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Oral Sessions

302. Vascular Wall Biology, Endothelial Progenitor Cells and Platelet Adhesion: Endothelium, von Willebrand factor, and Blood Coagulation

Protein Disulfide Isomerase Inhibitors: A New Class of Antithrombotic Agents

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Protein disulfide isomerase (PDI) is a prototypical member of a large family of oxidoreductases that catalyze posttranslational disulfide exchange necessary for proper protein folding. Despite having an ER retention sequence, PDI has been identified at cellular locations outside the ER. PDI is secreted from platelets and endothelial cells upon agonist stimulation or vascular injury. Secreted PDI is essential for platelet thrombus formation and fibrin generation *in vivo*. Inhibition of PDI with a non specific thiol inhibitor bacitracin A or a specific inhibitory anti-PDI antibody RL90 leads to decreased thrombus formation and fibrin generation *in vivo* in the laser injury model of thrombosis in mice (Cho J. et al, 2008, J. Clin. Invest. 118:1123; Jasuja R. et al, 2010 Blood116:4665). We screened a 5000 compound library of known bioactive compounds using an insulin reduction assay with turbidimetric end point to identify potent and selective small molecule inhibitors of PDI. The screen identified 18 inhibitory compounds representative of 13 separate chemical scaffolds, including 3 flavonols. Rutin, a glycoside of the flavonol quercetin, was the most effective inhibitor and inhibited PDI reductase activity with an IC_{50} of 6.1 μM. Inhibition of PDI by rutin was confirmed in an additional fluorescence-based reductase assay using oxidized glutathione coupled to di-eosin (Di-E-GSSG). Rutin specifically inhibited PDI activity and did not affect reductase activity of other thiol isomerases ERp57, ERp72, ERp5, thioredoxin or thioredoxin reductase. PDI inhibition by rutin was fully and rapidly reversible, indicating that rutin does not covalently bind PDI. Evaluation of rutin binding to immobilized PDI using surface plasmon resonance indicated a K_D of 2.8 μM. Quercetin-3-glucuronide, an abundant metabolite of rutin found in plasma, demonstrated an IC_{50} of 5.9 μM (3.5–10.1 μM, 95% confidence interval). Isoquercetin, hyperoside, and datiscin, other flavonols with a 3-O-glycosidic linkage also inhibited PDI reductase activity. Metabolites of rutin that lack a 3-O-glycoside such as tamarixetin, isorhamnetin, diosmetin, or quercetin did not inhibit PDI reductase activity, whether or not they are hydroxylated or methoxylated at the 3' and 4' positions on ring B of the flavonol backbone. Activation of washed human platelets induced by 50 μM AYPGKF, a PAR4 agonist, was reversibly inhibited by rutin in a dose-dependent manner. Rutin effectively blocked fibrin generation from laser activated human umbilical vein endothelial cells bathed in plasma with an $IC_{50}$of approximately 5 μM and 95 % reduction in fibrin formation at 10 μM rutin ($P<0.001$). Intravenous infusion of rutin prior to vessel wall injury in a mouse laser injury model of thrombosis showed a dose dependent inhibition of both platelet thrombus formation and fibrin generation *in vivo*. Platelet thrombus size was reduced by 71% at 0.1 mg/kg and fibrin deposition was inhibited by 68% with an intravenous dose of 0.3 mg/kg. Orally administered rutin also demonstrated antithrombotic activity. However, diosmetin, a non derivatizable form of flavonol that cannot under glycosylation at position 3 of the C ring did not affect platelet thrombus size or fibrin deposition. