Feasibility of Intermittent Fish Oil Infusions in Outpatients with Graft-Versus-Host Disease of the Digestive Tract

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Abstract

N-3 polyunsaturated fatty acids from fish oil may have immune-modulating effects in Graft-versus-Host Disease of the digestive tract (GVHD-DT). The objective of this pilot study was to investigate feasibility, safety and effects on fatty acid composition of plasma lipids and white blood cells (WBC), following intermittent fish oil infusion in outpatients with chronic GVHD-DT. Four outpatients received intermittent infusion of a 10% fish oil emulsion (Omegaven) during 4 hours, at day 1 (1.5 mL/kg), 3 (2.25 mL/kg) and 5, 8, 10 and 12 (3 mL/kg). At baseline and consecutive visits, fatty acid composition of plasma triglycerides (TG), plasma phospholipids (PL) and WBC, serum TG concentrations, routine laboratory tests, as well as adverse events were monitored. During the fish oil infusions, serum TG increased, but decreased 2 h after termination of infusion. In 3 patients, the dose of Omegaven® needed to be reduced. EPA was incorporated into plasma PL, plasma TG and WBC as of 2 days after the first infusion; peak levels of EPA were reached at the final infusion, or 2 days after. In conclusion, intermittent fish oil infusions result in incorporation of EPA in plasma and WBC, but can be complicated by a reversible increase in serum triglycerides.

Keywords: Fish Oil Emulsion; Omegaven®

Introduction

Allogeneic haematopoietic stem cell transplantation (allo-SCT) is a commonly used modality in the treatment of haematological malignancies. A significant, but rare complication of this treatment is Graft-versus-Host Disease (GVHD), when donor T cells mediate cytotoxic damage to host target organs, such as the skin, liver or digestive tract. The prevalence of GVHD is 30-40% after myeloablative allo-SCT, and at least 50% after reduced intensity conditioning stem cell transplantation (RIC) [1,2].

Two main categories of GVHD are recognized; acute and chronic GVHD [1]. By definition, acute GVHD presents within the first 100 days after the conventional myeloablative allo-SCT, and chronic GVHD emerges 100 days or longer after allo-SCT. The distinction between chronic and acute GVHD after RIC appears to be less clear.

When GVHD involves the digestive tract (GVHD-DT), symptoms include abdominal pain and cramps, nausea, gastrointestinal bleeding and dysphagia, as a result of inflammation and crypt cell degeneration [3,4]. These symptoms can cause malabsorption, with severe fluid and electrolyte losses and weight loss. Treatment of chronic GVHD-DT often requires long-term use of immunosuppressive agents (e.g. corticosteroids), supplementation of fluids, electrolytes and artificial nutrition, mostly in the outpatient setting. N-3 polyunsaturated fatty acids (PUFA) from fish oil are claimed to have immune-modulating effects, and may attenuate graft-versus-host responses. In animals, diets rich in fish oil resulted in diminished graft versus host responses [5]. Takatsuka et al. [6] described the effect of oral administration of fish oil during conventional bone marrow transplantation in humans. Fish oil capsules, containing 1.8 g/24 h of EPA, were administered from 3 weeks before transplantation up to 180 days after transplantation, while a matched control group did not receive fish oil. The results showed a lower complication rate and better overall survival in the EPA group, and at the time of maximum GVHD symptoms, the EPA group showed significantly lower levels of TNF-α, IFN-γ and IL-10, compared to the control group. As shown in another study by Takatsuka et al. [7], oral

supplementation of fish oil could also have immune-modulating effects in patients with GVHD-DT. Thus, far comparable clinical studies are not available. Given the degree of intestinal failure in patients with chronic GVHD-DT, parenteral supplementation of fish oil might be preferable over oral or enteral supplementation. However, little is known about feasibility and safety of intermittent infusion of n-3 PUFA in the outpatient setting.

The objective of this pilot study was to investigate feasibility, safety and dose-response effects on fatty acid composition of plasma lipids and WBC, following intermittent infusion of n-3 PUFA in outpatients with chronic GVHD-DT.

Materials and Methods

This study was conducted as a pilot study in outpatients with chronic GVHD-DT, after RIC allo-SCT of a human leukocyte antigen (HLA) identical sibling. The Medical Ethics Committee of the VU University Medical Center Amsterdam approved of the protocol, and written informed consent was obtained from all patients.

