Interaction of ω-3 polyunsaturated fatty acids with radiation therapy in two different colorectal cancer cell lines

CAI, Fang, et al.

Abstract

Background & aims: This study aims at evaluating if docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA) increases the efficacy of radiation therapy (RT) on two human colorectal cancer cell lines with different radio-sensitivity. Methods: LS174T and HT-29 cells were treated with 20 or 50 mmol/L EPA or DHA followed by single X-ray RT of 0, 2 or 4 Gy, to evaluate cell survival, apoptosis, peroxide and malondialdehyde productions. Inflammation- and apoptosis-related proteins were analyzed by Western Blot. ANOVAs were used for statistical analysis. Results: LS174T was more sensitive to RT than HT-29. DHA and to a lesser extent EPA increased cell death, apoptosis and peroxide production after RT in LS174T and to a lesser extent in HT-29 (p < 0.05). This was associated with increased expression of heat shock protein 70, decreased expression of NF-kB p65, COX-2 and Bcl-2 proteins. Conclusions: The effect of RT combination with DHA and to a lesser extent EPA was synergistic in the radio-sensitive LS174T cells, but additive in the radio-resistant HT-29 cells. This enhanced cytotoxicity was provoked at least partly by lipid [...]
Interaction of \(\omega-3\) polyunsaturated fatty acids with radiation therapy in two different colorectal cancer cell lines

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**Summary**

**Background & aims:** This study aims at evaluating if docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA) increases the efficacy of radiation therapy (RT) on two human colorectal cancer cell lines with different radio-sensitivity.

**Methods:** LS174T and HT-29 cells were treated with 20 or 50 \(\mu\)mol/L EPA or DHA followed by single X-ray RT of 0, 2 or 4 Gy, to evaluate cell survival, apoptosis, peroxide and malondialdehyde productions. Inflammation- and apoptosis-related proteins were analyzed by Western Blot. ANOVAs were used for statistical analysis.

**Results:** LS174T was more sensitive to RT than HT-29. DHA and to a lesser extent EPA increased cell death, apoptosis and peroxide production after RT in LS174T and to a lesser extent in HT-29 (\(p < 0.05\)). This was associated with increased expression of heat shock protein 70, decreased expression of NF-kB p65, Cox-2 and Bcl-2 proteins.

**Conclusions:** The effect of RT combination with DHA and to a lesser extent EPA was synergistic in the radio-sensitive LS174T cells, but additive in the radio-resistant HT-29 cells. This enhanced cytotoxicity was provoked at least partly by lipid peroxidation, which consequently modulated inflammatory response and induced apoptosis.

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**1. Introduction**

Colorectal cancer (CRC) is currently one of the most prevalent causes of cancer death.\(^1\) CRC treatment consists mainly in complete surgical resection with or without adjuvant radiation therapy (RT) and/or chemotherapy. Even though, CRC frequently remains refractory to these conventional treatments. More than 50% of patients develop local relapse or metastasis within two years after surgery.\(^2\) New modalities to improve CRC treatment outcome are therefore of great interest.

**Abbreviations:** CRC, colorectal adenocarcinoma; DHA, docosahexaenoic acid; DNPH, 2,4-dinitrophenylhydrazine; EDTA, Ethylenediaminetetraacetic acid; EPA, eicosapentaenoic acid; FACS, fluorescence-activated cell sorting; FITC, fluorescein isothiocyanate; HPLC, high-performance liquid chromatography; MDA, malondialdehyde; PBS, phosphate-buffered saline; RT, radiation therapy; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; \(\omega-3\) PUFAs, \(\omega-3\) polyunsaturated fatty acids.

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\(\omega-3\) Polyunsaturated fatty acids (PUFAs) might be used as adjuvant for cancer therapy. They could inhibit tumor progression in breast, skin and prostate cancers.\(^3,4\) The adjuvant use of \(\omega-3\) PUFAs increased chemo-\(^5\) and radio-sensitivity in different cancers.\(^6,7\) In our study, we investigated if \(\omega-3\) PUFAs could enhance radio-sensitivity in CRC cell lines.

