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A randomized trial of the effects of ezetimibe on the absorption of omega-3 fatty acids in cardiac disease patients: A pilot study

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SUMMARY

Background and aims: Elevated levels of circulating omega-3 polyunsaturated fatty acids like alpha linolenic acid (ALA) may be beneficial for cardiovascular health. Circulating ALA concentrations are elevated dramatically by a cholesterol supplemented diet which increases ALA bioavailability through enhanced micelle formation in the intestines. Conversely, it is possible that drugs which inhibit cholesterol metabolism in the intestine may also inhibit fatty acid absorption. The purpose of this study is to determine if a cholesterol absorption inhibitor, ezetimibe, will decrease circulating levels of ALA.
Methods and results: Cardiac patients (n = 34) between 44 and 80 years old, requiring statin therapy to regulate blood cholesterol levels, were randomly assigned to one of four groups for a 6 week trial: 1) placebo; 2) ezetimibe therapy; 3) a supplement of flaxseed oil (containing 1.0 g ALA in 2.0 g of flaxseed oil); or 4) ezetimibe and flaxseed oil supplementation. Ingestion of flaxseed oil resulted in a significant increase in circulating ALA levels (6 ug/dl) in patients who were not given ezetimibe. However, in the presence of ezetimibe, circulating ALA levels did not increase significantly even in the presence of flax oil supplementation (a decrease of 4 ug/dl). There were no significant differences amongst the groups in terms of circulating total cholesterol, LDL, HDL, triglyceride levels in the blood.
Conclusion: Ezetimibe therapy inhibited the absorption of omega-3 fatty acids. Patients receiving ezetimibe therapy may not receive the expected cardiovascular benefits from dietary supplementation with omega-3 fatty acids.
Clinical Trial Registration: NCT00955227.

1. Introduction

Cardiovascular disease (CVD) remains the leading cause of death worldwide [1]. While pharmacotherapy has become the leading primary therapy for the treatment of various cardiovascular diseases, there is a recent growing interest in elucidating the effects of natural health products for the prevention and treatment of CVD. One kind of natural health product which has demonstrated desirable effects for the treatment of CVD is omega-3 fatty acids. Alpha linolenic acid (ALA) obtained from flaxseed is an omega-3 fatty acid that possesses several cardioprotective properties in animal studies. This includes anti-atherogenic, anti-inflammatory and anti-arrhythmic capabilities and it is protective against vascular dysfunction [2–4]. Many of these cardioprotective effects have been demonstrated in human populations as well [5,6]. Furthermore, several human trials have shown a correlation of circulating ALA levels with a reduction in both fatal and non-fatal myocardial infarction [7–9]. The Lyon heart study [9], for example, found that a diet rich in ALA was associated with a significantly lower rate of recurrence of the major adverse cardiac events of cardiac death or non-fatal myocardial infarction.

If ALA is important to our cardiovascular health for primary and/or secondary prevention, then understanding factors that influence its bioavailability are critical. Previous studies have demonstrated that 2–4 weeks were required to observe an increase in ALA levels in the blood in response to a dietary supplementation with ALA [10,11]. ALA was best absorbed from the diet when ingested as flaxseed oil in comparison to whole or

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ground seed [11]. The age of the person does not appear to influence ALA bioavailability [12]. However, in animal studies, an increase in dietary cholesterol facilitated a dramatic increase in circulating ALA concentrations. It has been postulated that this effect may be due to a cholesterol-mediated increase in micelle formation in the gut [2–4,13].

If increasing the levels of cholesterol in the gut will increase omega-3 fatty acid absorption, then it is possible that the opposite is also true: inhibiting dietary cholesterol absorption in the intestine will interfere with ALA absorption. Ezetimibe is a cholesterol-lowering drug that is commonly used to treat patients at risk for CVD. Ezetimibe inhibits cholesterol absorption in the human intestine. It selectively targets and inhibits the Niemann-Pick C1-like 1 (NPC1L1) receptor [13]. The NPC1L1 is located in the jejunal intestinal brush border and is the primary regulator of sterol transport from the gut lumen to within enterocytes [14]. As a result, ezetimibe can lower LDL cholesterol, either as a monotherapy or in conjunction with statin pharmacotherapy. Clinically, ezetimibe may be utilized as an add-on therapy to statin pharmacotherapy when additional lipid lowering or a lower statin dosing is required [15–17]. While ezetimibe can reduce LDL cholesterol, several trials have demonstrated ezetimibe's failure to offer a superior reduction of clinical endpoints such as atherosclerotic lesions or mortality [18–20]. It is possible that this failure is due to other negative side effects that ezetimibe may have on risk factors associated with CVD. Recent emerging data from the IMProved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) study suggests a combination therapy of simvastatin and ezetimibe may lead to a 6.4% reduction in adverse cardiovascular events. There was no lowering of mortality rates compared to statin therapy alone, however [21]. Thus, the true role of ezetimibe in effective prevention and treatment of cardiovascular disease remains intriguing but unclear.