Patients

Patients with GVHD-DT, at least grade B International Bone Marrow Transplant Registry (IBMTR) [8], histopathological proven by biopsies, at least 3 weeks after initial conditioning of GVHD-treatment,

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using steady levels of immunosuppressive medication and low dose corticosteroids (<40 mg/24 h prednisone), n-6 PUFA intake of at least 50 g (n-6/n-3 PUFA ratio, of at least 2:1 as recommended [9,10], WHO performance status <3, fasting triglyceride level <7 mmol/L, and age 18 to 75 years, were asked to participate. Patients were excluded if they had active CMV disease, thrombocytes <50×10⁹/L, cardiac dysfunction New York Hearth Association (NYHA) classification II-IV, renal failure (creatinine clearance <40 mL/min), uncontrolled infections, or when they were HIV-positive, or allergic to fish or egg protein.

Fish oil infusions

The infusion schedule was designed to gradually reach an average dose of at least 2 g EPA per day in the second infusion week [6,7,11]. For this purpose, a 10% fish oil emulsion (Omegaven®, Fresenius-Kabi) was infused intermittently *via* a peripheral venous catheter. The fatty acid composition of Omegaven® is displayed in supplementary table 1. The aim was to infuse 1.5 mL/kg at day 1, 2.25 mL/kg at day 3, and 3 mL/kg of Omegaven® at day 5, 8, 10 and 12, during 4 hours. The infusion volume was calculated according to actual body weight, rounded off to tens. The estimated weight of oedema, and/or ascites was deducted, and in case of obesity (BMI >30), body weight at BMI of 22.5 was used.

Feasibility and safety parameters

We measured vital signs (blood pressure, body temperature and heart rate) before infusion, and every hour from the start of infusion until 2 hours after infusion. Assessments of blood pressure and heart rate were performed using the Maxi Stabil 3® (Welch Allyn, Skaneateles Falls, NY, USA); body temperature was measured in the axillae by a digital thermometer (Thermoval rapid®, Hartmann, Heidenheim, Germany).

During infusion, a nurse inspected the injection site of the peripheral venous catheter. The physician recorded and classified adverse events according to Common Toxicity Criteria for Adverse Events (CTCAE v3.0) [12], during infusion and at all study visits. Routine laboratory tests were performed at baseline and once a week thereafter.

During the first 3 infusions, plasma elimination of Omegaven® was evaluated by measuring serum triglycerides at t=0 h, t=2 h, t=4 h and t=6 h. The triglyceride half-life was calculated from these plasma triglyceride concentrations, and corrected for pre-infusion concentration (t=0 h). For the remainder of the study, non-fasting triglyceride levels were analysed.

Haemostasis was evaluated by the bleeding score (CTCAE v3.0 hemorrhage/bleeding) [12], and PT and APTT tests. After the final infusion, follow-up measurements were taken twice a week, during 2 weeks.

Considerations for dose escalation or study discontinuation

The dose of Omegaven® was increased according to the protocol, if no adverse events were observed, and if serum triglyceride concentrations 2 hours after infusion were <10 mmol/L, and the infusion-related increase was <4 mmol/L. If not, the dose of the consecutive infusion was reduced by 0.75 mL/kg.

In case of a fish oil related adverse event exceeding CTCAE level 3 [12], haemostatic abnormalities (defined as APTT>50 s, PT>1.4 INR), local intolerance (e.g. erythema, oedema, swelling at injection site), or the decision to stop GVHD treatment because of therapy resistant GVHD, and/or deteriorating clinical performance, the study in the concerning patient was discontinued.

Clinical parameters

Once a week, patients filled out the Edmonton Symptoms Assessment System (ESAS), a tool that was developed to assist in the assessment of nine symptoms that are common in palliative care patients: pain, tiredness, drowsiness, nausea, lack of appetite, depression, anxiety, shortness of breath, and wellbeing. Patients were instructed to rate the severity of each symptom on a 0 to 10 scale, where 0 represented absence of the symptom and 10 represented the worst possible severity. The sum of the scores for all symptoms is defined as the symptom distress score [13].

The course of GVHD was scored once a week by standardised instruments [8,14].

Fatty acid composition of plasma lipids and WBC

At baseline and every study visit, blood samples were collected into

Patient	1	2	3	4
Age (y)	60	59	63	55
Gender	Male	Male	Male	Male
Diagnosis	MM	MM	MDS	MM
Conditioning treatment	TBI (2 Gy)	Fludarabine, Cyclofosfamide	Fludarabine, TBI (2 Gy)	TBI (2 Gy)
Allo-RIC donor	HLA-matched sibling	HLA identical sibling	HLA identical sibling	HLA identical sibling
Serum triglycerides (mmol/L) at baseline	2.9	2.1	4.7	2.2
Body weight (kg)	82.0	74.7	68.4	96.8
Diarrhoea (mL/24 h)	1000	500	500-1000	700
GVHD Liver	B	A	B	A
GVHD Skin	A	A	A	A
Amylase (U/L)[1]	102	95	34	70
Lipase (U/L)[1]	56	26	28	42
ALAT (U/L)[1]	94	63	112	131
ASAT (U/L)[1]	39	82	72	70
GGT (<U/L)[1]	776	300	1139	307
AP (U/L)[1]	165	177	212	99