Among the \(\omega-3\) PUFAs, eicosapentaenoic acid (EPA, 20:5\(n\)-3) and docosahexaenoic acid (DHA, 22:6\(n\)-3) are the most studied. Both have been described to increase the therapeutic efficacy of RT in breast cancer cell lines,\(^6\) rodent model\(^8\) and also in patients.\(^9\) In CRC, EPA or DHA, on its own, inhibits HT-29 cell growth, but DHA is more efficient than EPA due to its rapid cellular absorption and integration into cell membrane.\(^10\) However, the combined effect of EPA/DHA and RT is rarely reported. We previously observed that EPA and DHA were able to increase radio-toxicity in different CRC cell lines, i.e. LS174T, Caco-2 and CO112. This synergic toxicity was negatively correlated with the differentiation degree of the cell lines.\(^6\)

Oxidative stress might contribute to the cytotoxicity of PUFAs, because of an increase in oxidative stress markers, such as glutathione, disulfide glutathione and malondialdehyde (MDA) observed...
during DHA or EPA combination with chemotherapy in breast carcinoma cells. When combined with RT, the mechanism of cytotoxicity of ω-3 PUFAs has not yet been evaluated in depth, especially in CRC. This study therefore aimed at evaluating the effect of RT combination with EPA or DHA on LS174T and HT29 CRC cell survival. These two cell lines have different proliferation rates and genetic backgrounds, especially in p53 and Bax protein expressions. The p53 and Bax positive HT29 cell line is more radioresistant than the p53 and Bax negative LS174T cell line. Lipid peroxidation and its downstream pathways such as inflammation and apoptosis, which cause cell survival inhibition, were also investigated.

2. Materials and methods

2.1. Reagents

EPA, DHA were obtained from Sigma–Aldrich (Buchs, Switzerland), diluted in ethanol and stored at 10 g/L at −20 °C under argon. All other chemicals were also purchased from Sigma–Aldrich (Buchs, Switzerland) unless otherwise stated.

2.2. Cell line

The human CRC cell line LS174T (ATCC no. CL-188™) and HT-29 (ATCC no. HTB-38™, USA) were cultivated in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated foetal bovine serum (Invitrogen, Switzerland). The medium was changed twice a week to keep the cells in exponential growth phase and apoptosis, which cause cell survival inhibition, were also investigated.

2.3. Lipid peroxidation

Cell treatment with EPA or DHA in combination with RT. Four hours after irradiation, cells were collected and 50 μL of RIPA lysate buffer (NaCl 150 mmol/L, Tris–HCl pH 8.0, 50 mmol/L NaF, 50 mmol/L Na3VO4, 1% Triton X-100, 1% SDS, 1% PMSF) were added to each sample and incubated at room temperature (protected from light) for 30 min. The absorbance of the mixture was read using a colorimeter (Model 680, Bio-Rad Laboratories AG) at 595 nm.

MDA level was measured following a derivatization with 2,4-dinitrophenylhydrazine (DNPH) and HPLC analysis with minor modifications. Fifty μL of reconstructed samples were injected into HPLC system (Agilent 1100 series) equipped with a quaternary pump, a UV diad-array detector, and a C18 column (Nucleodur® C18 Pyridin 3 μmol/L, Macherey–Nagel). The flow-rate of the mobile phase was 1 ml/min. The DNPH derivatization products of MDA (1-(2,4-dinitrophenyl) pyrazole) and methyl-MDA (4-methyl-1-(2,4-dinitrophenyl) pyrazole, internal standard, 100 μmol/L, synthesized by heating a mixture of 0.25 g 3-dimethylamino-2-methyl-2-propenal, 0.1 g sodium hydroxide and 350 μL HPLC grade water at 70 °C for 25 min) were analyzed at 306 nm and 322 nm, respectively. The calibration of the derivatization products was done using that of MDA and methyl-MDA standard solutions with molar extinction coefficients of 12800 (M−1 cm−1) at 249 nm for MDA in acidic medium and 22800 (M−1 cm−1) at 279 nm for methyl-MDA in alkaline medium.

