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Eicosapentaenoic acid and docosahexaenoic acid are antagonists at the thromboxane A_2/prostaglandin H_2 receptor in human platelets

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The present study investigated the mechanism by which eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) inhibit platelet activation induced by thromboxane A_2. DHA was found to be more potent than EPA in blocking platelet aggregation induced by the stable thromboxane A_2 mimetic, U46619. Furthermore, this inhibition by DHA or EPA was competitive. Binding studies using ^{3}H-U46619 demonstrated that both EPA and DHA interact with the platelet thromboxane receptor. The potency of the inhibition of binding corresponded with that seen for the inhibition of aggregation. These results suggest that thromboxane receptor antagonism may be an important mechanism by which EPA and DHA modulate platelet reactivity in vivo.

Platelet; Thromboxane receptor; Fatty acid

1. INTRODUCTION

Epidemiological studies suggest that populations whose diet contains increased amounts of seafood have a decreased incidence of occlusive vascular disease [1,2]. A possible basis for such a preventive effect could be an inhibition of blood platelet involvement in thrombosis by the marine fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Since thromboxane is considered to be an important vector in the genesis of thromboembolic disorders [3], marine fatty acid inhibition of thromboxane-mediated platelet aggregation may in part explain the reduction in the incidence of occlusive vascular disease. Support for this idea has come from in vitro studies which have shown that EPA and DHA inhibit platelet activation induced by thromboxane analogs like U46619 [4–6]. While EPA and DHA are known to inhibit cyclo-oxygenase [7], inhibition of U46619-mediated platelet activation would suggest that EPA and DHA may also act as thromboxane receptor antagonists. Based on these considerations, we investigated the ability of EPA and DHA to directly interact at the level of the platelet thromboxane receptor.

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2. MATERIALS AND METHODS

Human platelet-rich plasma was obtained from LifeSource Blood Services (Chicago). Free fatty acids were purchased from either Sigma (St. Louis) or Cayman Chemical (Ann Arbor). Platelets were isolated as previously described [8] with the exception that 10 mM Hepes was substituted for 25 mM Tris.

Platelet aggregation was measured by a turbidometric procedure [9]. Fatty acid or ethanol (final ethanol concentration was 0.1%) was added to platelets (approx. 90 s before aggregation was induced) followed by fibrinogen (100 μg/ml) and $CaCl_2$ (100 μM). The measurement of the specific binding of ^{3}H-U46619 (22.4 Ci/mmol, gift of Dr E. Do, New England Nuclear, Boston, MA) to washed human platelets was as previously described [10].

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3. RESULTS

Initially, the relative ability of EPA and DHA to inhibit platelet aggregation induced by U46619 was quantitated. It was found that both EPA and DHA inhibited U46619-induced platelet aggregation in a dose-dependent manner with DHA more potent than EPA (fig.1). The concentration of EPA necessary for 50% inhibition (IC_{50}) was 5.9 ± 0.8 μM (n = 3) while the IC_{50} for DHA was 2.2 ± 0.6 μM (n = 4). A saturated fatty acid, eicosanoic acid (20:0, EA), had little effect on aggregation (fig.1).

We next determined whether the observed inhibition of aggregation was competitive or non-competitive. The extent of aggregation was plotted as a function of the dose of U46619, and these dose-response curves were constructed in the absence and presence of various doses of EPA or DHA. These data were transformed by the method of Arunlakshana and Schild [11]. This analysis revealed slopes not significantly different from one suggesting that DHA and EPA are competitive inhibitors of U46619-induced aggregation at the level of the thromboxane receptor (fig.2). Thromboxane receptor antagonism was further evaluated by performing direct binding studies using radiolabeled U46619.

EPA and DHA both inhibited specific binding of ^{3}H-U46619 in a dose-dependent manner (fig.3). Again, DHA was more potent (IC_{50} = 1.5 ± 0.22 μM; n = 4) than EPA (IC_{50} = 5.6 ± 1.02 μM; n = 5). These IC_{50} values for inhibition of binding are not significantly different from the IC_{50} values seen for inhibition of aggregation. Hence, a large part of the functional inhibition can be explained by the inhibition of receptor binding. Again, the saturated fatty acid, eicosanoic acid (EA), had little effect on binding.