MM: Multiple Myeloma; MDS: Myelodysplastic Syndrome; TBI: Total Body Irradiation; RIC: Reduced Intensity Conditioning stem cell transplantation; HLA: Human Leukocyte Antigen; GVHD: Graft-Versus-Host Disease; ALAT: Alanine Aminotransferase; ASAT: Aspartate Aminotransferase; GGT: Gamma Glutamyl Transferase; AP: Alkaline Phosphatase.

[1]Reference values: amylase: <100 U/L, lipase: <70 U/L, ALAT: <50 U/L, ASAT: <45 U/L, GGT: <45 U/L, AP: <125 U/L.

Table 1: Baseline characteristics of 4 patients with chronic GVHD-DT grade B.

7 mL glass tubes containing disodium EDTA (1 mg/mL). Plasma was immediately separated from blood cells by low speed centrifugation at 228 g for 10 min (10°C), and stored for fatty acid composition analyses. A dextran sedimentation procedure was used to isolate WBC from RBC. In brief, after the addition of Tris (pH 8.4) and 10% of a Dextran solution (50 mg/L), tubes were gently mixed and incubated at 37°C for 20 min, to allow the red blood cells to settle. The WBC-rich

Figure 1: Serum triglycerides concentration in 4 patients with GVHD-DT during intermittent fish oil infusions.

Figure 2: EPA relative weight content (%) in plasma phospholipids (PL) of 4 patients with GVHD-DT receiving intermittent fish oil infusions.

supernatant was collected (with care to avoid disturbance of the RBC layer) and centrifuged at 1430 g for 5 min. After which WBC pellet was resuspended in Tris, and again centrifuged. Finally, WBC pellet was resuspended in 1 mL of Tris and used for fatty acid composition analyses.

Analysis of fatty acid composition of plasma lipids and WBC were performed by Nutrisub (Bruxelles), in collaboration with Prof. Y.A. Carpentier, using gas chromatography, as described by Richelle et al. [15].

Statistics

Data analysis was performed using SPSS for Windows, Release 15. Descriptive statistics were carried out for evaluation of parameters at each time point.

Results

We screened 12 patients with GVHD-DT complaints after allo-RIC of an HLA-matched sibling; 4 of them deceased, 3 patients were not willing to participate, and 1 patient was not suffering from GVHD-DT, but diagnosed with an oesophageal ulcer. The 4 remaining eligible patients were included. General characteristics of patients are displayed in table 1; Table 2 summarises the administered doses of Omegaven® per patient. Results of serum triglycerides and PUFA concentrations of plasma and WBC are depicted in figures 1-5.