2.6. Apoptosis quantification

Cells were seeded at a density of 50 000/well in 24-well plates for 24 h before treatment of 50 μmol/L EPA or DHA for 4 h followed by a 4-Gy RT. Apoptosis was quantified 2, 4 or 24 h after RT using two-parameter fluorescence-activated cell sorting (FACS) analysis with annexin V/propidium iodide detection kit according to manufacturer’s instructions (BD Biosciences, Switzerland). Briefly, cells were detached with trypsin, washed with 1× PBS and then resuspended in binding buffer (10 mmol/L HEPES/NaOH (pH 7.4), 140 mmol/L NaCl, 2.5 mmol/L CaCl2) at a concentration of 1 × 106 cells/ml. Samples were stained with 5 μL annexin V conjugated with fluorescein isothiocyanate (FITC) and 5 μL propidium iodide at room temperature for 15 min in the dark. They were then diluted in 400 μL of binding buffer and analyzed within 1 h using a flow cytometer (Accuri C6 personal flow cytometer, BD Biosciences; excitation 488 nm; emission 530 nm).

2.7. Western Blot

Two hours after a 4-Gy RT, cells were washed with PBS and collected with 100 μL of RIPA lysate buffer (NaCl 150 mmol/L, Tris–HCl pH 8.0, 50 mmol/L, NP40 1%, Triton X100 1%, SDS 0.1%), and supplemented with proteinase inhibitors. The supernatant was then collected and denatured at 95 °C for 5 min. 25 μg of protein from each sample were loaded on 9%–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels, transferred, and immunoblotted onto PVDF membrane. Anti-COX-2, anti-HSP-70, anti-Bcl-2, anti-Bax, anti-p53 antibodies (Santa-Cruz, USA) were used as primary antibodies. Anti-GAPDH antibody (Millipore, USA) was used as internal standard for protein.

2.8. Data analysis

For apoptosis analysis, data were acquired with CFlow® Plus software (BD biosciences, USA). All experiments were reproduced in triplicate and results were presented as means ± SD. Normality of data was tested using Shapiro–Wilks. Three-way ANOVA was used for clonogenic assay, apoptosis and xylalen orange, while two-way
ANOVA was performed for MDA, apoptosis for LS174T at 2 h, HT29 at 4 h, xylene orange for LS174T at 4 h and HT29 at 24 h. All the ANOVA were followed by Tukey test using SPSS 11.5 (USA). Student’s t-test was used to compare means between two specific conditions. P-value ≤ 0.05 was considered as statistically significant.

3. Results

3.1. DHA and EPA decrease cell survival after RT

Clonogenic assays were performed to evaluate the cytotoxic effect of different treatments on LS174T (Fig. 1A) and HT29 cells (Fig. 1B). Cell survival depended on the addition of ω-3 PUFAs (p < 0.001), the dose of ω-3 PUFAs (p < 0.001) and RT (p < 0.001). DHA was more efficient than EPA (p < 0.001) to decrease cell survival in both LS174T (DHA, 39.7 ± 29.9%; EPA, 88.2 ± 71%). While HT29 cells (2 Gy, 75.5%, p = 0.02; 4 Gy, 66.7%, p < 0.001) were more resistant to RT than LS174T cells (2 Gy, 61.2%, p < 0.001; 4 Gy, 28.2%, p < 0.001), the effect of RT combination with different doses of ω-3 PUFAs was more evident in LS174T (p = 0.052) than in HT29 cells (p = 0.1). A tendency of synergy between ω-3 PUFAs and RT was observed in LS174T (p = 0.09) but not in HT29 cells (p = 0.5). SER induced by 50 μmol/L EPA was 1.4 and 1.5 in LS174T and HT29 cells, respectively, while SER induced by 50 μmol/L DHA was 1.6 and 1.7 in LS174T and HT29 cells, respectively. These results suggested that DHA is a better radio-sensitizer than EPA in both LS174T and HT29 cells.

