Modulation of human platelet function by food flavonoids

COLLEEN KELLY, KIRSTY HUNTER, LYNN CROSBIE, MARGARET J. GORDON and ASIM K. DUTTA-ROY

Division of Biochemical Sciences, Rowett Research Institute, Aberdeen, AB2 9SB, Scotland, U.K.

Flavonoids, a group of polyphenolic natural compounds, represent an important source of antioxidants in the human diet [1,2]. Although the mechanism of action of flavonoids is not well understood, these compounds have been found to be inversely related to mortality from coronary heart disease[3]. Aggregation of platelet is fundamental to a wide range of physiological processes, i.e. in the events ranging from normal blood coagulation to the extremes of thrombosis and atherosclerosis [4,5], therefore the aim of this study was to investigate the effects of different dietary flavonoids (catechin, myricetin, quercetin, apigenin and morin) and α-tocopherol on platelet function. Platelet rich plasma (PRP) was prepared from blood collected in sodium citrate (10% v/v of 3.8% citrate solution) from healthy volunteers who had not taken any medication for at least 14 days prior to phlebotomy. Platelets were counted in PRP and adjusted to a constant platelet count (2.5x10^5/ml) with platelet poor-plasma (PPP), obtained by further centrifugation of the blood remaining after removal of PRP. Washed platelets (WP) were prepared from PPP as described [6].

Aggregation of platelets was induced by either collagen or ADP. Maximum amplitude of aggregation of PRP was obtained with 10μg/ml collagen and 10 μM ADP, whereas WP were aggregated with collagen only. The inhibitory effects of flavonoids and vitaomin E and C were initially evaluated at different times of preincubation either with PRP or WP with the compound dissolved in ethanol-water at a final ethanol concentration of 0.13%. Control samples were preincubated with vehicle alone.

Aggregometric responses in the absence or presence of these compounds were evaluated by measuring the amplitude curves with different concentrations of these compounds in three separate experiments using platelets from different subjects. Plasma levels of fibrinogen, vitamin E and vitamin C of these volunteers were determined and were found to be within the normal range. Apigenin, quercetin, morin and α-tocopherol did not inhibit platelet aggregation in PRP induced by collagen, ADP, or arachidonic acid (AA) (n=5). Conversely, catechin inhibited collagen-induced aggregation by approximately 4.3% (n=7) whereas myricetin inhibited AA-induced aggregation by 75%, ADP-induced aggregation by 21% and collagen-induced aggregation by 5% in PRP (n=2). In contrast to PRP, aggregation of WP was significantly inhibited by these flavonoids except apigenin and α-tocopherol (Table). Minimum concentration of flavonoids required to inhibit 50% platelet aggregation (IC50) induced by collagen was determined for these flavonoids. The IC50 value for myricetin was 37.6 μM whereas for morin and catechin the values were very high, 322278 μM and 5912 65 μM (n=3), respectively.

Table 1. % Inhibition of platelet aggregation by various flavonoids in WP

<table>
<thead>
<tr>
<th>Flavonoids</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen (10μM)</td>
<td>Thrombin (1.5 U/ml)</td>
</tr>
<tr>
<td>Catechin (420 μM)</td>
<td>63.9±7.2 (n=5) 0 (n=3)</td>
</tr>
<tr>
<td>Morin (420μM)</td>
<td>85.1±3.0 (n=3) 0 (n=2)</td>
</tr>
<tr>
<td>Myricetin (100μM)</td>
<td>79.5 ± 5.1 (n=3) 6.5±6.5 (n=2)</td>
</tr>
<tr>
<td>Quercetin (40μM)</td>
<td>32.5±12.5 (n=2) Not investigated</td>
</tr>
</tbody>
</table>

To determine whether the inhibitory effect on platelet aggregation was due to the reduced synthesis of thromboxane A2 (TXA2), the levels of TxB2, the inactive metabolite of TXA2, were measured in WP in the presence and absence of flavonoids. TxB2 levels in collagen stimulated WP were markedly elevated above basal levels (without collagen). Incubation of WP with these flavonoids inhibited TxB2 production induced by collagen to different degrees; catechin (420μM) was least effective in inhibiting TxB2 production (48%) whereas morin (420 μM) and myricetin (100μM) inhibited TxB2 production by 65% and 95%, respectively, compared with the controls. Our data show that some of the flavonoids used in this study inhibited platelet aggregation in WP very strongly but had little effect in PRP. This could be the results of the proteins present in PRP which may reduce the inhibitory effects of these flavonoids possibly through binding. Among the flavonoids, myricetin is the most potent inhibitor of platelet aggregation whereas apigenin and α-tocopherol had no affect. The inhibition of platelet aggregation in WP by the flavonoids was associated with reduced TxA2 production. To determine whether the modification of platelet membrane properties by the flavonoids was associated with the inhibition of platelet aggregation, membrane anisotropy of WP in the presence and absence of flavonoids was investigated using 1,6-diphenyl-1,3,5-hexatriene as a fluorescence probe, as described [7]. There was no significant change observed in the membrane fluorescence anisotropy in catechin-treated membranes (0.23±0.004, n=2) compared with that of control membranes (0.22±0.005, n=2). These data indicate that membrane fluidity is not involved in the flavonoid-mediated inhibition of platelet aggregation.

Flavonoids have very complex effects on cell metabolism [1,2]. Many of the flavonoids are capable of modifying AA metabolism due to their anti-oxidant properties[1,2]. Oxidative processes, resulting in the formation of free radicals and the generation of lipid peroxides, occur in tissues and cells under various conditions. Lipid peroxides are also produced during the reactions involved in eicosanoid metabolism in platelets and other tissues. The major lipid soluble antioxidant with vitamin properties present in tissues, α-tocopherol [8], has been shown to affect eicosanoid synthesis by platelets very marginally [9]. However, the effect of vitamin E on platelet function is highly controversial [10,11]. In fact, several antioxidants with potent inhibitory activity on the non-enzymatic peroxidation of lipids, such as flavonoids and various simple phenols, have been shown to inhibit the 5-lipoxigenase pathway and cyclooxygenase pathways with a potency that is not correlated with their antioxidant activity [9]. Flavonoids have also been reported to exert their anti-platelet action through modulating platelet prostacyclin (PGI2/PGE1) receptor activity as well as the cAMP phosphodiesterase activity [1,10,12].

In conclusion, our data clearly indicate that pure flavonoids inhibit platelet aggregation by reducing TxA2 formation at different degrees. However, plasma proteins may significantly reduce the inhibitory effect of the pure compounds.

This work was supported by the Scottish Office Agriculture and Fisheries Department.