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Original Article

The effect of three lipid emulsions differing in fatty acid composition on growth, apoptosis and cell cycle arrest in the HT-29 colorectal cancer cell line

Aleix Sala-Vila, Jacqueline Folkes, Philip C. Calder*

Institute of Human Nutrition and Developmental Origins of Health and Disease Division, School of Medicine, University of Southampton, Southampton SO16 6YD, United Kingdom

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SUMMARY

Background & aims: An *in vitro* study showed that a lipid emulsion containing fish oil (FO) slows the growth of colon cancer cells and enhances their sensitivity to 5-fluorouracil (FU). The aim was to confirm this finding and to compare such an emulsion with an alternative to lowered n-6 fatty acid exposure.
Methods: We determined the number of viable cells, apoptosis and cell cycle distribution of HT-29 cells after exposure to one of three lipid emulsions. Cell cycle distribution was also assessed after treatment with lipid emulsions and FU.
Results: The lipid emulsion containing FO induced a significant growth inhibitory effect without changing the percentage of apoptotic cells. Exposure to the other lipid emulsions had no effect on growth and decreased apoptosis. Each lipid emulsion potentiated the S phase-halting effect of 1 and 10 μM FU. This effect also occurred at 0.1 μM FU when the cells were exposed to the FO containing lipid emulsion.
Conclusions: A lipid emulsion containing FO has a growth inhibitory effect on a human colon adenocarcinoma cell line, an effect not due to the induction of apoptosis, and potentiated the S phase-halting effect of FU. Thus, an FO lipid emulsion may be of benefit in colorectal cancer.

1. Introduction

Colon cancer ranks third amongst cancers in terms of incidence and mortality worldwide, affecting men and women equally.[1] Epidemiological studies suggest an association between quantity and quality of dietary fat and colon cancer risk.[2,3] Experimental studies using chemically induced colon cancer in rats and mice, implanted human colon cancer cells in athymic mice, and genetically predisposed ("min") mice have enabled the influence of the type of fat to be carefully studied.[4–13] One consistent observation is that diets rich in long-chain n-3 polyunsaturated fatty acids (PUFA) found in fish oils (FO) slow the development of colon cancer in these models.[14] Current evidence indicates that dietary n-3 PUFA may be helpful in preventing inflammatory bowel diseases, which are linked to colorectal cancer, and can modulate the balance between colonic epithelial cell proliferation and apoptosis (reviewed in[15,16]). This effect of n-3 PUFA is supported by a cell culture study using the CaCo-2 human colorectal adenocarcinoma cell line that showed that the two n-3 PUFA found in fish and FO (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) dose-dependently slowed the growth of the cells, in association with induction of apoptosis.[17] In addition, it has been suggested that n-3 PUFA may enhance the influence of ionising radiation on the cells[17] and the sensitivity of tumours to 5-fluorouracil (FU),[18,19] although the mechanisms by which this action is exerted remain to be fully elucidated.

Parenteral nutrition containing n-3 PUFA can be beneficial to post-operative colon cancer patients,[20–25] having been shown to modify inflammation and immune function and to speed recovery, seen as a reduction in hospital stay. Given the effects of EPA and DHA and of FO in model systems,[17–19] it is possible that parenteral nutrition containing n-3 PUFA, alone or in combination with FU, in colon cancer patients after surgery to remove the tumour could be beneficial to increase recovery and decrease the chances of recurrence. An earlier study compared the effects of two lipid emulsions on CaCo-2 cell growth; the emulsions examined were Lipovenos®, based on soybean oil (SO), and Omegaven®, based on FO.[18] The FO containing emulsion slowed cell growth, trapped cells in the G_2/M phase of the cell cycle and increased the sensitivity of the cells to FU.[18] There is a need to confirm the findings made with the FO based emulsion and to compare such an emulsion with an alternative to lowered n-6 PUFA exposure e.g. use of a medium-chain

Non-standard abbreviations: EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid; FO, Fish oil; FU, 5-fluorouracil; MCT, Medium-chain triacylglycerols; PUFA, Polyunsaturated fatty acids; SO, Soybean oil.

* Corresponding author at: Institute of Developmental Sciences Building, Mailpoint 887, Southampton General Hospital, Tremona Road, Southamtpon SO16 6YD, United Kingdom. Tel.: +44 23 8079 5250; fax: +44 23 8079 5255.
E-mail address: pcc@soton.ac.uk (P.C. Calder).

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triacylglycerol (MCT)-containing emulsion. Therefore we compared the *in vitro* effect of a new FO containing lipid emulsion (Lipoplus®, also known as Lipidem®) with emulsions based on SO (Lipofundin®) and SO/MCT (Lipofundin MCT®). We studied effects on HT-29 cells, a human colon adenocarcinoma cell line.

2. Materials and methods

2.1. Cell lines and culture

The HT-29 cell line was obtained from European Collection of Cell Cultures, Porton Down, UK. HT-29 cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum, 2 mM glutamine and 100 μg/ml antibiotic cocktail containing penicillin and streptomycin (all obtained from Sigma, Poole, UK), at 37 °C in a humidified atmosphere containing 5% carbon dioxide in air. The medium was changed every two days. When the cells reached 80–90% confluence they were sub-cultured and seeded at a density of 1 in 6.