3.2. DHA and EPA increase peroxide production after RT

In order to investigate the time-dependent production of peroxides after a 4-Gy RT, total peroxide concentration was measured 4, 24, and 48 h after a 4-h pre-treatment with 50 μmol/L ω-3 PUFAs in these two cell lines (Table 1). Total peroxide production in both cell lines depended not only on the addition of fatty acids (p < 0.05) but also on the exposure time (p < 0.05). Regardless of the combination with RT, DHA and to a lesser extent EPA increased peroxide production in LS174T (p < 0.001) and lesser extent in HT29 cells (p < 0.001). Without RT, the peroxide production reached a peak between 4 h and 24 h after incubation with EPA or DHA in both cell lines (p < 0.001), and then decreased at 48 h in LS174T and remained stable in HT29 cells (p = 0.22). Whereas no significant effect could be observed within the first 24 h after RT alone, total peroxide production was increased significantly when RT was combined with ω-3 PUFA pre-treatment (p < 0.05). The peak production of peroxides was observed within the first 4 h after RT in LS174T cells and between 24 h and 48 h after RT in HT29 cells, DHA being more efficient than EPA to increase total peroxide production (Table 1).

3.3. DHA and EPA increase malondialdehyde production after RT

Cytoplasmic MDA level was measured by HPLC analysis 4 h and 24 h after RT in LS174T (Fig. 2A) and HT29 cells (Fig. 2B), respectively, to fit with the timing of peroxide production in these two cell lines. Again, the stronger effects of ω-3 PUFAs with or without RT were observed in LS174T compared to HT29 cells. DHA was more efficient than EPA to increase MDA production, regardless of RT combination (p < 0.01), whereas EPA showed a significant effect only when combined with RT in LS174T cells (Fig. 2C; p = 0.01), but not in HT29 cells (Fig. 2D; p = 0.3).

3.4. DHA and EPA accelerate early apoptosis after RT

FACS analysis with Annexin V/propidium iodide indicated that ω-3 PUFAs could increase early apoptosis in both LS174T (p < 0.001) and HT29 cells (p = 0.01). EPA induced apoptosis in a time-dependent manner in LS174T (Fig. 3A) and HT29 cells (Fig. 3B), while DHA caused a peak induction of 2.1 ± 0.5 fold (p < 0.01) and 1.8 ± 0.7 fold (p = 0.07) at 4 h in LS174T (Fig. 3C) and HT29 cells (Fig. 3D), respectively. Whereas RT alone stimulated apoptosis only in LS174T (p = 0.01), but not in HT29 cells (p = 0.3), RT combination with ω-3 PUFAs caused a time-dependent induction of apoptosis in both LS174T (p = 0.04) and HT29 cells (p = 0.01). In LS174T cells, a peak induction of 2.1 ± 0.3 fold (p = 0.001) and 2.5 ± 0.6 fold (p < 0.05) was observed at 2 h in the presence of RT combination with EPA (Fig. 3A, Table 2) and DHA (Fig. 3C, Table 2). In HT29 cells, a peak induction of 1.9 ± 0.6 (p < 0.05) and 2.4 ± 1.4 fold (p < 0.05) was rather observed at 4 h in the presence of RT combination with EPA (Fig. 3B) and DHA (Fig. 3D).

### Table 1

<table>
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<tr>
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<th>4 h</th>
<th>24 h</th>
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<td><strong>LS174T</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
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<td>12.8 ± 4.9</td>
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<td>1.1 ± 0.5</td>
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<tr>
<td>4 Gy + EPA</td>
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<td>10.5 ± 3.8h</td>
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<tr>
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<td>19.0 ± 6.3h</td>
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<td><strong>HT29</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.5</td>
<td>1.0 ± 0.1h</td>
</tr>
<tr>
<td>EPA</td>
<td>0.9 ± 0.5</td>
<td>5.6 ± 2.6h</td>
<td>8.1 ± 4.8h</td>
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<tr>
<td>DHA</td>
<td>3.2 ± 1.0h</td>
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<td>12.0 ± 7.5h</td>
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<tr>
<td>4 Gy</td>
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<td>0.8 ± 0.4</td>
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<tr>
<td>4 Gy + EPA</td>
<td>0.7 ± 0.5</td>
<td>4.6 ± 2.3h</td>
<td>6.2 ± 3.7h</td>
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<tr>
<td>4 Gy + DHA</td>
<td>2.3 ± 1.2h</td>
<td>17.1 ± 8.6h</td>
<td>16.3 ± 2.9h</td>
</tr>
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</table>

MDA, malondialdehyde; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid. Values are calculated as fold to control and expressed as means ± SD (n > 3).

