# Omega-3 fatty acid ethyl esters do not improve clopidogrel associated P2Y12 inhibition in stroke patients

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## Abstract

The specific action of omega-3 fatty acid ethyl esters (OFA) in preventing cerebrovascular disease remains unknown, but research has demonstrated multiple possible mechanisms. In addition to altering lipid profiles, OFA may inhibit platelet aggregation. Clopidogrel inhibits platelets via the P2Y12 receptor. OFA may alter clopidogrel-associated platelet-inhibition via a possible combined effect on P2Y12 inhibition. To determine if OFA affects clopidogrel associated P2Y12 platelet receptor inhibition by comparing the percentage of responders in patients with cerebrovascular disease who were taking clopidogrel with or without OFA. We retrospectively reviewed data from adult patients with cerebrovascular disease or cerebral aneurysms and taking clopidogrel, who were seen at a single hospital between March 2010 to September 2011. We included 438 subjects in the study. For the 67 subjects who received loading doses of both clopidogrel and OFA, 71.6% had a P2Y12 inhibition response more than 20%, which is considered a positive response. For the 55 subjects who received just clopidogrel load, 67.2% of subjects were responders. There were 70.4% responders in the 274 subjects who were taking 75 mg of clopidogrel alone at home, and 73.8% responders in the 42 subjects who were taking both clopidogrel and OFA at home. However, these percentage differences were not statistically significant. This study did not find additional P2Y12 platelet inhibition when patients were given OFA, either given as a loading dose or taking it daily.

## Introduction

Several trials have confirmed that antiplatelet agents like aspirin, clopidogrel, and aspirin-dipyridamole do provide secondary stroke prevention.[1,2] Therefore, the use of antiplatelet agents is a mainstay of stroke management.[3] Although the readily available platelet function tests have made the determination of patients' responses to certain antiplatelets more objective, their use in management of stroke prevention remains a continued area of research.

Additionally, the use of other agents for secondary stroke prevention such as HMG-CoA inhibitors is also considered a valid addition to a post-stroke prevention regime.[3,4] In addition to these medications, other over the counter and complementary medicines have been investigated concurrently with these medications. One such class of complementary medicines includes omega-3 fatty acid ethyl esters (OFA), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

Omega-3 fatty acids are a class of fatty acids of essential nutrients, since they cannot be synthesized by humans and must be obtained in the diet. However, most western diets are rich in omega-6 fatty acids and not largely fish derived omage-3 fatty acids. The hypothesized result is that low omega-3 fatty acid diets result in high rates of poor serum lipid profiles and subsequent vascular disease, including stroke and coronary artery disease.[5-7]

The mechanism of how these fats improve serum lipid proportions and how they contribute to a less inflammatory state continues to be elucidated. One area where omega-3 fatty acids have an effect appears to be platelet aggregation. Omega-3 fatty acids appear to compete with omega-6 fatty acid enzymes that convert omega-3 fatty acids into category 3 thromboxanes and prostaglandins instead of category 1 and 2 thromboxanes and prostaglangins from omega-6 fatty acids. With less thromboxane A2 and more A3, this ultimately leads to less glycoprotein IIb/IIIa signaling resulting less in platelet activation and binding to fibrinogen.[5] Also, the P2Y12 receptor on platelets is another mechanism that can amplify the glycoprotein IIb/IIIa integrin signaling. Antiplatelet agents such as aspirin and clopidogrel inhibit the cox-2 enzyme and P2Y12 receptor, respectively, which decreases platelet-platelet aggregation.[8]

Data in the cardiac literature has shown that there was lower rate of clopidogrel resistance (non-responders) after taking OFA with clopidogrel and aspirin for 30 days compared to those taking clopidogrel and aspirin without OFA.[9] Although it is not a common practice, it has been extrapolated to give OFA to clopidogrel non-responders with cerebrovascular disease. The purpose of this study is to determine if OFA use in patients with cerebrovascular disease can improve clopidogrel associated P2Y12 platelet inhibition by comparing: i) the percentage of responders in cerebrovascular disease patients given clopidogrel load along with OFA and patients only receiving loading doses of clopidogrel; ii) the percentage of responders in cerebrovascular disease patients who were taking both a standard dose (75 mg daily) of clopidogrel and OFA with patients only taking the standard dose of clopidogrel at home.

## Materials and Methods

This is a retrospective chart review study of patients (19 to 98 years old) with cerebrovascular disease or cerebral aneurysms who received clopidogrel in a single high volume stroke center. The study was approved by Health Science IRB of the University at Buffalo. These patients were seen consecutively between March 2010 and September 2011. Only patients who were on clopidogrel prior to arrival to the hospital or started at the hospital for cerebrovascular disease were included in the study. Patients who were taking clopidogrel only for cardiac reasons, patients whose P2Y12 platelet inhibition tests were done prior to the administration of clopidogrel, and those who did not have P2Y12 platelet inhibition test were excluded.