Patient 1

The first participant was a 60-yr-old male with stage III multiple myeloma (MM), also affecting L4 (plasmocytoma). One month after allo-SCT, he developed GVHD of the skin, liver and digestive tract, in spite of prophylactic treatment. Donor chimerism by that time was almost 100%. After 2 months on high dose prednisone treatment, chronic GVHD remained, with 3 to 9 stools daily (approximately 1000 mL/24 h), hypo-albuminenia, severe oedema
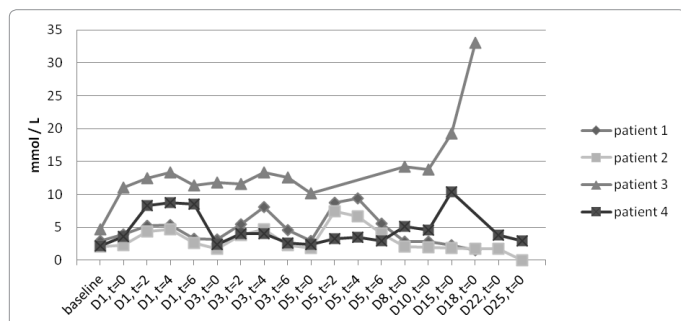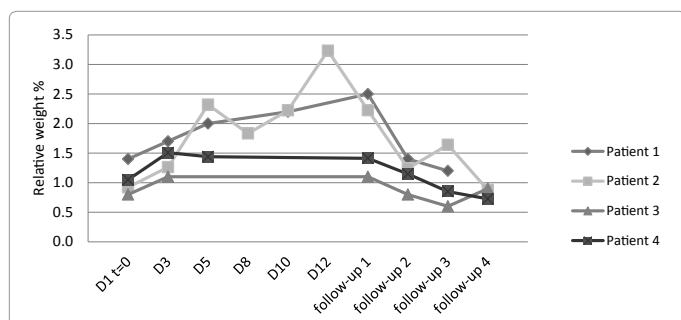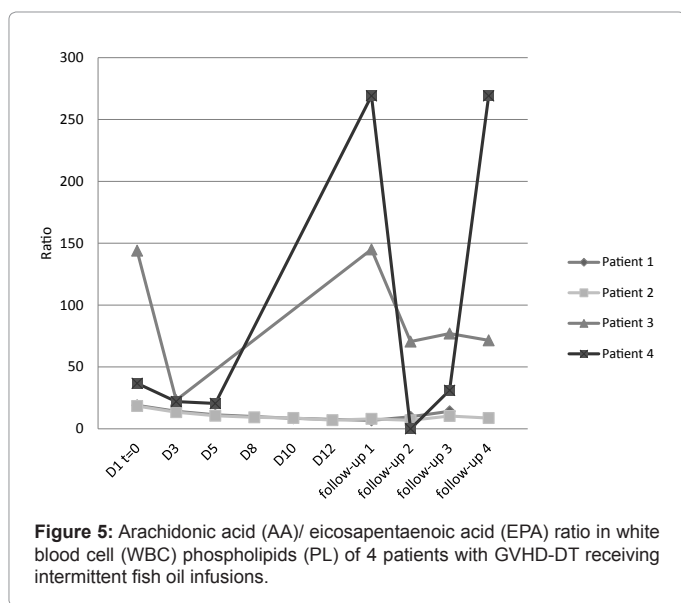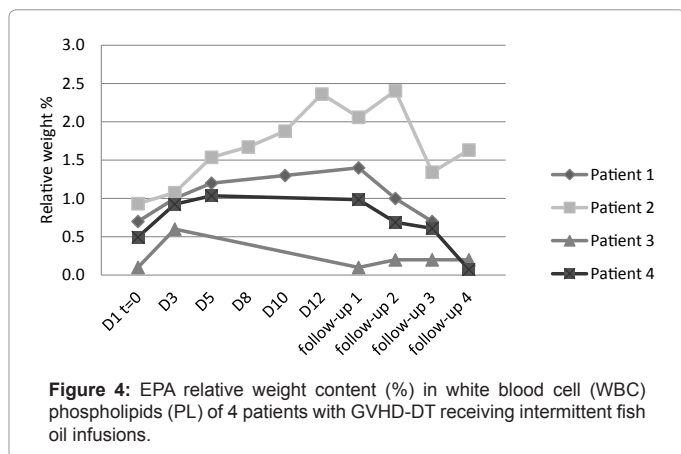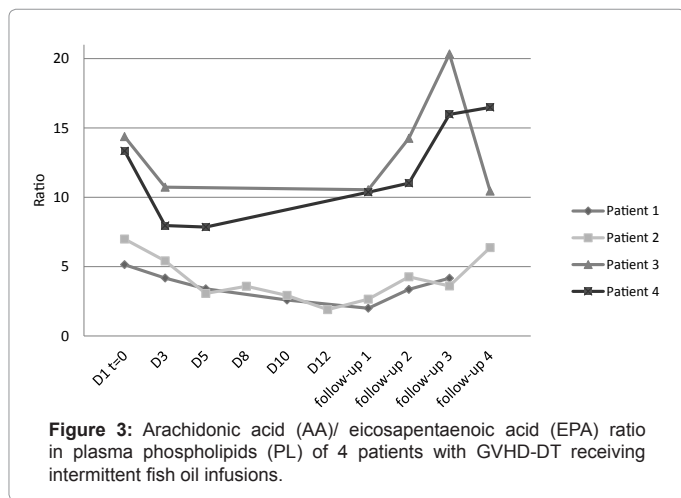
		Patient			
	Infusion dose	1	2	3	4
D1	Omegaven® (mL)	125	110	100	145
	Protocol (mL/kg): 1.5	1.5	1.5	1.5	1.5
	EPA (g)	2.6 (1.6-3.5)	2.2 (1.4-3.1)	2.0 (1.3-2.8)	3.0 (1.8-4.1)
D3	Omegaven® (mL)	185	170	50	70
	Protocol (mL/kg): 2.25	2.25	2.25	0.75	0.75
	EPA (g)	3.8 (2.3-5.2)	3.5 (2.1 – 4.8)	1.0 (0.6 – 1.4)	2.9 (0.9 – 2.0)
D5	Omegaven® (mL)	245	225	0	70
	Protocol (mL/kg): 3	3	3	0	0.75
	EPA (g)	5.0 (3.1-6.9)	4.6 (2.8-6.4)	0	1.4 (0.9 – 2.0)
D8	Omegaven® (mL)	245	225	0	0
	Protocol (mL/kg): 3	3	3	0	0
	EPA (g)	5.0 (3.1-6.9)	4.6 (2.8-6.4)	0	0
D10	Omegaven® (mL)	245	225	0	0
	Protocol (mL/kg): 3	3	3	0	0
	EPA (g)	5.0 (3.1-6.9)	4.6 (2.8-6.4)	0	0
D12	Omegaven® (mL)	0	225	0	0
	Protocol (mL/kg): 3	0	3	0	0
	EPA (g)	0	4.6 (2.8-6.4)	0	0
	Total EPA (g)	21.3 (13.1-29.5)	24.0 (14.8-33.3)	3.1 (1.9-4.2)	5.8 (3.4-8.0)
	Total EPA (g/24 h)	2.1 (1.3 – 3.0)	1.7 (1.1-2.4)	1.0 (0.6-1.4)	1.2 (0.7-1.6)