* P < 0.05 vs. control.  
b P < 0.05 vs. RT.  
c P < 0.05 EPA vs. DHA.  
d P < 0.05 vs. 4 h.  
e P < 0.05 vs. 24 h (Student’s t-test).
apoptotic effect of RT only at 2 h after RT in LS174T cells \((p = 0.01)\), while there was a tendency of synergy between \(\omega-3\) PUFAs and RT in HT29 cells 24 h after treatment \((p = 0.07)\).

3.5. **DHA and EPA affect protein expression after RT**

As shown in Fig. 4, Heat shock protein 70 (HSP70), a biomarker of oxidative stress, increased with the addition of EPA, DHA and RT in LS174T and HT29 cells, while the combination of these two treatments decrease HSP70 expression compared to RT alone. The activated form of nuclear factor, NF-\(\kappa B\) p65, decreased with DHA and to a lesser extent EPA, in both cell lines. RT also decreased NF-\(\kappa B\) p65 expression. RT combination with \(\omega-3\) PUFAs, especially with DHA, considerably decreased NF-\(\kappa B\) p65 expression in both LS174T and HT29 cells. A decrease in COX-2 with DHA with or without RT was observed in both cell lines. EPA, RT, but not DHA increased COX-2 expression in HT29 cells. Similar to DHA, EPA combination with RT decreased COX-2 expression in HT29 cells.
RT and EPA or DHA decreased Bcl-2 expression, a member of apoptosis regulatory and pro-survival protein family, in both cell lines. The combination of EPA or DHA with RT had an additive effect especially when DHA was combined with 4 Gy. Increase in Bax expression, one of the pro-apoptotic proteins, was observed with the addition of EPA, DHA and RT in HT29 cells. Increase in p53 and also its small molecular isoforms were observed especially when RT was combined with DHA.

4. Discussion

DHA and to a lesser extent EPA had a strong dose-dependent additive effect with RT to suppress cell survival in the radiosensitive LS174T cells. An additive effect was also observed in the radio-resistant HT29 cells. This suppressive effect could be explained by the induction of lipid peroxidation by fatty acids, which was enhanced by the addition of RT and consequently modified protein expression leading to apoptosis in these two cell lines.

ω-3 PUFAs have been shown to inhibit cell growth of several malignant cancer cells. Habermann et al. found that DHA was the most cytotoxic among the fatty acids, including EPA. This is in agreement with our findings that DHA was more efficient than EPA in suppressing cell survival in both LS174T and HT29 cells, possibly due to a better intracellular absorption rate of DHA than EPA in CRC cell lines. DHA (22 carbons, 6-double-bonds) is more lipophilic and has thus more facility to be incorporated in cell membrane than EPA (20 carbons, 5-double-bonds). Therefore, DHA would be oxidized more quickly than EPA. Bougnoux et al. have revealed the exiting clinical benefit of DHA in breast cancer patients under chemotherapy. This tumor progression inhibition and survival prolongation is correlated with DHA plasma absorption level. The biochemical characteristics of ω-3 PUFAs contributed to their different tumor-suppressive effects in breast cancer, which was proportional to their number of double bonds. ω-3 PUFAs increased the anti-tumor effect of RT in animal models of head and neck cancers and mammary tumors. RT is currently one of the main components of CRC treatment, especially for rectal cancer. A synergistic effect of PUFAs and RT in a radio-sensitive LS174T cell line and also an additive effect in a more radio-resistant HT29 cell line is therefore encouraging for a potential utilization in clinical practice.