The purpose of our study was to determine if ezetimibe therapy will alter the bioavailability of the beneficial omega-3 fatty acid, ALA. It is hypothesized that ezetimibe administration to cardiac patients with CVD will attenuate the rise in circulating ALA levels in response to ALA supplementation with flax oil.

2. Methods

2.1. Study population

This clinical trial was registered at clinicaltrials.gov (NCT00955227). Cardiac patients were recruited from the Bergen Cardiac Centre at St. Boniface Hospital. Male and female subjects aged 18–80 years old were included in the study. Patients were further included/excluded from the study based on several criteria. For inclusion in this study, patients were required to be undergoing statin therapy at the time of initial screening. Furthermore, patients were required to attend two study visits (baseline and six weeks) and donate 10 ml of blood. Patients were also required to adhere to dietary restrictions as described below. The majority of subjects enrolled in the study had experienced a prior acute cardiac or cerebrovascular event and were undergoing aspirin, ACEi/ARB and beta-blocker therapy for the secondary prevention of CVD at time of enrollment. From the 34 subjects, 6 had diabetes (17.65%) (4 took drugs to control the diabetes, 1 controlled glucose levels with diet and 1 was pre-diabetic). All patients were undergoing statin pharmacotherapy (25 were medicated with Lipitor from 10 to 80 mg), 6 took Crestor (from 10 to 40 mg), 2 were taking Zocor (both 40 mg) and 1 person was taking Pravachol (20 mg).

2.2. Study design

This was a four arm, parallel group, randomly controlled, open-label clinical trial. The study design was approved by the University of Manitoba Research Ethics Board and the St. Boniface Hospital Research Review Committee. Cardiac patients were randomized by computer program into one of four treatment groups (all patients were maintained on statin therapy and all other regular medications throughout the study): 1) control (statins alone), or 2) plus 10 mg ezetimibe pharmacotherapy, or 3) plus 2 capsules of flax oil (containing a total of 1 g ALA in 2 g of flaxseed oil), or 4) plus ezetimibe and flax oil treatment for a total of 6 weeks. Over the time period, patients receiving flax oil ingested a total of 42 g ALA. Ezetimibe was taken orally (10 mg/day). The timing of the ingestion was not controlled. Each subject provided written informed consent prior to beginning the study. The study schedule included two blood draws, one at baseline and one at follow-up after six weeks of treatment. At each appointment, medical history, height, weight and blood were collected from each patient (Fig. 1).

Subjects were fasted for 12 h prior to their blood draw. During treatment, subjects were not allowed to ingest oils or salad dressings containing oils, as well as seafood. In addition, subjects were required to abstain from any additional source of omega-3 fatty acid supplementation. Subjects who had been ingesting omega-3 supplementation prior to beginning the study were required to undergo a one month period of abstinence from omega-3 ingestion prior to beginning treatment. Subjects were free to withdraw from participation at any time, for any reason, without penalty. This study was conducted on a volunteer basis. No reward (financial or otherwise) was given for participation.

2.3. Study procedures

2.3.1. Blood analysis

During each visit, 10 ml of blood was collected by venipuncture in tubes containing 1 mg EDTA/ml from subjects who had fasted for 12 h. Blood samples were centrifuged at 1000× *g* at 4 °C for 10 min, and plasma was then stored at −80 °C until analysis at a later date.

2.3.2. Preparation of plasma fatty acid Methyl esters

Plasma fatty acids methyl esters were measured by gas chromatography coupled with flame ionization detection as described in detail elsewhere [2–4]. In summary, plasma samples were derivatized directly to fatty acid methyl esters using 4:1 methanol:toluene as described by Lepage and Roy [22]. A Varian CP-3800 GC w/flame ionization detector and CP-Sil 88 capillary column 60 m × 0.25 mm × 0.20 μm GC apparatus was used for analysis. The oven temperature was maintained at 111 °C for 1 min, increased by 20 °C/min to 170 °C, raised again by 5 °C/min to 190 °C and finally increased by 3 °C/min to 225 °C. It was maintained at that temperature for 10 min, for a total run time of 29.62 min. Equipment was standardized using GLC 462 (Nu-Chek Prep, Inc.). The internal standard used was C19:0 (Nu-Check Prep, Inc.).

2.3.3. Cholesterol and triglyceride assays

Commercial assay kits (Thermo Electron Corporation, Waltham, MA) were used to determine total cholesterol and triglyceride levels in plasma. A separate assay kit (Biovision Inc., Mountain View, CA) was used to analyze plasma HDL levels. The remaining fraction of LDL/VLDL was calculated from total cholesterol and HDL measured values, as per assay protocol.

2.3.4. Adverse effects monitoring

During the six week follow-up visit, secondary effects associated with the treatments were monitored via questionnaire.