

Effects of Combination Treatment with Policosanol and Omega-3 Fatty Acids on Platelet Aggregation: A Randomized, Double-Blind Clinical Study

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ABSTRACT

Background: Policosanol is a mixture of long-chain primary aliphatic alcohols purified from sugar cane wax that has cholesterol lowering and antiplatelet effects. Omega-3 fatty acids (FA) have triglyceride lowering and antiplatelet effects. Combination treatment with policosanol and omega-3 FA (Ω 3FA) has been associated with significant inhibition of platelet aggregation in rabbits compared with either drug alone.

Objective: The aim of this study was to investigate the effects of combination treatment with Ω 3FA (1 g/d) and policosanol (Ω 3FA+Poli) compared with Ω 3FA (1 g/d) plus placebo (Ω 3FA+Pla) on platelet aggregation in human patients with hypercholesterolemia.

Methods: This randomized, double-blind, clinical study at the Surgical Medical Research Center (Havana City, Cuba) recruited outpatients from lipid clinics, with some atherosclerotic risk factors. Outpatients of both sexes aged 20 to 75 years with serum total cholesterol (TC) levels ≥ 5 and < 6 mmol/L were eligible to enroll. They were included in the study at the end of a 4-week diet stabilization period if their platelet aggregation to arachidonic acid (AA) was $\geq 50\%$ and serum TC level remained ≥ 5 mmol/L. Patients were then evenly randomized to receive Ω 3FA (1 g/d) + placebo or Ω 3FA (1 g/d) + policosanol (10 mg/d) to be taken PO with the evening meal for 21 days. Treatment was assigned according to a randomization code using balanced blocks and a 1:1 allocation ratio. Inhibition of platelet aggregation to AA was the primary efficacy variable, while effects on platelet aggregation to collagen and epinephrine and on lipid profile were secondary variables. Drug compliance and adverse events (AEs) were monitored. Tolerability was assessed using physical examinations and laboratory test results.

Results: Sixty-four subjects were initially enrolled. Fifty-four patients (30 women, 24 men; mean [SD] age, 58.4 [12] years, [range, 40–70 years]) met the inclusion criteria and were randomized to treatment; 2 groups of 27. After 21 days, platelet aggregation to AA was significantly inhibited in the 2 groups. Ω 3FA+Poli inhibited platelet aggregation to AA significantly by $\geq 20\%$. Platelet aggregation to AA 1.0 and 1.5 mM was inhibited with combination treatment (39.6% and 33.9%, respectively; both $P < 0.001$ vs baseline; $P < 0.001$ and $P < 0.01$, respectively, vs Ω 3FA+Pla) and with Ω 3FA+Pla (11.0% and 13.3%; both, $P < 0.001$). Combination treatment was more effective in inhibiting platelet aggregation to AA 1.0 and 1.5 mM in 28.6% ($P < 0.001$) and 20.6% ($P < 0.01$), respectively. Platelet aggregation to collagen 1 $\mu\text{g/mL}$ was significantly inhibited with combination treatment and with Ω 3FA+Pla compared with baseline (43.2% and 15.1%, respectively; both, $P < 0.001$), but the effects of combination treatment were significantly greater ($P < 0.01$). Platelet aggregation to epinephrine 0.1 mM was inhibited with Ω 3FA+Poli and Ω 3FA+Pla (34.8% and 20.1%; both, $P < 0.001$), with similar results for both groups. Bleeding time did not change significantly for either group and Ω 3FA+Pla did not significantly change the lipid profile. Combination treatment did significantly reduce levels of low-density lipoprotein cholesterol (LDL-C) (17.4%; $P < 0.001$ vs baseline, $P < 0.05$ vs Ω 3FA+Pla) and TC (10.1%; $P < 0.001$ vs baseline, $P < 0.05$ vs Ω 3FA+Pla), increase high-density lipoprotein cholesterol (HDL-C) levels (18.0%; $P < 0.001$ vs baseline), but did not significantly change triglyceride levels. Three patients (2 from the Ω 3FA+Poli group and 1 from the Ω 3FA+Pla group) withdrew from the trial, though none were due to AEs. Two patients receiving combination treatment reported mild AEs (headache). All treatments were well tolerated.

Conclusions: In these patients, policosanol (10 mg/d) administered concomitantly with Ω 3FA (1 g/d) enhanced the inhibition of platelet aggregation to AA and collagen, but not to epinephrine, compared with Ω 3FA+Pla, without significantly affecting bleeding time. Concomitant treatment was also associated with reduced levels of LDL-C and TC and raised HDL-C levels. All treatments were well tolerated. (*Curr Ther Res Clin Exp.* 2006;67:174–192) Copyright © 2006 Excerpta Medica, Inc.

Key words: policosanol, omega-3 fatty acids, platelet aggregation, antiplatelet drugs, cholesterol-lowering drugs.