Lipid peroxidation products, including primary products, peroxides (—C—O—OH) and also one of its end products, MDA, might be the main cause of PUFA-induced DNA damages, which may lead to apoptosis and then cell death. Lu et al. found an increase in plasma MDA concentration that might contribute to the inhibitory Table 2

<table>
<thead>
<tr>
<th>Early apoptosis (%)</th>
<th>LS174T</th>
<th>HT29</th>
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<tbody>
<tr>
<td>Control</td>
<td>7.5 ± 4.3</td>
<td>7.6 ± 4.3*</td>
</tr>
<tr>
<td>EPA</td>
<td>7.0 ± 4.5</td>
<td>7.6 ± 4.9**</td>
</tr>
<tr>
<td>DHA</td>
<td>7.5 ± 4.5</td>
<td>11.5 ± 2.6**</td>
</tr>
<tr>
<td>4 Gy</td>
<td>8.0 ± 5.7</td>
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<tr>
<td>4 Gy + EPA</td>
<td>13.0 ± 8.4*b,d</td>
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</tr>
<tr>
<td>4 Gy + DHA</td>
<td>14.2 ± 7.8*b,c</td>
<td>14.6 ± 2.3*a</td>
</tr>
</tbody>
</table>

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid. Values are presented as means ± SD % (n > 3).

<sup>a</sup> P < 0.05 vs. control.

<sup>b</sup> P < 0.05 vs. RT.

<sup>c</sup> P < 0.05 EPA vs. DHA.

<sup>d</sup> P < EPA/DHA vs. EPA/DHA+4Gy (Student’s-t test).

Fig. 4. Alteration of protein expression. Cells were pre-treated with DHA or EPA at 50 μmol/L and then exposed to X-rays of 4 Gy. 2 h after irradiation, cells were collected and lysed by RIPA solution (NaCl 150 mmol/L, Tris—HCl pH 8.0 50 mmol/L, NP40 1%, Triton × 100 1%, SDS 0.1%). Indicator of reactive oxidation species, HSP70 (70 kDa); inflammation related protein, NFκB p65 (65 kDa) and COX-2 (72 kDa); apoptosis related protein, Bax (21 kDa), Bcl-2 (26 kDa) and p53 (53 kDa), were detected in using specific antibodies. GAPDH (36 kDa) was used as the internal standard for protein dosage. GAPDH, small molecular weight isoforms of p53. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.
effect of the PUFAs at a relatively high dose (100–300 μmol/L). As shown in Fig. 2 and Table 1, addition of ω-3 PUFAs, especially DHA, increased production of peroxides and MDA in LS174T and to a lesser extent in HT29 cells. Moreover, a synergistic effect in peroxide production of PUFAs and RT existed in LS174T but not in HT29 cells, which corresponded to their strong additive effect in cell survival suppression in LS174T and to a lesser extent in HT29 cells.

The oxidative products would consequently modify several signaling pathways, including apoptosis. Apoptosis is a protective reaction for cells to fight against internal or external stimulation that will lead to aberrant cell transformation, which is one of the critical steps in cancer development and progression, and also one of the biological targets for cancer treatment. We found that DHA and EPA induced apoptosis in a time-dependent manner, which was consistent with peroxide production. DHA had more efficient pro-apoptotic effect than EPA in these two CRC cell lines (Fig. 3).

DHA induced apoptosis in different cancers both in vitro and in vivo. EPA supplementation reduced cell proliferation and increased apoptosis in the crypts of patients with CRC history by a selective increase in EPA uptake in the colonic mucosa. When combined with RT, the pro-apoptotic effect of PUFAs was less studied. We found that PUFAs had a synergistic effect in apoptosis induction 2 h after exposure to RT in LS174T (p = 0.01), while in HT29 cells, a tendency of synergy (p = 0.07) was observed 24 h after exposure. Decrease in Bcl-2, an anti-apoptotic protein of the pro-survival protein family whose abnormal increase leads to malignant transformation, together with an increase in pro-apoptotic Bax expression were observed in the presence of EPA or DHA using Western Blots. This effect was enhanced by the addition of RT. Therefore, DHA and to a lesser extent EPA enhanced the pro-apoptotic effect of RT in LS174T while in HT29 cells, the synergistic effect was not obvious. Similar results were found in cell survival and also in peroxide production as described before.

Addition of EPA or DHA increased intracellular reactive oxidation species (ROS) as indicated by an augmentation of HSP70 (Fig. 4). HSP70, a member of heat shock protein family, is usually induced by oxidative stress to protect cells from this challenge. In our study, we found an increase in HSP70 expression with ω-3 PUFAs or RT alone. Because our dose of ω-3 PUFAs or RT are not critical as we aimed at evaluating a potential synergy between these treatments. Decrease in HSP70 by the combination of these two treatments compared to RT alone suggests a radiosensitizer effect of DHA, because high expression of HSP70 can lead to radio-resistance.