Table 2: Administered amounts of Omegaven® and EPA per patient.

Figure 3: Arachidonic acid (AA)/ eicosapentaenoic acid (EPA) ratio in plasma phospholipids (PL) of 4 patients with GVHD-DT receiving intermittent fish oil infusions.

Figure 4: EPA relative weight content (%) in white blood cell (WBC) phospholipids (PL) of 4 patients with GVHD-DT receiving intermittent fish oil infusions.

Figure 5: Arachidonic acid (AA)/ eicosapentaenoic acid (EPA) ratio in white blood cell (WBC) phospholipids (PL) of 4 patients with GVHD-DT receiving intermittent fish oil infusions.

(approximately 3 L), insulin-dependent diabetes mellitus and 7.9% involuntary weight loss. Resting energy expenditure (REE), assessed by indirect calorimetry, was 1912 kcal/24 h, and total energy intake was approximately 3000 kcal/24 h, of which 50% was delivered by nasogastric tube feeding. His total dietary fat intake was 150 g/24 h, of which 3 g of n-3 PUFA.

The patient received respectively 1.5, 2.25, 3, 3, and 3 mL/kg of Omegaven® (Table 2).

Omegaven® appeared to be well tolerated, but after the first 3 infusions, the patient experienced continuous hyperglycaemia, which also led to dehydration. By that time, blood levels of cyclosporine were too high (364 and 479 ug/L at day 5 and 8, reference value 150-300 ug/L), and cyclosporine dosage was decreased. The hyperglycaemias were treated by adaptation of the insulin dosage algorithm. The 6th infusion of Omegaven® was not administered, because of severe dyspnoea and clinical deterioration, and diagnosis of bronchiolitis obliterans and aspergillosis of the lungs, classified as a complication of immune-suppressive treatment and GVHD.

Laboratory tests showed anaemia, leucopoenia, and increased levels of ALAT, ASAT, GGT and AP at baseline and throughout the study, probably caused by GVHD of the liver. Blood amylase concentrations were slightly above reference values throughout the study, and lipase concentrations remained below reference values. With regard to hemostasis/bleeding, the patient reported grade 1 hematoma and grade 2 petechiae at all time points, and PT and APTT remained within reference ranges. The patients' perceived symptom distress scores at day 1, 8, 15 and 22 were respectively 7, 44, 20, and 31.

Patient 2

Subsequently, a 59-y-old male with MM, in complete remission 2 yr after allo-SCT, was included. At enrolment, he had insulin-dependent diabetes mellitus, chronic GVHD of the skin, liver and digestive tract, and sicca syndrome. The chronic GVHD was treated by a low dose of prednisone (5 mg/24 h). Gastrointestinal complaints consisted of 4 fatty stools daily, and a diarrhoea volume of approximately 500 mL/24 h. REE was 1697 kcal/24 h, and daily oral intake approximately 1700 kcal and 69 g of fat (of which 2 g of n-3 PUFA), without the use of ONS, tube feeding or TPV. The patients' body weight was 10.4% below his usual body weight. He had minimal oedema.

After inclusion, the patient received 6 infusions of Omegaven®, in accordance with the protocol. The patient experienced minimal adverse events: slight RR increases during the first infusion, grade 1 hematoma and grade 1 petechiae at all time points and grade 1 nosebleeds at the last study visit. His triglyceride levels remained within the described ranges. Laboratory tests showed increased levels of ALAT, ASAT, GGT and AP at baseline, and throughout the study. Blood amylase concentrations were slightly above reference values throughout the study, and lipase concentrations remained rather low. PT and APTT remained within reference ranges throughout the study. Bleeding time was 6.5 min at baseline, and at day 15, after having received all Omegaven® infusions, bleeding time was 7 min. Perceived symptom distress scores at day 1, 8, 15 and 22 were respectively 5, 7, 4, and 3.

