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Effects of low-dose aspirin and fish oil on platelet function and NF-kappaB in adults with diabetes mellitus ☆

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ABSTRACT

Introduction: Many diabetics are insensitive to aspirin's platelet anti-aggregation effects. The possible modulating effects of co-administration of aspirin and fish oil in subjects with diabetes are poorly characterized.

Participants and methods: Thirty adults with type 2 diabetes mellitus were treated with aspirin 81 mg/d for 7 days, then with fish oil 4 g/day for 28 days, then the combination of fish oil and aspirin for another 7 days.

Results: Aspirin alone and in combination with fish oil reduced platelet aggregation in most participants. Five of 7 participants classified as aspirin insensitive 1 week after daily aspirin ingestion were sensitive after the combination. Although some platelet aggregation measures correlated positively after aspirin and fish oil ingestion alone and (in combination) in all individuals, correlation was only observed in those who were aspirin insensitive after ingestion of the combination.

Conclusions: Co-administration of aspirin and fish oil may reduce platelet aggregation more than aspirin alone in adults with diabetes mellitus.

1. Introduction

Aspirin has long been a stalwart and inexpensive therapy for the prevention of cardiovascular disease (CVD) as well as in the acute treatment of acute myocardial ischemia [1]. It is well established that low-dose aspirin (81–162 mg/day) has the potential to reduce the rate of recurrent vascular events with a 44% reduction in risk of myocardial infarction [2,3], and is associated with a much lower risk of gastrointestinal bleeding than higher doses [4]. In the primary prevention setting, however, evidence supporting a benefit is much less clear for all individuals [5] and those with diabetes mellitus [6]. Its benefits in preventing CVD have been most widely ascribed to its antiplatelet effects as aspirin reduces the production of the very potent platelet aggregation agonist thromboxane A2 [4] through the acetylation of cyclooxygenase-1 (COX-1). However, excess thromboxane release has been shown to occur in type 2 diabetic patients with CVD [7].

Many individuals with, and at risk of, CVD events, including those with type 2 diabetes mellitus, do not derive the anticipated anti-platelet aggregation benefits of aspirin. This issue, generally referred to as aspirin insensitivity, is common and associated with an increased risk of CVD events [8]. Unfortunately, aspirin insensitivity is more common in those with diabetes mellitus than for healthy individuals [9]. Krasopoulos et al. systematically reviewed the frequency of biochemical

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insensitivity to aspirin, defined by a variety of platelet function assays, within 20 studies including a total of 2930 patients with CVD [8]. Their study concluded that 28% of individuals have biochemical aspirin insensitivity, meaning that its effects on reducing platelet function are absent. The risk for a CVD-related event in individuals with aspirin insensitivity was significantly higher than those with a normal aspirin effect (odds ratio (OR)= 3.85; 95% CI: 3.08 to 4.80), with a higher incidence of death (OR=5.99; 95% CI: 2.28 to 15.72), and an acute coronary syndrome (OR=4.06; 95% CI: 2.96 to 5.56). The molecular basis of aspirin insensitivity is poorly understood, although genetic variants including the PlA1/A2 polymorphism in the GPIIIa platelet receptor [10] and platelet activation via pathways that are not modified by aspirin [11] have been suggested to play a role. In addition, a recent study of non-diabetics found that resistance to aspirin's anti-platelet effects could be attributed to enteric coating [12].

Current evidence suggests that patients with aspirin insensitivity do not benefit from other antiplatelet drugs [8]. In addition, the combination of aspirin with other anti-platelet drugs, such as clopidogrel, is associated with a significantly higher risk of major bleeding than with aspirin alone [13–15]. In contrast, emerging evidence from clinical trials and observational studies suggests that combining the omega-3 (ω3) fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) together with aspirin may be more beneficial than using aspirin alone [16]. EPA and DHA have known anti-inflammatory, anti-platelet aggregation, and CVD preventive effects [17,18]. EPA and DHA may be especially beneficial for individuals with insulin resistance due to the underlying abnormal fatty acid and lipoprotein milieu [19], characterized by elevated circulating free fatty acids, low blood and tissue levels of ω3 fatty acids, and increased concentrations of highly atherogenic oxidized low density lipoprotein (LDL) [19–21].