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Additionally, patients on proton pump inhibitor medications (known competitive inhibitor of clopidogrel metabolism),[10] and those who did not have a mediation list on record were excluded. Whether patients were on omega-3 supplementation (either prescription or Over-The-Counter) prior to arrival to the hospital or started in combination with clopidogrel were recorded. From the medical records, demographic information, the dosages of clopidogrel and OFA, and patients' significant past medical histories were recorded.

The VerifyNow P2Y12 point-of-care assay (Accumetrics, San Diego, CA, USA) was used to measure platelet response. VerifyNow P2Y12 is a rapid platelet function assay designed to measure directly the effects of drugs on the P2Y12 receptor. Results of 20% or greater inhibition (PRNL237) was considered as adequate response of platelet inhibition.[11] The percentage inhibition was based on P2Y12 Reaction Units (PRU) and Base PRU, with Base as maximal platelet aggregation via thrombin receptor activating peptide pathway.

Statistical analysis was performed using Chi-square test for the categorical variables and student's t-test for the continuous variables. Multiple logistic regression model was used to investigate the effects of OFA on P2Y12 inhibition controlling hypertension (HTN), coronary artery disease (CAD), and dyslipidemia. Significance was defined as $P<0.05$. All statistics were performed by using SAS version 9.1 (SAS Inc, Chicago, IL, USA).

## Results

Among 2681 consecutive patients who were given clopidogrel during the study period, 438 patients were included in the study. Among the patients who were excluded, 1808 patients did not have clopidogrel platelet inhibition test, 15 did not receive any clopidogrel before the P2Y12 inhibition test, 109 did not have a detailed medication list on record, 268 were on clopidogrel for cardiac reasons only, and 43 were taking proton pump inhibitor. The included subjects were taking clopidogrel for either secondary stroke prevention (203, 46.3%) or stent placement in carotid or vertebral-basilar arteries (235, 53.7%).

In the 122 patients who received loading doses of clopidogrel, 67 (54.9%) received both loading doses of clopidogrel and OFA, and 55 (45.1%) received only clopidogrel loading dose. The patients in the two groups were around the same age (66.6±12.7 *versus* 67.8±12.9, respectively; Table 1). There were no significant differences in the responder rates either with OFA or without (71.6% *versus* 67.2%), or in the mean percentage platelet inhibitions (29.1% *versus* 32.5%). The frequency of patients with hypertension, dyslipidemia, diabetes mellitus, coronary artery disease, taking aspirin concurrently, and current smoking status did not differ between the two groups as well (Table 1). The loading doses of clopidogrel in the prior 24-hour and 48-hour of P2Y12 platelet inhibition test were comparable between the two groups (619.8±358.4 mg *versus* 568.6±347.2 mg for 24-hour, and 901.2±443.5 mg *versus* 785.5±342.1 mg for 48-hour).

In the 316 patients who were taking daily dose of clopidogrel (75 mg per day) at home, 42 (13.3%) were taking OFA daily (ranging from 300 mg to 4000 mg per day) as well. The age and gender between the two groups were similar (Table 2). Although the responder rate was higher in patients who were taking both clopidogrel and OFA at home (73.8%) than in patients who were not (70.4%), there was no significant difference after controlling for different variables (hypertension, dyslipidemia, and coronary artery disease) using multiple logistic regression model (P=0.522). The mean percentage of platelet inhibitions between the two groups were also not significant (41.6±28.8 for those who were taking both clopidogrel and OFA at home vs. 38.9±27.6 for those who were taking clopidogrel but not OFA at home). There were more subjects who were on both medications at home having hypertension or coronary artery disease, which were likely the reasons for them to take OFA chronically (Table 2). The frequency of patients with dyslipidemia, diabetes mellitus, on concurrent aspirin, and current smoking status did not differ between the two groups.

## Discussion

The use of OFA in patients with vascular diseases remains controversial. However, it is prescribed in patients with vascular risk factors including dyslipidemia, coronary artery disease, and ischemic stroke with some evidence showing beneficial results.[9,12-15] Our study demonstrates that OFA do not appear to impair platelet function via the P2Y12 receptor; therefore taking OFA cannot lower the rate

**Table 1. The characteristics of subjects who received clopidogrel loads.**

| | With OFA | Without OFA |
|---|---|---|
| Number | 67 (54.9%) | 55 (45.1%) |
| Age | 66.6±12.7 | 67.8±12.9 |
| Male | 40 (59.7%) | 31 (56.4%) |
| Percentage inhibition | 29.9±19.7 | 32.5±24.6 |
| Responders | 47 (70.1%) | 37 (67.2%) |
| Hypertension | 55 (82.1%) | 46 (83.6%) |
| Dyslipidemia | 52 (77.6%) | 46 (83.6%) |
| Diabetes mellitus | 30 (44.8%) | 19 (34.5%) |
| Coronary artery disease | 30 (44.8%) | 24 (43.6%) |
| On aspirin concomitantly | 62 (92.5%) | 50 (90.9%) |
| Current smoker | 16 (23.9%) | 21 (38.2%) |

OFA, omega-3 fatty acid ethyl esters.