INTRODUCTION

Coronary, cerebrovascular, and peripheral arterial diseases are major causes of morbidity and mortality in adults, with atherosclerosis being the main pathologic process involved in their etiology.^{1,2} Hypercholesterolemia, mainly elevated serum levels of low-density lipoprotein cholesterol (LDL-C), is a major atherosclerotic risk factor.^{3,4} Reducing LDL-C levels significantly prevents coronary events in patients with and without coronary heart disease (CHD).^{5–11} Guidelines for preventing coronary events include achieving target LDL-C levels.^{12,13}

In arterial circulation, activation of platelet aggregation is the initial event leading to blood clotting. Platelet aggregation is activated by several factors

(eg, hypercholesterolemia, hypertension, diabetes, blood turbulence), with induced functional changes in endothelial cells triggering the accumulation of lipids and monocytes and additional platelet aggregation.¹⁴⁻¹⁶ Arterial thrombi commonly develop along a ruptured atherosclerotic plaque or in areas of turbulent blood flow where platelets aggregate and are held by strands of fibrin.¹⁴ Damage to the intimal surface with denuding endothelium induces platelet adhesion, and the release of growth factors stimulates smooth muscle cell proliferation, which leads to the development of unstable, easily ruptured lesions on which thrombi form.¹⁴⁻¹⁸

Therefore, decreasing platelet aggregation can reduce the risk of thrombosis and acute coronary syndromes (eg, myocardial infarction, sudden death, unstable angina). The prophylactic use of antiplatelet drugs in patients at high risk of atherosclerosis from CHD and stroke has been clinically demonstrated, with risk reductions of major coronary and cerebrovascular events of 20% to 29%.¹⁹⁻²²

Although a variety of antiplatelet drugs is available, aspirin remains the gold standard due to its efficacy, relative safety, and low cost.¹⁵⁻²² Aspirin reduces the coronary risk of secondary prevention patients by ~25%.¹⁹ The effects of aspirin on platelet aggregation are moderate, mediated only by the inhibition of thromboxane A₂ (TxA₂) synthesis through the irreversible inhibition of cyclooxygenase activity, leaving platelet recruitment unaffected in other pathways.^{15,21} Ten percent to 20% of patients with arterial thrombosis treated with aspirin have experienced recurrent vascular events during long-term follow-up.²¹ Antiplatelet drugs (eg, ticlopidine and clopidogrel) act by inhibiting the binding of adenosine diphosphate (ADP) to its platelet receptors without involving other pathways of platelet activation.^{15,19-22} A limitation of currently available antiplatelet drugs is that none inhibits platelet aggregation against all agonists.

Policosanol is a mixture of long-chain primary aliphatic alcohols purified from sugar cane wax (*Saccharum officinarum* L) that has experimentally and clinically demonstrated cholesterol-lowering effects.²³⁻³² Policosanol inhibits cholesterol biosynthesis through the indirect regulation of hydroxymethylglutaryl-coenzyme A reductase³³⁻³⁵ by suppressing enzyme upregulation in cells cultured in a lipid-depleted medium through the inhibition of de novo synthesis and/or enzyme degradation.³⁵ In addition to its cholesterol-lowering effects, policosanol inhibits platelet aggregation^{23,26,32,36,37} and LDL-C lipid peroxidation,^{38,39} both of which help prevent atherosclerosis and thrombosis. Furthermore, policosanol is well tolerated.^{23-32,40-45} Therefore, it might be useful to investigate the possible benefits of combination treatment with policosanol and other antiplatelet or lipid-modifying drugs.

Fish oils are rich in omega-3 long-chain polyunsaturated fatty acids (FA).⁴⁶⁻⁴⁸ Consumption of omega-3 FA (Ω 3FA) is recommended for secondary coronary prevention, with antiarrhythmic mechanisms being the most convincing explanation for such preventive effects. The triglyceride (TG) lowering and antiplatelet effects of Ω -3FA could also work toward prevention.⁴⁶⁻⁵²

Considering their effects on lipid profile and platelet function, combination treatment with policosanol and Ω 3FA should be more beneficial than monotherapy with either drug, and some experimental and clinical evidence supports this idea.^{53,54} Concurrent treatment with policosanol and Ω 3FA significantly decreased levels of LDL-C, total cholesterol (TC), and TG (all, $P < 0.01$) and significantly increased high-density lipoprotein cholesterol (HDL-C) levels in rabbits ($P < 0.01$); the effects of the 2 treatments were additive.⁵³ All treatments inhibited platelet aggregation to arachidonic acid (AA), but greater effects were achieved with policosanol + Ω 3FA than respective monotherapies.⁵³ A previous clinical study demonstrated that policosanol (5 or 10 mg/d) administered with Ω 3FA (1 g/d) significantly increased the changes in LDL-C, TC, and HDL-C levels (all, $P < 0.001$), while maintaining the reduction in TG induced by Ω 3FA monotherapy.⁵⁴ Clinical studies of the effects of such combination treatment on platelet aggregation in humans, however, have not been conducted (MEDLINE search through 2006, key terms: *policosanol*, and *omega-3 fatty acids*).

The aim of this study was to investigate whether treatment with Ω 3FA 1 g/d + policosanol 10 mg/d (Ω 3FA+Poli) inhibits platelet aggregation more than Ω 3FA + placebo (Ω 3FA+Pla) in human patients with type II hypercholesterolemia.