In order to determine whether the observed inhibition of ^{3}H-U46619 binding by EPA and DHA was due to cyclo-oxygenase [12] or lipoxygenase [13] products, additional binding studies were performed in the presence of the cyclo-oxygenase inhibitor indomethacin or the lipoxygenase inhibitor BW755c. We observed that neither indomethacin nor BW755c significantly reduced the inhibitory potency of EPA or DHA demonstrating that the

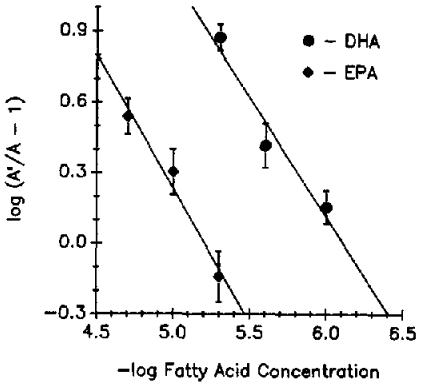

Fig.2. Schild transformation of DHA (circles) and EPA (diamonds) effects on U46619 induced-aggregation dose-response curves. Individual plot slopes were −1.01 ± 0.099 for DHA and −1.13 ± 0.255 for EPA.

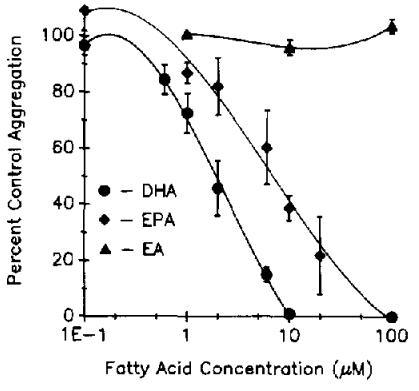

Fig.1. Inhibition of U46619-induced platelet aggregation by DHA (circles), EPA (diamonds) and EA (triangles). The dose of U46619 was that which just caused maximal aggregation and varied between 40 and 160 nM.

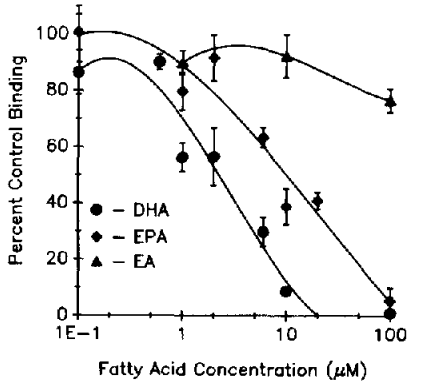

Fig.3. Inhibition of specific ^{3}H-U46619 binding by DHA (circles), EPA (diamonds), and EA (triangles).

parent fatty acids are responsible for the observed effect (not shown).

4. DISCUSSION

The present studies demonstrate that the unsaturated fatty acids DHA and EPA inhibit U46619-induced platelet aggregation in a competitive manner and block the interaction of ^{3}H-U46619 with the platelet thromboxane receptor. One possible explanation for the observed inhibition of aggregation is that unsaturated fatty acids cause a generalized membrane perturbation such as an increase in membrane fluidity [14,15]. However, not all studies have been able to show an association between increased membrane fluidity and inhibition of platelet aggregation [16]. Furthermore, a generalized membrane perturbation would not seem to explain the unique effects EPA and DHA have on thromboxane-mediated platelet activation. For example, EPA is more effective in inhibiting U46619-induced platelet aggregation than aggregation induced by other agonists [4]. In addition, the competitive nature of the functional inhibition as revealed by Schild analysis suggests a direct interaction of EPA and DHA with the platelet thromboxane receptor. These arguments do not exclude the possibility that at higher doses and with other platelet aggregating agents, the inhibitory action of unsaturated fatty acid is based on the ability to increase membrane fluidity. However, the data do suggest that there is a particular sensitivity of thromboxane-mediated platelet activation to the presence of unsaturated fatty acids like EPA and DHA.

In conclusion, EPA and DHA are capable of directly inhibiting agonist interaction with the thromboxane receptor. This inhibition of binding is presumably responsible for a major portion of the functional effects of these fatty acids on thromboxane-mediated platelet activation and may serve as a mechanism for their antithrombotic effects.

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