**Table 2. The characteristics of subjects who were taking clopidogrel at home.**

| | With OFA | Without OFA |
|---|---|---|
| Number | 42 (13.3%) | 274 (86.7%) |
| Age | 72.1±12.5 | 69.5±13.6 |
| Male | 19 (45.2%) | 156 (56.9%) |
| Percentage inhibition | 41.6±28.8 | 38.9±27.6 |
| Responders | 31 (73.8%) | 193 (70.4%) |
| Hypertension | 40 (95.2%) | 224 (81.8%)* |
| Dyslipidemia | 34 (80.9%) | 187 (68.2%) |
| Diabetes mellitus | 10 (23.8%) | 89 (32.5%) |
| Coronary artery disease | 26 (61.9%) | 107 (39.1%)* |
| On aspirin concomitantly | 38 (90.5%) | 230 (83.9%) |
| Current smoker | 6 (14.3%) | 39 (14.2%) |

OFA, omega-3 fatty acid ethyl esters. *$P<0.05$.

of clopidogrel resistance. Gajos *et al*. reported that the addition of OFA to the combination of aspirin and clopidogrel significantly potentiates platelet response to clopidogrel after percutaneous coronary intervention.[9] However, in this study 5 mol/L ADP was used as the agonist when determining the clopidogrel platelet inhibition, which had been shown having higher coefficients of variation for light transmission aggregation comparing to 20 mol/L ADP as used in VerifyNow assay P2Y12 assay.[16] The definition of low responders to clopidogrel was also different. The difference of the methodology between the studies besides different patient populations might explain the difference in the results. One prior cardiac study was able to show ADP and glycoprotein IIb/IIIa inhibition with omega-3 fatty acid supplementation, but like our study in stroke patients, it was not able to show a statistically significant increase in platelet inhibition using the VerifyNow assay test.[17] The VerifyNow P2Y12 point-of-care assay calculates inhibition using measurements of light transmittance. Low light transmittance is associated with inhibited platelet function, while high light transmittance is associated with normal platelet function. It may be that light transmittance is unchanged in the type of platelet inhibition associated with omega-3 fatty acids. There appears to be conflicting evidence in the cardiac literature as to whether OFA affects platelet function depending on the tests used in the study.[17-19] Some research has indicated gender specific effects.[20] There are different hypotheses of the mechanism as to whether OFA affects platelet function, such as through blocking protein kinase signal transduction,[21] through decreasing CD40-ligand,[22] or through modulating the fatty acid composition of platelet membranes.[23] The data in the stroke or cerebrovascular literature in this area is little, if any. OFA may affect platelet function through a different mechanism which cannot be shown by checking P2Y12 inhibition test. It is also unclear whether there is a robust conversion of the OFA to resolvin and protectin molecules, as seen when OFA are used in combination with aspirin.[24-26] It is these molecules that may inhibit platelet aggregation. There are also other factors which may contribute to OFA effectiveness. It is unclear whether the pathophysiology in the pre-stroke state versus the post-stroke state would alter OFA effectiveness. It is also not clear whether the duration of OFA therapy contributes to greater platelet inhibition. Although not statistically significant, home use, and therefore longer duration of use, prior to hospitalization did result in a higher percentage of patients having adequate P2Y12 platelet inhibition. OFA may not inhibit platelet aggregation directly in that omega-3 fatty acids may have to first incorporate into the cell membranes in order to affect platelet function as previously suggested,[27,28] which will take at least 1-2 weeks for one complete platelet to turnover.

One of the limitations of the study is the different time period between the clopidogrel load and the P2Y12 platelet inhibition test. A study had shown that the achievement of sufficiently maximal platelet inhibition with clopidogrel load may not be possible in less than 3 to 4 hours, regardless of the initial loading dose.[29] By retrospective nature of this study, the formula of OFA which the subjects were taking at home were different. This could also confound the study as EPA and DHA appear to affect platelet function through different mechanisms and have different potencies.[25] Patients' compliance could have also confounded the study. Further research is needed to determine if clopidogrel and/or OFA dose and/or duration has an impact on platelet function. Other measures of platelet function, including bleeding time, immune and impedance aggregometry will be useful to measure as part of future studies. Also, aspirin along with OFA use in stroke patients should be investigated as well. Lastly, the long-term outcome of stroke patients on these combined therapies should also be studied to determine if the activity at the cellular and biochemical level is associated with an improved clinical outcome of secondary stroke prevention.

## Conclusions

This study does not completely rule out the possibility of omega-3 fatty acid inhibiting platelet function in stroke patients. However, the concomitant use of omega-3 fatty acid does not appear to improve P2Y12 associated platelet inhibition using the VerifyNow assay.

### Strengths and limitations of the study

I) Omega-3 fatty acids did not provide additional P2Y12 receptor inhibition upon platelets of patients given clopidogrel.

II) Only a single method of inhibition was measured (VerifyNow assay P2Y12 assay).

III) Another mechanism may be involved in platelet inhibition by omega-3 fatty acids

IV) Further study is need to determine if omega-3 fatty acids can have additional platelet function inhibition on patients already on an antiplatelet function medication such as aspirin or clopidogrel.

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