubicin by DHA, we have used three cell lines: a doxorubicin-resistant one (MCF-7dox) and two sensitive lines (MCF-7 and MDA-MB-231), and took advantage of their differing properties. We found that DHA was able to enhance doxorubicin cytotoxicity in MDA-MB-231 or in MCF-7dox cell lines, but not in the parental MCF-7 cell line. In order to understand the differential effects of DHA on these three cell lines, fatty acid composition of membrane phospholipids and intracellular accumulation of doxorubicin were examined. The cellular oxidative status was also evaluated through the measure of two parameters of oxidative stress, malondialdehyde level (a final product of lipid peroxidation) and glutathione levels, cofactors for major antioxidant enzymes which modulate the cell's response to redox changes and vitamin E level.

Materials and methods

Drugs and chemicals

Unless otherwise stated, all chemicals were purchased from Sigma (Sigma-Aldrich Chimie, France). Doxorubicin (chlorhydrate de doxorubicine Teva®, 10 mg/5 ml) was purchased from Teva Pharma S.A. (France) and [^{14}C] doxorubicin hydrochloride (2 GBq/mmol) was purchased from Amersham Pharmacia Biotech (France). Stock solutions of doxorubicin (1 mM) were stored at -80°C and dilutions of doxorubicin were freshly prepared in Hanks' medium (Hanks' balanced salts without sodium bicarbonate and phenol red) buffered with 10 mM Hepes (pH 7.2 at 37°C) or in appropriate media. [^{14}C]Doxorubicin hydrochloride was used at 5 μM (final concentration with isotopic dilution ≈1/11). Oleic acid (OA, 18:1n-9) and docosahexaenoic acid (22:6n-3) were used as methyl esters. The fatty acid was dissolved in 99% ethanol and stored as stock solution (150 mM) under nitrogen at −80°C. For all experiments, fatty acid was prepared freshly from stock solution and diluted with growth culture medium (final ethanol concentration: 0.02%).

Cell culture

The human breast carcinoma cell lines MDA-MB-231 and MCF-7 were obtained from American Type Culture Collection (LGC Promochem, France) and the MCF-7dox cell line was a gift of Dr. K. Cowan (National Cancer Institute, Bethesda, MD). MCF-7 is as drug-sensitive, P-gp-negative cell line. The MCF-7dox was originally established by in vitro selection with increasing concentrations of doxorubicin [31]. Cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 5% heat-inactivated fetal calf serum, 50 UI/ml penicillin, and 50 μg/ml streptomycin (Cambrex, France). MCF-7dox cells were