Patient 3

The third patient was a 63 yr-old male with myelodysplasia (MDS), GVHD of the skin and digestive tract, onset 10 wk after allo-SCT, and donor chimerism almost 100%. Treatment of GVHD consisted of high dose prednisone and intensive supportive care. At enrolment, the patient had chronic GVHD with stools 5-7 times daily (diarrhoea volume 500 to 1000 mL/24 h). He had lost a substantial amount of body weight over the previous 6 months (24.2%). REE was 1500 kcal/24 h, and oral intake, supported by 2 oral nutritional supplements (600 kcal)

daily, contained approximately 2150 kcal/24 h and 83 g of fat (of which 2 g of n-3 PUFA).

During the first infusion of Omegaven® (1.5 mL/kg), systolic blood pressure increased by 20 to 35 mm Hg and heart rate decreased by 25 beats/min. Serum triglycerides increased from 11.0 to 13.4 mmol/L during infusion, and were >10 mmol/L at t=6 h. At day 3, the dose was reduced to 0.75 mL/kg. During this infusion, systolic blood pressure increased by 20 mm Hg, and triglycerides exceeded 10 mmol/L at t=6 h; therefore, we discontinued the study and switched to the follow-up schedule. During the first follow up week, the patient reported more gastrointestinal complaints and fatigue/malaise, and in the second follow-up week, he developed a pressure ulcer on the rump, increased abdominal pain and abdominal discomfort. He reported grade 1 hematoma throughout the study; ALAT, ASAT, GGT and AP fluctuated around levels beyond upper limit of reference values. Blood amylase and lipase concentrations remained far below reference values throughout the study. His perceived symptom distress scores were 37, 49, and 28 at day 1, 8, and 15.

Patient 4

The fourth patient was a 55-yr old male with MM, who was in complete remission after allo-SCT, with 100% donor chimerism. Nine months post allo-SCT, he experienced severe diarrhoea and vomiting; GVHD of the digestive tract was histopathologically proven by biopsies. Due to weight loss and intolerance for oral and enteral nutrition, total parenteral nutrition was started. Within 2 weeks, the gastrointestinal complaints reduced and the patient shifted to oral nutrition and nasogastric tube feeding.

At enrolment, the patient had diarrhoea (2-4 stools daily, approximately 700 mL/24 h), and a weight loss of 19.3% in the previous 6 months. REE was 2001 kcal/24 h, and his oral intake, supported by 2 oral nutritional supplements (600 kcal), contained around 3700 kcal/24 h and 173 g of fat (of which 5 g of n-3 PUFA).

The patient received the first dose of Omegaven® according to the protocol (145 mL), but due to hypertriglyceridemia during and after infusion, the dose was reduced to 70 mL at day 3 and 5. Adverse events included grade 1 hematoma throughout study and grade I hypertension (and reduced heart rate), at day 1, 3 and 5, which was successfully treated by one dose of Norvasc (5 mg) at day 5.

In the second week, the patient was admitted to the hospital with cyclosporin intoxication; he accidentally used 3×5 mg/24 h instead of 3×1 mg/24 h of cyclosporin, and experienced nausea and trembling. Liver function tests showed considerable increases. For this reason, it was not possible to continue the infusions. In the first follow-up week, he was dizzy, tired, hyperglycaemic, oedemateus, anorexic and depressive. Blood amylase and lipase concentrations fluctuated below reference values throughout the study. His perceived symptom distress scores were 28, 39, 54, and 54 at respectively day 1, 8, 15, and 22.

Serum triglycerides

Figure 1 displays the individual curves of serum triglycerides during the study. As expected, serum triglycerides increased during infusion of Omegaven®. Triglyceride half-life varied between patients, and depended on the administered dose of Omegaven®. Higher amounts of Omegaven® resulted in higher serum triglycerides, and a longer half-life.

At day 1, serum trigylceride concentrations returned to baseline at t=6 h. After dose increases, triglyceride concentrations at t=6 h were slightly higher than baseline at day 3, and at day 5, half-life was roughly reached at t=6 h. On subsequent visits (2 to 3 days later), serum triglycerides had returned to individual baseline concentrations. Triglyceride half-life was 42, 69, 72 and 1040 minutes in the 4 consecutive patients at day 1. The extreme high half-life in the fourth patient can be explained by the extreme small decrease (0.3 mmol/L), after the first 2 hours following infusion. At day 3 and 5, triglyceride half-life varied from respectively 84 to 129 minutes, and 101 to 132 minutes at day 5.