PATIENTS AND METHODS

Study Design

This randomized, double-blind study was conducted at the Surgical Medical Research Center (Havana City, Cuba). An independent ethics review committee (Ethics & Institutional Review Board, Havana City, Cuba) approved the study protocol. Before recruitment, each patient provided informed written consent. According to study protocol, outpatients of both sexes with an atherosclerotic risk factor (CHD, stroke or transient ischemic attack, peripheral artery disease, hypercholesterolemia, hypertension, diabetes, smoking, age >45 years for men, age >55 years for women, obesity [body mass index ≥ 30], and/or family history of premature CHD) were recruited from lipid clinics and enrolled in the trial (visit 1).

Patients were advised to follow a cholesterol-lowering diet for a baseline period of 4 weeks.¹² Thereafter, their serum lipid profile was recorded at weeks 4 and 5 on diet. When blood was withdrawn the second time (week 5 on diet), aliquots were kept to determine platelet aggregation and blood safety. Eligible patients were included (visit 2) and randomized to Ω 3FA+Pla or Ω 3FA+Poli for 21 days. Patients also visited the clinic at the end of treatment (visit 3).

The Ω 3FA+Pla group was included to assess whether policosanol enhanced the inhibition of platelet aggregation induced with Ω 3FA. The double-blind design was selected to reduce subjective bias of patients and investigators.

Dosage and Administration

Eligible patients were randomized to receive Ω 3FA 1 g capsules + placebo or Ω 3FA 1 g capsules + policosanol 10 mg. Treatments were consumed PO once a day with the evening meal for 21 days. Ω 3FA capsules were provided by Rainbow & Nature, Ltd. (Sydney, Australia); their content was assessed using gas chromatography. They mainly contained eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHEA), with other Ω 3FA being present at lower concentrations.

Patients were assigned to treatment according to a randomization code using balanced blocks and a 1:1 allocation ratio. Patients received Ω 3FA in bottles and policosanol or placebo tablets in identical coded packages. The chosen dose of Ω 3FA was low to assess the potential benefits of combination treatment on platelet aggregation while avoiding the risk of a potential increase in bleeding time. The dose of policosanol was based on the results of a previous clinical study.⁵⁴ Because platelet half-life ranges from 7 to 10 days, 21 days of treatment would be sufficient to explore the antiplatelet effects of the treatment.

Enrollment Criteria

Outpatients of both sexes aged 20 to 75 years with serum TC levels ≥ 5 and < 6 mmol/L were eligible to enroll in the trial.

Inclusion Criteria

Patients were included in the study at the end of the diet-only baseline period if their platelet aggregation to AA was $\geq 50\%$ and serum TC level remained ≥ 5 mmol/L.

Exclusion Criteria

Patients were excluded at enrollment if they routinely took antiplatelet drugs or if they had a recurrent history of bleeding episodes indicating increased susceptibility to hemorrhage. Patients whose bleeding time at baseline (prior to the second visit) was ≥ 4 minutes were excluded from the trial as were pregnant or lactating women and women of childbearing potential who were not using contraceptives. Patients were also excluded if they had hepatic or renal diseases, diagnosed neoplastic diseases, inadequately controlled hypertension (diastolic blood pressure ≥ 100 mm Hg), or diabetes mellitus. Patients who had experienced any adverse event (AE) that led to hospitalization within the 3 months prior to the study were also excluded.

The reasons for study withdrawals were predefined in the study protocol and were defined as being attributable or not to AEs. Withdrawals due to AEs included all discontinuations due to AEs that occurred during the trial. Bleeding time ≥ 4 minutes was predefined as a withdrawal due to an AE. Withdrawals not attributable to AEs included unwillingness to follow-up, travel abroad, address changes impeding attendance at planned visits, and major protocol violations (ie, no consumption of study drugs for ≥ 3 consecutive days and/or consumption of any concomitant drug with recognized antiplatelet, anticoagulant, or lipid-lowering effects).

Assessment of Compliance

Compliance with study drug protocol was assessed by counting the tablets remaining at the end of the study and by patient interviews. *Satisfactory compliance* was defined as having taken $\geq 85\%$ of the scheduled tablets. Compliance with diet was assessed by interview and by weighing each patient.

Patient Assessment

At enrollment, a complete medical history was taken. Physical examinations were conducted at clinic visits 1, 2, and 3, and laboratory tests were done prior to the 3 visits. Assessment of drug compliance and AE interviews were done weekly.

Efficacy Variables

The primary efficacy variable was the reduction of platelet aggregation to at least 1 of the 2 doses of AA. Treatment was a priori and considered effective if the reduction in AA was significant and was $\geq 20\%$ compared with baseline. One treatment was considered more effective than the other if the final values and percentage changes in platelet aggregation to AA were significantly different.

Reductions in platelet aggregation to collagen and epinephrine were considered secondary efficacy variables. Each treatment was considered effective if the reductions were significant and were $\geq 20\%$ versus baseline. One treatment was considered more effective than the other if the final values and percentage changes in platelet aggregation to collagen or epinephrine were significantly different.

