Abstract

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Title of Doctoral Thesis:	The influence of polyphenolic compounds and their metabolites on platelet activation

Blood aggregation is a very difficult and complex process, in which platelets play crucial role. On the other hand, their activation is excessive and therefore undesirable under pathophysiological conditions. Cardiovascular diseases are the most common cause of mortality in developed countries and unfortunately, despite significant advances in treatment, the Czech Republic remains in mortality from these diseases behind most Western European countries. Epidemiological studies suggest, that higher intake of (iso)flavonoids is associated with reduced cardiovascular mortality. It is also worth mentioning, that the Czech Republic is among European countries with the lowest content of flavonoids in diet.

The aim of this doctoral thesis was to find out, if polyphenolic compounds, especially (iso)flavonoids and/or their metabolites, are able to influence platelet aggregation and thus indirectly to support epidemiological findings, i.e. whether there may be a link between these substances and possible positive cardiovascular effect at experimental level.

In summary, 16 isoflavonoids, 4 isoflavonoid metabolites, 29 flavonoid metabolites and 12 synthetic xanthen-3-ones were tested in this thesis. At first, the screening was performed on aggregation induced by arachidonic acid (AA) and collagen with using of impedance aggregometry in whole human blood. In the case of very active substances, the mechanism of action was also tested: determination of cyclooxygenase-1 and thromboxane synthase inhibition, antagonism at thromboxane receptors, calcium signalling cascade interference or serotonin release inhibition. These experiments were performed principally by ELISA determination of final products or again by impedance aggregometry.

First, the antiplatelet potential of isoflavonoids, which were the most effective flavonoids in previous studies, was tested. The good inhibitory effect of genistein and daidzein has been confirmed, but now also in whole human blood, i.e. under more clinically relevant biological conditions. Subsequently, these compounds inhibited platelet cyclooxygenase-1 and acted as antagonists at thromboxane receptors. Antagonism at these receptors has also been established as the major mechanism for the most potent compound, tectorigenin, whose IC_{50} values were three times lower than that of the standard, acetylsalicylic acid (ASA). Due to the poor bioavailability of parent compounds after oral administration due to their poor penetration into the gastrointestinal cells and/or their rapid metabolism in these cells, the proven isoflavonoid metabolites were tested. Of these, *S*-equol has shown the greatest effect that was similar to ASA on collagen induced aggregation. Another active metabolite was 4-ethylphenol, which was able to partially inhibit cyclooxygenase-1, antagonize thromboxane receptors and affect calcium homeostasis. The metabolite O-desmethylanolensin showed a lower effect, but shares the same mechanisms of action with 4-ethylphenol. In contrast, no antiplatelet effect was observed for (2RS)-2-(4-hydroxyphenyl) propionic acid.

Based on pharmacokinetics of flavonoids and the fact, that these metabolites reach higher concentrations than parent flavonoids in plasma, these GIT metabolites were also tested. Only four compounds from 29 were able to inhibit platelet aggregation induced by AA; 4-methylcatechol, pyrogallol, resorcinol and phloroglucinol. IC_{50} of 4-methylcatechol were approximately ten times lower than for ASA, both for aggregation induced by AA and by collagen. This compound was able to partially inhibit thromboxane synthase, but we assume that the main mechanism of action is based on influence on calcium kinetics. 4-methylcatechol and phloroglucinol are among other proven metabolites of the most commonly occurring flavonol quercetin. The effects of 4-methylcatechol and pyrogallol were also confirmed in a new *in vivo (ex ovo)* model of thrombosis. In this experiment, platelet aggregation was induced by local (topical) administration of AA. This resulted in 46 % mortality of the control samples after the first hour and 60 % after 24 hours. I.v. premedication with ASA reduced the mortality to 7 % and 27 %, respectively. Premedication with 4-methylcatechol and pyrogallol was not associated with any mortality in the first hour. The corresponding values after 24 hours were 7 % and 31 %, respectively.

A part of this thesis was also testing of the antiplatelet effects of newly synthesized compounds from the group of xanthene-3-one derivatives. From 12 tested substances, almost all were able to significantly inhibit AA induced platelet aggregation at a concentration of 80 µM. Only two compounds were active in lower concentrations and were subsequently tested more in depth. 9-(2'-hydroxy-5'-bromophenyl)-2,6,7-trihydroxyxanthene-3-one inhibited ovine recombinant cyclooxygenase-1, but this effect was not confirmed in human platelet rich plasma. The second

compound, (9-(4'-dimethylamino-phenyl)-2,6,7-trihydroxy-xanthen-3-one, was very active on collagen induced aggregation. In fact, its activity was even comparable with ASA. The mechanism of action was subsequently established as antagonism at thromboxane receptors.

In conclusion, these published outcomes on polyphenolic substances, that form part of our diet and their gastrointestinal metabolites, suggest possible protective cardiovascular effects. However, for the most active substances there is still space for new information, especially their pharmacokinetics, and then their testing *ex vivo* and *in vivo* in combinations with clinically used antiplatelet agents.