Fatty acid composition of plasma lipids and WBC

Figures 2-5 display the incorporation of EPA in plasma PL and WBC. In all patients, WBC and plasma PL, and TG fatty acid analyses showed dose-dependent increases in the concentrations of EPA and DHA, and decreases of AA/EPA ratios as of 2 days after the first infusion of Omegaven® (data of DHA and plasma TG not shown). Peak concentrations of EPA in plasma PL and TG, as well as WBC, were reached either at the final infusion or the first follow-up assessment (2 days after the final infusion) (Figures 2 and 4). Peak DHA in plasma PL and TG were reached during follow-up, after 2 to 8 days (data not shown). Likewise, the ratio of arachidonic acid (AA)/EPA of plasma PL, plasma TG and WBC decreased until the final infusion (Figures 3 and 5). In 2 patients, the AA/EPA ratio of plasma PL, plasma TG and WBC progressively increased during the follow-up period. In these patients, EPA concentrations during follow-up decreased to almost zero, while AA concentrations remained stable, which could be related to infections or general clinical deterioration. After the final infusion, wash out of EPA was seen after 5 to 13 days in plasma TG and PL, and after 2 to 13 days in WBC. For DHA, washout from plasma and WBC appeared to be longer, and varied from 5 to more than 13 days. Fatty acid concentrations of plasma TG showed comparable curves as those of plasma PL.

Discussion

This pilot study investigated the feasibility and dose-response effects of intermittent infusion of Omegaven® in patients with chronic GVHD-DT, and shows the rate of incorporation and washout of n-3 PUFA in plasma and WBC, after infusion of various doses of fish oil. Although only a small group of patients was able to participate, this pilot study obtains useful information on n-3 PUFA administration in a complex patient group, suffering from malabsorption and nutritional issues.

The idea of n-3 PUFA administration in patients with GVHD originated from the supposed immune-modulating effects, which could translate into clinical benefits with regard to nutritional status, morbidity and mortality. Animal and human studies showed incorporation of n-3 FA to be associated with a reduction of pro-inflammatory cytokines and PGE2, after supplementation of at least 2 g of EPA per day in healthy subjects [16,17], patients with cancer [18-20], sepsis [21] and ARDS [22-24]. Supplementation of a lower dose of EPA did not modulate immune function in humans [25-27]. Small clinical studies from a Japanese group showed the relationship of GVHD and inflammation, and the probable beneficial effects of EPA [6,7]. Apart from these studies, clinical trials supplementing n-3 PUFA in patients with GVHD-DT have not yet been performed.

A major concern in patients with GDVHD-DT is the intestinal malabsorption of nutrients [3,28,29], also resulting in a reduced intestinal absorption of n-3 PUFA, when supplemented by oral or enteral nutrition. Parenteral administration of n-3 PUFA is not hindered by malabsorption, and appeared to be a better option for patients with

GVHD-DT. These patients are often in need of intensive medical support, and need to visit the outpatient day-care unit twice a week, or are admitted to the hospital. Therefore, it appeared to be possible to carry out intermittent fish oil infusions in outpatients 3 times a week. To administer an average infusion dose of 2 g EPA per day, dose escalation to 4 g EPA per 4-h infusion was required. To reach this amount in 4 hours, an infusion rate above the recommended rate described in the SPC (summary of product characteristics), was required.

In the current pilot study, 2 out of 4 patients reached the average dose of 2 g EPA per day, and the protocol could be followed in only 1 patient. After Omegaven® infusions, the concentrations of EPA in plasma and WBC increased, and the AA/EPA ratio in plasma and WBC phospholipids decreased. The increase of EPA in plasma PL in this patient (around 2%) was comparable with the increase resulting in immune-modulation in earlier studies [30], but was lower than the increase reached in other studies in cancer patients [20,31]. In patient 2, monocytes were isolated to explore the incorporation and washout of n-3 PUFA from these cells. In this patient, we observed an increase of monocytes EPA after 2 weeks of Omegaven® infusions, and washout of EPA from monocytes was more extended than washout from plasma and WBC; after 2 weeks, baseline concentrations were not yet reached.