Due to the short duration of treatment, changes in serum lipid profile variables (LDL-C, TC, HDL-C, the ratios of LDL-C/HDL-C and TC/HDL-C) and TG were considered secondary efficacy variables. Treatments were considered effective in lowering cholesterol if the LDL-C level was reduced by $\geq 15\%$ versus baseline.⁵⁵

Tolerability

Tolerability was assessed using physical examination findings and laboratory test results. Physical safety indicators included changes in weight, pulse rate, and arterial pressure. Laboratory tests included determination of bleeding time, serum alanine aminotransferase and aspartate aminotransferase activities, and fasting serum glucose and serum creatinine levels.

Analysis of drug tolerability also included AE reports. Any undesirable experience occurring during the study, whether or not it was drug related, was considered an AE; this included AEs not present at baseline and those that worsened during the study. AEs were classified as mild, moderate, or serious according to their intensity. An AE was considered mild if it did not require discontinuation of study drug or specific treatment of the AE, and moderate if it required discontinuation of study drug and/or specific treatment of the AE. Serious AEs were those that resulted in hospitalization or death.

AEs were also classified according to their causal relationship to drug treatment as definite, probable, possible, or doubtful according to the Naranjo Adverse Drug Reaction algorithm using the following scale: doubtful = 0, possible = 1 to 4; probable = 5 to 8; definite ≥ 9 .⁵⁶ Because only 1 previous clinical study had investigated the effects of the combination treatment with $\Omega 3FA+Poli$, any AE that occurred in the study was considered related to treatment.

Laboratory Analysis

Venous blood samples were withdrawn after an overnight fast of 12 hours at weeks 4 and 5 of the baseline period, and after 21 days on treatment.

Approximately 9 mL of venous blood was collected into tubes containing 3.8% sodium citrate, with a final citrate/blood volume ratio of 1:9. Blood samples were centrifuged for 10 minutes at 900 rpm, and the layer of platelet-rich plasma (PRP) was removed. The remaining blood sample was centrifuged for another 10 minutes at 3000 rpm, and the thin layer of platelet-poor plasma (PPP) was removed.

Platelet aggregation responses to AA 1.0 mM, AA 1.5 mM, collagen 1 $\mu\text{g/mL}$, and epinephrine 0.1 mM were evaluated according to the light transmission method⁵⁷ using a dual-channel optical aggregometer (BIO/DATA Corporation, Horsham, Pennsylvania) calibrated for maximum light transmission; 500 μL samples of PPP were used from each patient at each point. Subsequently, 450 μL of PRP was added to the aggregometer at 37°C for 3 minutes. The extent of platelet aggregation was evaluated as the percentage of maximal light transmission reached within 5 minutes of the addition of each agonist.

TC and TG levels were determined using enzymatic reagent kits from Roche (Basel, Switzerland). HDL-C levels were determined from the cholesterol content in the supernatant obtained after β -lipoprotein precipitation.⁵⁸ LDL-C levels were calculated using the Friedewald equation.⁵⁹ Blood biochemistry safety indicators were determined using reagent kits (Roche). Analyses were done in a Hitachi 719 autoanalyzer (Hitachi, Tokyo, Japan) at the Surgical Medical Research Center. Systematic quality control was performed throughout the study.

Blood safety indicators were determined using reagent kits (Roche), and the analyses were performed using the same equipment used for the lipid profile.

Bleeding time was determined using a routine method. Briefly, a disposable template was placed in the center of the ear lobule. Using the template, a longitudinal cut of approximately 1 \times 5 mm was made and the blood was gently blotted from near the cut without disturbing the platelet plug. The time required to stop the blood flow was recorded, with each value rounded to the nearest 15 seconds.

Statistical Analysis

Analyses were performed according to intent-to-treat. Data of all randomized patients were included in all analyses, disregarding the degree of compliance with treatments or if they withdrew from the trial. Sample size was calculated

to detect a significant relation for a 20% difference between the reduction of platelet aggregation to AA with Ω 3FA+Pla compared with Ω 3FA+Poli. The power of the test was 80%, and $P < 0.05$ was considered significant. A sample size of 50 patients (25 per group) was found to be sufficient. Assuming a dropout rate of 5%, 53 patients needed to be included in the study.

Baseline lipid levels were the mean of the 2 consecutive values obtained after the diet-only period. Considering that platelet aggregation data are commonly variable, within-group comparisons of continuous data were determined using the nonparametric Wilcoxon signed rank test for paired samples, and comparisons between groups were determined using the Mann-Whitney U test. Categorical data were compared using the Fisher exact test. Statistical analyses were performed using Statistics for Windows release 4.2 (StatSoft, Inc., Tulsa, Oklahoma).

RESULTS

Baseline Characteristics

Of the 64 patients enrolled, 54 (30 women, 24 men; mean [SD] age, 58.4 [12] years, [range, 40–70 years]) were randomized. Ten enrolled patients were not included due to platelet aggregation values to AA 1.5 mM $<50\%$ (5 patients) and TC level below the inclusion criterion (5 patients). Fifty-one (94.4%) of the randomized patients completed the trial.