However, the benefits of combining EPA+DHA with aspirin on platelet function or other inflammatory parameters have not received much attention. We hypothesized that the combination of low-dose aspirin and ω3 fatty acids of fish oil would be more effective than aspirin alone in reducing platelet aggregation and related mechanisms that potentiate the atherosclerotic process in subjects with type II diabetes.

2. Participants and methods

2.1. Participants

We enrolled 30 adults aged 40 to 80 years with type 2 diabetes mellitus based on the criteria from the Executive Committee of the American Diabetes Association Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [22]. These criteria include having symptoms of diabetes plus casual plasma glucose concentration ≥200 mg/dl (11.1 mmol/l), a plasma glucose ≥126 mg/dl (7.0 mmol/l) after an 8 h fast, or a 2-h plasma glucose ≥200 mg/dl during an oral glucose tolerance test. Glucose measurements were performed as described by the World Health Organization [22]. Participants could not take vitamins, nutritional supplements, or herbal preparations for the study duration. Exclusion Criteria were: a diagnosis of CVD including coronary heart disease, congestive heart failure, peripheral vascular disease, stroke, or atrial fibrillation; history of malignancy, except subjects who have been disease-free for greater than 10 years, or whose only malignancy has been basal or squamous cell skin carcinoma; history of peptic ulcer or gastrointestinal bleeding in the past 5 years, diagnosed bleeding disorder, use of antiplatelet or antithrombotic therapy, defined as clopidogrel, ticlopidine, cilostazol, dipyridamole, trapidil, warfarin, and argatroban, oral contraceptive use, and daily use of NSAIDs. Other exclusion criteria were a calculated creatinine clearance <60 mg/dl, signs of obstructive hepatic disease, or any other obvious metabolic disease that would influence lipid metabolism, based upon a screening complete blood count and comprehensive metabolic profile, pregnancy, surgery within 30 days of screening, history of drug or alcohol abuse, or current weekly alcohol consumption >14 units/week (1 unit=1 beer, 1 glass of wine, 1 mixed cocktail containing 1 ounce of alcohol), allergy to aspirin or fish/fish oil, and tobacco use. The use of any diabetes medications was permitted, including insulin.

2.2. Protocol

This was an 8-week sequential-therapy clinical trial. The clinical trial timeline for each subject is outlined in Table 1. All study visits were conducted at the University of Rochester's Clinical Research Center (CRC). Our study was designed to examine

Table 1
Study timeline of activities.

Day	Study visit	Activity
1		• Recruitment and screening • Collection of baseline demographic and clinical data
1–10		• Aspirin-free period of 10 days prior to Study Visit 1
Aspirin only		
11	1	• **Blood draw 1** (baseline, before aspirin) • Ingestion single 81 mg dose aspirin • **Blood draw 2** (4 h post aspirin ingestion)
12–17		• Single 81 mg aspirin/day
Fish oil only		
18	2	• **Blood draw 3** (7 days aspirin ingestion) • Begin fish oil 4g/day and discontinue aspirin unless subject takes it as prescribed by their doctor
19–45		• 28 days of fish oil 4g/day
36–45		• Aspirin-free period of 10 days prior to Study Visit 3
Aspirin+fish oil		
46	3	• **Blood draw 4** (4 weeks fish oil ingestion, before aspirin) • Continue fish oil and a single dose 81 mg aspirin • **Blood draw 5** (4 h post aspirin+fish oil ingestion)
47–52		• Continue aspirin and fish oil 4g/day
53	4	• **Blood draw 6** (7 days aspirin+fish oil ingestion) • Discontinue fish oil and aspirin