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Original article

Plasma and erythrocyte uptake of omega-3 fatty acids from an intravenous fish oil based lipid emulsion in patients with advanced oesophagogastric cancer

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SUMMARY

Background: It has been demonstrated that short term intravenous (IV) administration of omega-3 polyunsaturated fatty acids (PUFAs) is more effective than oral supplementation at promoting incorporation of the bioactive omega-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) into plasma, blood cells and tissues. The effect of repeated short term IV infusion of omega-3 PUFAs was investigated in patients with advanced oesophagogastric cancer during palliative chemotherapy.

Methods: Patients with advanced oesophagogastric cancer (n = 21) were recruited into a phase II pilot clinical trial. All patients were scheduled for an intravenous infusion of Omegaven® (fish oil supplement containing EPA and DHA) at a rate of 2 ml/kg body weight for 4 h once a week for up to six months. Blood samples were collected to assess omega-3 PUFA uptake into plasma non-esterified fatty acids (NEFAs) and phosphatidylcholine (PC) and into red blood cell (RBC) membranes. Fatty acid profiles were analysed by gas chromatography.

Results: Twenty patients received at least one Omegaven® treatment and were included in the analysis. Each infusion of omega-3 PUFAs resulted in increased EPA and DHA in plasma NEFAs, but there was little effect on PUFAs within plasma PC during the infusions. However, with repeated weekly infusion of omega-3 PUFAs, the EPA content of plasma PC and of RBC membranes increased.

Conclusion: Repeated weekly omega-3 PUFA infusion is effective in enriching plasma PC and RBC membranes in EPA in patients with advanced oesophagogastric cancer receiving palliative chemotherapy.

Trial Registration: Clinical Trials.Gov NCT01870791.

1. Introduction

As long ago as 1863 Rudolf Virchow, after noting the presence of leukocytes in cancer specimens, proposed a link between inflammation and cancer development [1–4]. Chronic inflammation leads to release of pro-inflammatory eicosanoids which are metabolites of the omega-6 polyunsaturated fatty acid (PUFA) arachidonic acid (AA). These metabolites, which include prostaglandin E_2 and leukotriene B_4, play key roles in the initiation and propagation of colorectal, prostate, breast and pancreatic cancer [2,3,5,6]. In contrast, there is now much evidence that the omega-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have anti-inflammatory and anti-cancer properties [1,7–10].

Fish is the major dietary source of EPA and DHA and they are also found in fish oil supplements. One of the main mechanisms of their anti-inflammatory action involves opposing the production and effects of the AA-derived eicosanoids [7]. This mechanism of action

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is linked to the incorporation of EPA and DHA into cell membranes [11]. Because of the opposing actions of omega-6 and omega-3 PUFAs, both the content of omega-3 PUFAs and the ratio of omega-6 to omega-3 PUFAs in cell membranes are important determinants of their anti-inflammatory effects [12,13].

Omega-3 PUFAs may be administered by oral, enteral or parenteral means [14–16]. Carpentier et al. reported that intravenous (IV) infusion of a blend of 80% medium-chain triacylglycerol and 20% fish oil into healthy volunteers led to an increase in EPA in platelet and white blood cell phospholipids within 60 min and that the observed enrichment remained for 48 h [17]. Another study demonstrated incorporation of omega-3 PUFAs from IV fish oil into plasma lipids and red blood cell (RBC) membranes in patients with advanced pancreatic cancer [18]. A study in rats showed that short term IV infusion of omega-3 PUFAs is more effective than oral supplementation at promoting incorporation of the bioactive omega-3 PUFAs EPA and DHA into plasma, blood cells and tissues [19]. Hence, we investigated the effect of once-weekly infusions of a fish oil-based lipid emulsion (Omegaven®) for six months in patients with advanced oesophagogastric cancer receiving palliative chemotherapy. The outcomes were appearance of EPA and DHA in two plasma lipid fractions (i.e., non-esterified fatty acids (NEFAs) and phosphatidylcholine (PC)), and in RBC membranes.

2. Methods

2.1. Study design

This was a prospective, single arm clinical trial, evaluating the effect of using intravenous (IV) omega-3 PUFAs in patients with advanced oesophagogastric cancer receiving conventional platinum-based palliative chemotherapy.

2.2. Participants and setting

The study recruited adult patients referred to the University Hospitals of Leicester NHS Trust Oesophagogastric Cancer Service, Leicester, United Kingdom with confirmed diagnoses of oesophageal or gastric cancer. Inclusion criteria were, amongst others, patients with inoperable oesophageal, junctional or gastric cancer eligible for palliative platinum-based chemotherapy. Treatment intent along palliative lines was determined after discussion at the weekly multi-disciplinary team meeting by the clinical team.