The baseline characteristics were similar in the 2 treatment groups (Table I). In addition to TC level >5.0 mmol/L, study patients were also associated with other coronary risk factors, the most frequent ($>15\%$) being age (men >45 , and women >55 years); arterial hypertension (18 patients, 33.3%); family history of premature CHD (17, 31.4%); and current smoking (9, 16.7%). The concomitant drugs used were also similar in the 2 groups.

Effects of Treatment on Platelet Aggregation

Compliance with study medication was good. With the exception of the 3 patients who withdrew from the study (all due to protocol violations), all patients fulfilled the criterion of satisfactory compliance.

Table II summarizes the effects of treatment on platelet aggregation and bleeding time. Baseline values were similar in the 2 treatment groups. After 21 days of treatment, platelet aggregation to AA 1.0 and 1.5 mM was significantly inhibited in the 2 groups. However, a reduction from baseline in platelet aggregation to AA $\geq 20\%$, the primary efficacy variable, was reached only with combination treatment.

Platelet aggregation to AA 1.0 and 1.5 mM was significantly inhibited with Ω 3FA+Poli (39.6% and 33.9%, respectively; both, $P < 0.001$ vs baseline; $P < 0.001$ and $P < 0.01$, respectively, vs Ω 3FA+Pla) and with Ω 3FA+Pla (11.0% and 13.3%, respectively; both $P < 0.001$ vs baseline). Combination treatment was more effective in inhibiting platelet aggregation to AA 1.0 and 1.5 mM in 28.6% ($P < 0.001$) and 20.6% ($P < 0.01$), respectively.

Table I. Baseline demographic and clinical characteristics of study patients (N = 54).*

Characteristic	Ω3FA+Pla (n = 27)	Ω3FA+Poli (n = 27)
Age, mean (SD), y [†]	58 (14)	58 (11)
Body mass index, mean (SD), kg/m ²	26.4 (4.8)	26.1 (3.1)
Sex, no. (%)		
Female	16 (59.3)	14 (51.9)
Male	11 (40.7)	13 (48.1)
Risk factors for atherosclerosis, no. (%)		
Males aged >45 years	10 (37.0)	9 (33.3)
Females aged >55 years	10 (37.0)	9 (33.3)
Arterial hypertension	9 (33.3)	9 (33.3)
Current cigarette smoking	5 (18.5)	4 (14.8)
Obesity	3 (11.1)	2 (7.4)
Family history of premature CHD	11 (40.7)	6 (22.2)
Concomitant drug use [‡]		
Diuretics	6 (22.2)	8 (29.6)
ACEI	4 (14.8)	2 (7.4)
β-Blockers	2 (7.4)	2 (7.4)

Ω3FA+Pla = omega-3 fatty acids 1g/d + placebo; Ω3F+Poli = Ω3FA 1g/d + 10 mg/d policosanol; CHD = coronary heart disease; ACEI = angiotensin-converting enzyme inhibitors.

*No significant between-group differences were found.

[†]Range 40 to 70 years.

[‡]Concomitant drugs used by ≥2 patients/group.

Compared with baseline, Ω3FA+Poli and Ω3FA+Pla significantly inhibited platelet aggregation to collagen (43.2% and 15.1%, respectively; both, $P < 0.001$), with combination treatment being significantly more effective than Ω3FA+Pla ($P < 0.01$). Likewise, Ω3FA+Poli and Ω3FA+Pla significantly inhibited platelet aggregation to epinephrine (34.8% and 20.1%, respectively; both, $P < 0.001$), but the between-group differences were not significant. Therefore, the effects of Ω3FA+Poli and Ω3FA+Pla on epinephrine-induced platelet aggregation were similar.

The reductions in platelet aggregation to all agonists induced with Ω3FA+Poli, including aggregation to epinephrine, were ≥20%, satisfying the efficacy criterion. In contrast, although significant, the reductions of aggregation to AA and collagen with Ω3FA+Pla were <20%. Therefore, combination treatment was more effective than Ω3FA alone in inhibiting platelet aggregation.

Bleeding times did not change significantly from baseline in either group, probably because of the variability in the final values. Two subjects in each group had final bleeding times >4 minutes.

Table II. Effects of omega-3 fatty acids (Ω 3FA) 1 g/d + placebo (Ω 3FA+Pla) and Ω 3FA 1 g/d + policosanol (Ω 3FA+Poli) 10 mg/d on platelet aggregation and bleeding time.