The incorporation of n-3 PUFA into plasma and cell membranes has been demonstrated in healthy subjects and various patient populations. The incorporation in cancer patients after enteral supplementation was thought to be at least 3 to 4 weeks [19,32], but an increasing number of studies show the incorporation to occur within a day [30,33,34]. Depending on the frequency of blood sampling, incorporation within one or more days was also demonstrated after parenteral supplementation of n-3 PUFA in patients with sepsis and around surgery [31]. We took blood samples 2 days after the first infusion of Omegaven®, and found incorporation of n-3 PUFA in WBC and plasma phospholipids.

The infusion rate in this pilot study (1.5 to 3 ml/kg in 4 h; 0.38 to 0.75 ml/kg/h) was beyond the maximal rate, as recommended by the producer of Omegaven® (0.5 mL/kg/h). In addition, all patients had increased serum triglycerides (>2 mmol/L) at baseline. One patient had serum triglycerides beyond the recommended level (4.7 mmol/L, recommended maximum level: 3 mmol/L).

Although triglycerides significantly increased during infusions, these increases were reversible within a few hours. Elevations in serum triglycerides could also be explained by other factors, such as GVHD of the liver. For example, the progressive increases of serum triglycerides in patients 3 and 4 occurred more than a week after their last Omegaven® infusion, and were unlikely to be related to Omegaven®. In addition, hyperlipidemia is a common side effect of cyclosporine, and was seen in 2 patients (3 out of 4 patients used cyclosporine). Moreover, inflammation is associated with alterations in lipid metabolism and a reduced expression of lipoprotein lipase (LPL), an enzyme that hydrolyses triglycerides into lipoproteins [35]. All patients experienced moderate to severe infectious complications, as a result of GVHD-DT. This could also explain hypertrigyceridemia at baseline, and the reduced clearance of trigylcerides during infusion. Hypertriglyceridemia can lead to pancreatitis, and may interfere with blood tests, and a serious complication of rapid fat infusion is the 'fat overload syndrome', characterized by sudden elevation of the serum triglyceride level associated with fever, hepatosplenomegaly, abnormal platelet function and bleeding disorders and variable end-organ dysfunction.

The hazards of transient hypertriglyceridemia (during a few hours) have not been well described; in a small group of infants, rapid infusions of a fish oil emulsion were well tolerated and no fat overload syndrome was observed [36]. Nevertheless, infusing fatty acid at a slower rate appears to be preferable.

Because our patients had a normal oral intake, we choose to supplement n-3 PUFA by a parenteral lipid emulsion, and the only option to do so was the 10% fish oil emulsion (Omegaven®). Omegaven®, a 10% n-3 PUFA emulsion with a high amount of phospholipids, is known to have a slow triglyceride clearance, and EPA and DHA may enter cells as triglycerides or partial glycerides within emulsion particles, and not as free fatty acids [9,37]. The use of a 20% n-3 lipid emulsion, which contains a lower amount of phospholipids, would already allow for more efficient triglyceride clearance [38]. Even more, emulsions with the lipid being a mix of MCT, soybean oil, olive oil and fish oil appear to have an increased plasma elimination, as compared to the standard soybean oil emulsion [37,39,40].

Other documented side effects of Omegaven® infusion are anemia, leukocytopenia, liver enzyme abnormalities and hyperglycemia, and are related to metabolic overload. We observed continuous hyperglycaemia in a patient with already known insulin-dependent diabetes, and temporary increased blood pressures during infusion, together with decreased heart rates. These adverse events were rated as mild, and could be adequately treated by insulin and Norvasc.

Administration of a high amount of n-3 PUFAs can also cause a prolonged bleeding time and an inhibited platelet aggregation. We monitored bleeding and hemostatis lab, and did not observe clinically significant changes after infusion of Omegaven®. More importantly, two patients also showed clinical deterioration due to complications associated with GVHD-DT or medicine use. Another issue was the experienced burden for the patients with GVHD-DT to visit the outpatient clinic 3 days a week, as compared with their normal schedule of 2 days a week.

Adequate treatment of GVHD-DT and participation in an intensive pilot study appears to be complicated by serious illness, fatigue and unexpected events. Intermittent intravenous supplementation of a 10% fish oil emulsion at a high infusion rate appears to be not feasible in this patient population. For patients with total parenteral nutrition, the continuous use of a formula containing n-3 PUFA would be an option, although clinical efficacy in this patient population has not yet been established.

In conclusion, intermittent fish oil infusions result in incorporation of EPA in plasma and WBC, but can be complicated by reversible increases in serum triglycerides, which may be caused by GVHD-related liver failure. Clinical implementation of n-3 PUFA administration in outpatients who do not tolerate or absorb enteral nutrition, such as patients with short bowel syndrome or GVHD-DT, deserves more extensive research, focusing on safety, clinical and immunological effects of n-3 PUFA infusion, either or not as part of total parenteral nutrition.

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