Abstract

Charles University, Faculty of Pharmacy in Hradec Králové

Department: Department of Pharmacology and Toxicology

Candidate: Mgr. Marcel Hrubša

Supervisor: Prof. PharmDr. Přemysl Mladěnka, Ph.D.

Title of dissertation thesis: The influence of metabolites and their parent phenolic compounds on blood platelets

Acute cardiovascular events (ACEs), such as stroke and acute coronary syndromes encompassing acute myocardial infarction, are one of the main causes of cardiovascular mortality in the world. Current pharmacotherapy focuses primarily on secondary prevention of their recurrence, where antiplatelet drugs play a key role. These drugs reduce platelet hyperreactivity, which is present in the majority of cardiovascular and metabolic diseases, and significantly decrease the risk of ACEs. However, the palette of currently available drugs faces many issues, such as resistance, high variability in both pharmacokinetics and pharmacodynamics, serious side effects and the route of administration.

This thesis firstly briefly summarizes the current knowledge of platelet physiology along with available antiplatelet drugs and their mechanism of action. Second part of the theoretical introduction is dedicated to polyphenolic compounds, as polyphenol-rich diet is associated with many beneficial effects, particularly on metabolic and cardiovascular diseases. However, polyphenolic compounds have, in general, low bioavailability. Therefore, small phenolic compounds formed by human intestinal microbiome have been suggested to be the cause of these benefits, as they achieve much higher plasma concentrations than the parent polyphenols. In this thesis, the antiplatelet effect of various metabolites of polyphenols has been evaluated and their mechanism of action was also investigated.

Six silymarin flavonolignan sulfates were tested as representatives of secondary phase metabolites of parent polyphenols along with their parent compounds. Their antiplatelet activity was, however, negligible.

For this reason, we have instead turned our attention to small phenolic metabolites of polyphenols. Four isoflavonoid-specific small metabolites and 29 known flavonoid small metabolites were examined in terms of their antiplatelet activity and mechanism of action. We have found that 3 out of 4 tested isoflavonoid metabolites can have relevant effects on platelet aggregation. 4-ethylphenol seemed to partially inhibit both cyclooxygenase and thromboxane synthase and interfered with calcium signalling, while equol was a thromboxane receptor antagonist.

O-desmethylangolensin was similar to 4-ethylphenol in terms of mechanism, but clearly inferior in terms of potency.

The study with 29 flavonoid metabolites has identified 4 compounds, which can contribute to antiplatelet effects of flavonoid-rich diet. All 4 of these compounds belonged to the group of hydroxybenzenes and were potent antiplatelet compounds: resorcinol, pyrogallol, phloroglucinol, and 4-methylcatechol (4-MC). Further experiments have shown that pyrogallol and 4-MC were by far the most potent of them. Pyrogallol was similar to acetylsalicylic acid (ASA) in terms of potency, while 4-MC was 10 times more potent than ASA. Their effect was also confirmed using a novel HET-CAV (hen's egg test on chick area vasculosa) *ex ovo in vivo-like* model; 4-MC was once again more potent than ASA. Initial mechanistic studies performed in this study have suggested, that 4-MC interferes with calcium signalling in platelets. Following studies performed by us have shed more light on its mechanism of action. 4-MC was able to block many aggregatory pathways, but the major mechanism was targeted to collagen and arachidonic acid derived pathways. In contrast to the previous compounds, 4-MC did neither block cyclooxygenase 1 and thromboxane synthase, nor acted as an antagonist at thromboxane receptors. Finally, it was found that it interferes with transformation of arachidonic acid to thromboxane A_2 likely by blockade of cyclooxygenase and thromboxane synthase coupling.

This, associated with the fact that 4-MC was more potent than ASA makes 4-MC an interesting candidate for antiplatelet drug development. The efficacy of 4-MC was therefore investigated using a heterogenous population sample of 53 healthy donors aged 20-66 years old. Using this relatively robust sample, we were able to confirm the potency and efficacy of 4-MC, which was confirmed to be more potent than ASA in both arachidonic acid and collagen induced aggregation. The effect of 10 µM of 4-MC lay between the effect of 30 µM and 70 µM of ASA on AA-triggered platelet aggregation. In terms of collagen triggered aggregation, 70 µM of ASA was ~17% more potent than 20 µM of 4-MC but ~12% less potent than the equimolar concentration of 4-MC. 4-MC is therefore a potent antiplatelet compound.

While the potency of 4-MC was clearly high, we have also wanted to explore the structure-activity relationship of this fairly small and structurally simple molecule. Hence, 22 structurally related compounds were investigated using human whole blood in terms of antiplatelet activity and *in vitro* toxicity. This study has provided interesting insights in terms of structural elements necessary to retain the antiplatelet activity, while also exploring the effect of substitution of the base structure.

This thesis has shown, that 4-MC, a small phenolic compound derived from microbial metabolism of polyphenols is an efficient antiplatelet compound and may present, along with its derivatives, a novel avenue in antiplatelet therapy.