Treatment	Baseline, Mean (SD)	21 Days, Mean (SD)	% Change
Platelet aggregation to AA 1.0 mM, %			
Ω 3FA+Pla	81.37 (10.29)	72.45 (15.65)*	-11.0
Ω 3FA+Poli	76.93 (10.21)	46.44 (29.09)*†	-39.6†
Platelet aggregation to AA 1.5 mM, %			
Ω 3FA+Pla	87.22 (9.12)	75.58 (16.59)*	-13.3
Ω 3FA+Poli	84.59 (8.45)	55.94 (29.70)*‡	-33.9§
Platelet aggregation to collagen 1 μ g/mL, %			
Ω 3FA+Pla	78.04 (12.40)	66.23 (19.64)*	-15.1
Ω 3FA+Poli	76.07 (10.88)	43.18 (28.88)*‡	-43.2‡
Platelet aggregation to epinephrine 0.1 mM, %			
Ω 3FA+Pla	78.44 (12.25)	62.65 (21.04)*	-20.1
Ω 3FA+Poli	79.93 (12.89)	52.12 (29.38)*	-34.8
Bleeding time, min			
Ω 3FA+Pla	2.25 (0.81)	2.51 (1.08)	-
Ω 3FA+Poli	2.25 (0.73)	2.64 (1.15)	-

AA = arachidonic acid.

* $P < 0.001$ versus baseline (Wilcoxon signed rank test).

† $P < 0.001$ versus Ω 3FA+Pla (Mann-Whitney U test).

‡ $P < 0.01$ versus Ω 3FA+Pla (Mann-Whitney U test).

§ $P < 0.05$ versus Ω 3FA+Pla (Mann-Whitney U test).

Effects of Treatment on Lipid Profile

Ω 3FA+Pla did not change the lipid profile. Despite the short treatment period (21 days), Ω 3FA+Poli significantly reduced levels of LDL-C (17.4%), TC (10.1%), LDL-C/HDL-C (28.2%), and TC/HDL-C (21.2%) (all, $P < 0.001$ vs baseline and $P < 0.05$ vs Ω 3FA+Pla) and significantly increased HDL-C (18.0%; $P < 0.001$ vs baseline), while TG levels remained unchanged (Table III).

Tolerability

Treatments were well tolerated. No significant changes in safety indicators were found (Table IV). Individual values remained within normal limits. Three subjects (2 from the Ω 3FA+Poli group, and 1 from the Ω 3FA+Pla group) discontinued the study, all due to protocol violations (refusal to continue treatment).

Table III. Effects of omega-3 fatty acids (Ω 3FA) 1 g/d + placebo (Ω 3FA+Pla) and Ω 3FA 1 g/d + policosanols (Ω 3FA+Poli) 10 mg/d on the lipid profile of the study patients.

Treatment	Baseline, Mean (SD)	21 Days, Mean (SD)	% Change
LDL-C, mmol/L			
Ω 3FA+Pla	4.07 (0.62)	3.88 (0.73)*	-4.7
Ω 3FA+Poli	4.02 (0.88)	3.32 (0.89) ^{†‡}	-17.4 [§]
TC, mmol/L			
Ω 3FA+Pla	5.95 (0.69)	5.80 (0.69)*	-2.5
Ω 3FA+Poli	5.93 (0.71)	5.33 (0.76) ^{†‡}	-10.1
HDL-C, mmol/L			
Ω 3FA+Pla	1.26 (0.32)	1.35 (0.35)	7.1
Ω 3FA+Poli	1.28 (0.32)	1.51 (0.45) [‡]	18.0
TG, mmol/L			
Ω 3FA+Pla	1.68 (0.69)	1.55 (0.67)	-7.7
Ω 3FA+Poli	1.69 (0.66)	1.62 (0.67)	-4.1
LDL-C/HDL-C			
Ω 3FA+Pla	3.47 (1.11)	3.15 (1.29)	-9.2
Ω 3FA+Poli	3.37 (1.22)	2.42 (0.97) ^{†‡}	-28.2 [§]
TC/HDL-C			
Ω 3FA+Pla	5.02 (1.38)	4.62 (1.44)	-8.0
Ω 3FA+Poli	4.90 (1.30)	3.86 (1.04) ^{†‡}	-21.2 [†]

LDL-C = low-density lipoprotein cholesterol; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; TG = triglycerides.

* $P < 0.05$ versus baseline (Wilcoxon signed rank test).

[†] $P < 0.05$ versus Ω 3FA+Pla (Mann-Whitney U test).

[‡] $P < 0.001$ versus baseline (Wilcoxon signed rank test).

[§] $P < 0.01$ versus Ω 3FA+Pla (Mann-Whitney U test).

Two patients, both treated with Ω 3FA+Poli, experienced mild AEs (headache) that were classified as possibly drug related.

DISCUSSION

This randomized, double-blind, clinical study is the first to compare the effects of combination treatment with Ω 3FA+Poli versus Ω 3FA+Pla on platelet aggregation. The study participants had serum TC levels >5.0 mmol/L and a variety of other atherosclerotic risk factors.

Both treatments inhibited platelet aggregation to AA and collagen, but Ω 3FA+Poli was more effective than Ω 3FA monotherapy. The enhancement of

Table IV. Effects of omega-3 fatty acids (Ω 3FA) 1 g/d + placebo (Ω 3FA+Pla) and Ω 3FA 1 g/d + policosanol (Ω 3FA+Poli) 10 mg/d on safety indicators of study patients. Values are mean (SD).