2.2. Cell treatment

To test the effect of lipid emulsions on the growth of HT-29 cells, the cells were exposed to concentrations of FO containing lipid emulsion (Lipoplus®, also known as Lipidem®) corresponding to concentrations of EPA from 10 to 100 μM as used by Jordan and Stein,[18] which resulted in the final concentration of lipid emulsion ranging from 1.41 to 14.13 ml/l of medium, equivalent to lipid concentrations of approximately 0.3–3 mM. This range of FO containing lipid emulsion concentrations corresponded to a range of DHA concentrations of 7–70 μM. To exclude a non-specific effect of lipid emulsion on the cells, two other lipid emulsions were used. The first was an emulsion containing a 50:50 (v/v) mixture of SO and MCT (Lipofundin MCT®) and containing predominantly linoleic, caprylic and capric acids; this emulsion is referred to as SO/MCT throughout this manuscript. The second was an emulsion based on SO (Lipofundin®) and containing oleic and linoleic acids as the main fatty acids. Neither the SO/MCT or SO lipid emulsions contained EPA, DHA or docosapentaenoic acid but the FO lipid emulsion contained all three. The fatty acid composition of the three emulsions used is shown in Table 1. The SO/MCT and SO lipid emulsions were used in cell culture at the same concentrations (ml/l of medium) as the FO emulsion. The range of final concentrations of linoleic and α-linolenic acids in the culture medium were approximately 65–650 and 7.7–77, 78–780 and 8.5–85, and 143–1430 and 16–160 μM for the FO, SO/MCT and SO emulsions, respectively. The lipid emulsions used differ not only in their fatty acid composition but also in their content of tocopherols and in the tocopherol isomer distribution. The concentrations of α-tocopherol in SO, SO/MCT and FO emulsions are in the range 0.11–0.21, 0.13–0.21 and 0.16–0.22 g/l, respectively, depending upon the batch (data supplied from the manufacturer).

Table 1
Fatty acid compositions (mg/ml) of the intravenous lipid emulsions[a].

	FO[b]	SO/MCT[b]	SO[b]
8:0	18.48 ± 0.99	18.64 ± 0.90	–
10:0	23.91 ± 0.83	13.43 ± 0.62	–
16:0	4.91 ± 0.28	5.33 ± 0.26	8.57 ± 0.16
16:1(n-7)	0.16 ± 0.02	–	–
18:0	2.06 ± 0.13	2.06 ± 0.10	3.34 ± 0.05
18:1(n-9)	7.20 ± 0.58	8.23 ± 0.38	14.18 ± 0.26
18:1(n-7)	0.68 ± 0.07	0.57 ± 0.04	0.96 ± 0.02
18:2(n-6)	13.05 ± 1.24	15.50 ± 0.70	28.30 ± 0.53
18:3(n-3)	1.52 ± 0.16	1.67 ± 0.07	3.19 ± 0.04
20:0	0.16 ± 0.01	0.16 ± 0.01	0.27 ± 0.01
20:1(n-9)	0.19 ± 0.01	0.17 ± 0.02	0.16 ± 0.01
20:4(n-6)	0.35 ± 0.01	0.29 ± 0.01	0.20 ± 0.00
22:1(n-9)	0.16 ± 0.00	–	–
20:5(n-3)	2.14 ± 0.15	–	–
22:5(n-3)	0.20 ± 0.03	–	–
22:6(n-3)	1.70 ± 0.07	–	–

[a] Values are expressed as mean ± SEM ($n = 3$ for each emulsion).
[b] FO, emulsion containing fish oil; SO/MCT, emulsion containing a mix of soybean oil and medium-chain triacylglycerols; SO, emulsion containing soybean oil.

2.3. Cell count

Cells were seeded in 24-well plates at a density of 3×10^4 cells/well in 400 μl of medium and were incubated at 37 °C for 24 h. The medium was then aspirated off and replaced with 400 μl of medium containing the appropriate concentration of lipid emulsion. At the end of the incubation period of 72 h, floating cells were discarded. Alive adherent cells were detached using trypsin–EDTA 0.05% and counted using a haemocytometer. The number of cells was expressed as a percentage of the number of cells in wells exposed to medium alone.

2.4. Cell cycle analysis and apoptosis

Cells were seeded in 12-well plates at a density of 1×10^5 cells/well in 1 ml of medium and were incubated at 37 °C for 24 h. The medium was then aspirated off and replaced with 1 ml of medium containing lipid emulsion at different concentrations. Cells were then incubated for 48 or 72 h; every 24 h 0.5 ml of fresh medium was added. At the end of the incubation period floating cells were collected. Adhering cells were detached using trypsin–EDTA 0.05% and they were pooled with floating cells. After fixation with methanol, the cells were stained with propidium iodide and staining was analysed using a FACScalibur flow cytometer (Becton Dickinson, Abingdon, UK). Data was acquired from 10,000 events where possible and analysed using CellQuestPro software (Becton Dickinson). Apoptotic cells were identified as those within the pre-G0/G1 region. For experiments with FU (obtained from Sigma, Poole, UK) the final concentrations of FU used were 0.1, 1 and 10 μM. Experiments with FU were conducted in the presence of 14.13 ml lipid emulsion/l of medium.

2.5. Data analysis

All experiments were performed in triplicate, and results were computed as mean ± SD. Statistical analysis was carried out with SPSS software using one-way analysis of variance (ANOVA) and Bonferroni's correction applied to the post-hoc test. A value of $P < 0.05$ was taken to indicate statistical significance.

3. Results

3.1. Cell growth

The percentage of live cells after 72 h of exposure to lipid emulsion with respect to the number of live cells after using medium alone is shown in Fig. 1. One-way ANOVA showed that only the FO emulsion induced a significant decrease in percent live cells ($P = 0.010$). FO emulsion at 7.06 ml/l caused the greatest decrease (approx. 50%) compared to untreated-control cells, while the same concentration of SO emulsion induced an unexpected growth promoting effect ($P < 0.05$ versus SO at 3.52, 10.57 and 14.13 ml/l). Although treatment with the SO/MCT emulsion tended to decrease the percentage of live cells, no statistical significance was observed ($P = 0.055$; one-way ANOVA). No differences were observed between emulsions at the same concentration except at 7.06 ml/l,