2.3. Recruitment

The study received approval from the National Research Ethics Service East Midlands - Nottingham 2 Committee (reference number 11/EM/0412). Eligible participants were offered a participant information sheet at their first oncology clinic visit. A minimum of 24 h later potential participants were contacted to enquire about trial participation. Participants were recruited between 1 May 2012 and 31 July 2013. Participant follow-up was continued for one year from the date of the last treatment, disease progression, or death. All participants provided written informed consent for trial inclusion.

2.4. Sample size

As this was a pilot and feasibility study, the sample size was selected on pragmatic grounds to make an estimate of recruitment, retention and drug toxicity. Using the Simon two stage model [20], the intention was to recruit 21 participants for the first stage of the study, perform interim analysis and proceed to recruitment of a further 24 participants, provided that eleven or more participants achieved a six month progression free survival. Here the findings for the first 21 trial participants are reported.

2.5. Intervention

Participants received palliative chemotherapy with IV epirubicin (50 mg/m^2) and oxaliplatin (130 mg/m^2) every 21 days and oral capecitabine (1250 mg/m^2) daily for 21 days [21]. This is standard practice for care of these patients in the UK. For the trial, this regimen was coupled with IV infusion of omega-3 PUFAs as Omegaven® (FreseniusKabi, Bad Homburg, Germany). Omegaven® was infused once weekly at a rate of 2 ml/kg body weight for 4 h (i.e., 140 ml over 4 h in a 70 kg patient). Omegaven® is a 10% fish oil lipid emulsion described by the manufacturer as containing 1.25–2.82 g/100 ml EPA and 1.44–3.09 g/100 ml DHA. Chemical analysis by gas chromatography revealed the EPA and DHA contents of the batch of Omegaven® used in the current study to be 2.0 and 2.3 g/100 ml, respectively. Thus, patients received 0.04 and 0.046 g EPA and DHA/kg body weight during each 4 h infusion; in a 70 kg patient this would equate to 2.8 g EPA and 3.2 g DHA during each infusion. Omegaven® was administered via a peripheral venous line immediately after the chemotherapy treatment on day 1 of each cycle and then again on days 8 and 15 of the cycle. Blood samples were collected prior to and immediately after each infusion for analysis of PUFAs in plasma NEFAs and plasma PC.

2.6. Outcome measures

The fatty acid composition of plasma NEFAs, plasma PC and RBC membranes was analysed over the entire treatment period of six months. Blood samples were taken immediately prior to and within 15 min of completion of each cycle of Omegaven® infusion (Fig. 1). Plasma was prepared from all blood samples while RBCs were prepared only from the pre-infusion blood samples. Blood was collected into EDTA and plasma isolated by centrifugation at 1300 × g for 10 min. RBC membranes were isolated from the pellet by addition of serial dilutions of phosphate buffer saline (PBS) and centrifugation after each at 1300 × g for 10 min. All samples were stored at −80 °C until analysed. Clinical outcomes will be reported separately.

2.7. Fatty acid analysis by gas chromatography

Total lipid was extracted from plasma and RBC membranes with chloroform:methanol (2:1 vol/vol); butylated hydroxytoluene (50 mg/l) was added as an antioxidant. NEFAs and PC were isolated from the plasma lipid extract by solid phase extraction (SPE) on Bond-Elute cartridges. The lipid extract was loaded onto the SPE cartridge and triacylglycerols and cholesteryl esters were eluted with chloroform and discarded. Next, PC was eluted with chloroform:methanol (60:40, vol/vol) under vacuum suction. Finally, NEFAs were eluted with chloroform:methanol:glacial acetic acid (100:2:2, vol/vol/vol) under vacuum suction. Plasma NEFAs, plasma PC and RBC membrane lipids were dried down under nitrogen at 40 °C and then redissolved in 0.5 ml of dry toluene. Then fatty acid methyl esters (FAMEs) were formed by reaction with methanol containing 2% (vol/vol) sulphuric acid and heating at 50 °C for two hours. After cooling and neutralisation with $KHCO_3$ and K_2CO_3, FAMEs were extracted into hexane.

FAMEs were separated and identified by gas chromatography on a Hewlett Packard 6890 gas chromatograph fitted with a BPX-70 column (30 m × 0.22 mm x 0.25 μm). The inlet temperature was 300 °C. The oven temperature was initially 115 °C and this was maintained for 2 min after injection. The oven temperature was programmed to increase to 200 °C at the rate of 10 °C/min to hold at

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