Treatment	Baseline	21 Days
Physical safety indicator		
Weight, kg		
Ω 3FA+Pla	69.78 (18.14)	69.71 (18.28)
Ω 3FA+Poli	70.39 (11.81)	70.24 (12.12)
Pulse rate, beats/min		
Ω 3FA+Pla	69.44 (1.53)	69.92 (2.17)
Ω 3FA+Poli	69.67 (1.64)	69.40 (1.22)
Diastolic blood pressure, mm Hg		
Ω 3FA+Pla	78.89 (9.74)	78.08 (6.34)
Ω 3FA+Poli	79.26 (6.75)	78.80 (6.00)
Systolic blood pressure, mm Hg		
Ω 3FA+Pla	126.30 (9.67)	125.77 (10.65)
Ω 3FA+Poli	127.04 (11.37)	124.80 (10.46)
Blood safety indicator		
ALT, UI		
Ω 3FA+Pla	19.33 (8.66)	17.19 (6.95)
Ω 3FA+Poli	21.56 (8.46)	17.32 (5.50)
AST, UI		
Ω 3FA+Pla	23.48 (7.05)	22.15 (8.37)
Ω 3FA+Poli	22.81 (6.99)	22.44 (10.87)
Serum glucose, mmol/L		
Ω 3FA+Pla	4.55 (0.52)	4.41 (0.61)
Ω 3FA+Poli	4.82 (0.76)	4.88 (1.19)
Serum creatinine, μ mol/L		
Ω 3FA+Pla	73.96 (16.47)	74.42 (16.34)
Ω 3FA+Poli	72.93 (14.51)	75.52 (17.32)

ALT = alanine aminotransferase; AST = aspartate aminotransferase.

inhibition was >20% for platelet aggregation to AA, the primary efficacy variable, and to collagen. In the case of platelet aggregation to epinephrine, however, the inhibition achieved with the combination treatment was only 14.8% greater than that induced with Ω 3FA+Pla. Therefore, we concluded that policosanol did not enhance the inhibition of platelet aggregation to epinephrine induced with Ω 3FA monotherapy. Except for the aggregation to epinephrine, only the combination treatment group achieved reductions in platelet aggregation \geq 20% compared with baseline values; future studies should explore the effect of longer treatment periods.

Ω 3FA+Pla significantly inhibited platelet aggregation to AA 1.0 and 1.5 mM (11.0% and 13.3%, respectively), collagen (15.1%), and epinephrine (20.1%). Considering that, for the purpose of the study, the treatments had to inhibit platelet aggregation by $\geq 20\%$ to be effective, this criterion was achieved for only epinephrine aggregation. With the addition of policosanol, platelet inhibition was enhanced to 39.6% (AA 1.0 mM), 33.9% (AA 1.5 mM), 43.2% (collagen), and 34.8% (epinephrine). Therefore, combination treatment enhanced inhibition of platelet aggregation to AA 1.0 and 1.5 mM by 28.6% and 20.6%, respectively, over Ω 3FA+Pla treatment. Combination treatment also enhanced inhibition of platelet aggregation to collagen and epinephrine by 28.1% and 14.7%, respectively.

This study was not designed to assess whether the enhancement of platelet inhibition with combination treatment was due to an additive or synergistic effect; however, the magnitude of the increases suggests that the effect was additive. To assess this, further studies including groups treated with policosanol monotherapy should be conducted.

The clinical data supporting the effects of Ω 3FA on platelet aggregation and its mediators are heterogeneous, depending on the nature and dose of the aggregating agent. Treatment with low doses of Ω 3FA induces modest antiplatelet effects, while higher doses induce marked effects⁶⁰⁻⁶⁴; the effects on fibrinolysis and blood coagulability are minor and inconsistent.⁶¹ The relevance of the antiplatelet/antithrombotic effects of Ω 3FA to cardiovascular risk reduction is currently being studied.

Treatment with Ω 3FA inhibits platelet aggregation by counteracting the role of omega-6 fatty acids (Ω 6FA). Linoleic acid, an Ω 6FA supplied by a normal diet, is a precursor of AA. In cell membranes, linoleic acid is converted to active Ω 6 eicosanoids, including thromboxanes, prostaglandins, and leukotrienes, which are cellular mediators of platelet aggregation and inflammation, both key processes in atherosclerosis and thrombosis development.

The fact that TxA_2 , which is derived in platelets from AA, is a potent aggregating agent could contribute to the reduction in coronary risk induced by Ω 3FA.⁵⁰ Ω 3FA compete with AA, inhibiting the synthesis of TxA_2 and shifting to production of TxA_3 , a weak aggregating agent derived from EPA. The prostacyclin produced in the arterial wall, which opposes the action of TxA_2 , is equally active whether it comes from AA or EPA. Therefore, the reduction of platelet TxA_2 appears to be relevant, at least partially, to the antiplatelet effects of Ω 3FA.