ROS consequently mediate activation of NF-κB, whose high constitutive expression is important to maintain cell viability in cancer cells. NF-κB is also the most important mediator in radio-resistance. Sandur et al. concluded that NF-κB dependent cell survival pathway was crucial for radio-resistance in CRC cell lines and that suppression of inducible NF-κB led to radiosensitivity. We found a decrease in NF-κB p65 expression after single DHA or EPA treatment. With the combination of PUFAs and RT, the decrease was more evident in both cell lines, especially when DHA was combined with RT. Zand et al. also found a synergistic apoptosis-inductive effect of DHA with RT via suppression of NF-κB pathway in a high radio-resistant cancer cell line. Therefore, NF-κB probably has a primordial effect in maintaining cell apoptosis induced by inflammatory challenge and at the same time modulating release of factors that are involved in cell survival.

Stabilization of NF-κB consequently led to change in expression of COX-2, a down-stream inflammatory protein that plays an essential role in CRC carcinogenesis and treatment. Long-term intake of COX-2 inhibitor was effective in decreasing colorectal cancer risk. These observations indicate that COX-2 does not only play an important role in promoting tumor cell growth but also is a potential target for anti-cancer treatment. In our study, decreased expression of COX-2 was observed in the presence of ω-3 PUFAs, especially when DHA was combined with RT. This is consistent with the cell survival inhibition and NF-κB p65 modulation as described before.

Tumor suppressor protein p53 is involved in conserving genome stability. Isoforms of p53 have recently been identified and their function is widely debated. Avery-Kiejda et al. found an aberrant increase in Δ40p53, a small molecular weight isoform of p53, in different cancer cells, which was related to chemotherapeutic treatment in a time-dependent manner. We also found an increase in this p53 isoform of about 40 kDa after the treatment combination of DHA and with RT (Fig. 4), which might possibly contribute to the radio-sensitive effect of the combined treatment.

Our study was carried out using two CRC cell lines with different genetic backgrounds, which affected their proliferation rate and also their different response to RT. Although the peroxide level of ω-3 PUFAs solution was not measured before use, both stock solutions were kept at –20 °C under argon to prevent oxidative degradation, and all the treatments were performed cautiously in parallel with EPA and DHA in order to compare their effect. The intracellular incorporation of ω-3 PUFAs was not quantified in our study. As reported by Habermann et al., intake of fatty acids into colon cells occurs continuously from 1 h to 72 h after incubation. This intake depends also on the cell type. Therefore, quantification of ω-3 PUFAs is of less important in our study, since the subsequent cascade for this incorporation, which are peroxyl production, peroxidation and apoptosis have been tested thereafter. The results suggest that ω-3 PUFAs strongly enhance the radio-cytotoxicity in radio-sensitive LS174T cells while an additive effect was observed in radio-resistant HT29 cells. Nevertheless animal experiment is needed to confirm these observations before its potential clinical application.

5. Conclusion

DHA and to a lesser extent EPA strongly enhance the cytotoxic effect of RT in radio-sensitive LS174T cells, while an additive effect of ω-3 PUFAs with RT was observed in the radio-resistant HT-29 cells. ω-3 PUFAs enhanced RT efficacy by inducing lipid peroxidation, with subsequent modulation of inflammatory response and finally led to cell apoptosis. Our observations suggest that ω-3 PUFAs might be used as radio-sensitizer for CRC treatment. Further investigations, especially experiments on animals, are needed to confirm these observations for potential clinical application.

Authors’ contributions

FC: data acquisition and analysis and manuscript drafting; OS: lipid peroxidation analysis processing and data interpretation; VG and EL: data acquisition; RM: irradiation protocol development; YMD: study conception and design, data interpretation, manuscript revision; CP: study conception and design, data interpretation, manuscript revision and funding. This work was supported by research grants from the Swiss National Science Foundation n° 310000-116738/1 and the Foundation Nutrition 2000Plus.

Conflict of interest statement

None of the authors had any personal or financial conflict of interest.
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