Ω 3FA from fish oil have been associated with significant inhibition of collagen-induced platelet aggregation.⁶⁰⁻⁶⁶ The effect of 10 g/d of fish oil is thought to be equivalent to that of 325 mg/d of aspirin on platelet function.⁶¹ One hundred twenty subjects, 30 to 60 years old, with mildly elevated blood pressure and cholesterol were randomly allocated in 5 high-fat or 2 low-fat groups for 12 weeks. The 5 high-fat groups consisted of those taking 6 fish oil capsules daily, 12 fish oil capsules daily, a regimen of fish, a combination of fish + fish oil, or placebo capsules. The 2 low-fat groups took either fish or placebo capsules. All groups taking Ω 3FA reduced platelet aggregation to collagen and platelet

TxB₂ responses to collagen-induced aggregation.⁶² In healthy young males, a fish-enriched diet + fish oil, but not a fish-enriched diet + DHEA, significantly decreased platelet aggregation to collagen without altering blood coagulation factors.⁶³ Although the doses of Ω3FA recommended for inhibiting platelet aggregation have generally been ≥1 g/d, a randomized crossover study conducted in 12 healthy male volunteers suggested that a low dose of fish oil (350 mg/d) administered for 6 weeks reduced platelet aggregation to ADP.⁶⁴

The effects of Ω3FA on TxA₂ production have been found to be variable. An open study conducted in 6 healthy volunteers who supplemented their normal Western diet with cod liver oil for 5 months, was associated with decreased platelet aggregation and lowered TxA₂ levels.⁶⁵ A randomized crossover study suggested that oral administration of purified EPA or DHEA (6 g/d) to healthy volunteers did not change TxA₂ synthesis but significantly decreased platelet aggregation to collagen.⁶⁶

The inhibition of platelet aggregation induced with policosanol has been associated with a reduction in plasma TxB₂, but the association is different from that of Ω3FA in that policosanol increases prostacyclin levels.^{67,68} The molecular mechanisms of such action are not understood, though they should not involve the irreversible inhibition of cyclooxygenase, because policosanol does not reduce prostacyclin levels.^{68,69}

The results of the present study are consistent with previous findings because the differential effects of both Ω3FA and policosanol on the vascular production and/or release of prostacyclin could contribute to shifting the balance between TxB₂ and prostacyclin as occurs with combined treatment policosanol + aspirin versus aspirin alone.^{23,67,68} In addition, the enhancement of inhibition of platelet aggregation was only moderate, suggesting an additive rather than a synergistic interaction, because both policosanol and Ω3FA use the same pathway of platelet recruitment and activation.

Policosanol is less effective in ADP-induced platelet aggregation than in aggregation to AA, collagen, or epinephrine, suggesting that its action on the binding of ADP with its platelet receptors is small, if it occurs at all.

Bleeding time did not change significantly in either treatment group. However, the lack of significance of intergroup variations could be associated with the high variability of posttreatment values. Further studies are needed to explore whether combination treatment with Ω3FA+Poli enhances bleeding time more than Ω3FA alone.

We did not expect significant changes in lipid profile because of the short duration of the study. The fact that treatment with Ω3FA+Pla did not change these findings, and particularly did not lower TG levels, is consistent with the documented effects of Ω3FA on lipid profile.^{50,52}

Combination treatment with Ω3FA+Poli significantly lowered levels of LDL-C and TC and the ratios of LDL-C/HDL-C and TC/HDL-C, while increasing HDL-C, supporting the hypothesis that administering policosanol with Ω3FA might improve the lipid profile. These findings are consistent with a previous study of

the effects of Ω 3FA+Poli.⁵⁴ However, we did not expect significant changes in only 21 days of treatment, because previous studies found such changes after 4 and 6 weeks of treatment with policosanol.^{23,29}

The mean reductions in levels of TC (10.1%) and LDL-C (17.4%) in the present study were significant, but they were less than those obtained in the previous study (TC [15.7%] and LDL-C [26.2%]),⁵⁴ probably because of the short duration of this study. However, the increase in HDL-C level achieved with combination treatment (18.0%) was similar to that found in the previous trial (15.5%),⁵⁴ suggesting that after 21 days the maximum effect on HDL-C had already been achieved. Nonetheless, because only 2 clinical studies have investigated the effects of this combination treatment on lipid levels, further studies, including longer-term studies, are needed.

The fact that policosanol improves the response of LDL-C to Ω 3FA supports the potential usefulness of this combination treatment. Because LDL-C correlates well with the risk of CHD, expert guidelines indicate that cholesterol-lowering strategies should set LDL-C targets based on the global risk of each individual patient.^{12,13} The potential for a significant increase in HDL-C level is another advantage of using policosanol with Ω 3FA.²³

Treatments were well tolerated, with no significant changes in the safety indicators compared with baseline. Only 3 patients discontinued the trial, none due to AEs. Only 2 patients, both administered combination treatment, experienced AEs. Both had mild headache episodes, which were considered to be possibly associated with treatment.

CONCLUSIONS

In this study, a combination of policosanol (10 mg/d) and Ω 3FA (1 g/d) administered to adults with type II hypercholesterolemia significantly enhanced the inhibition of platelet aggregation to AA and collagen without enhancing the effect of Ω 3FA on bleeding time. The treatment also significantly decreased levels of LDL-C and TC and increased levels of HDL-C. All treatments were well tolerated.

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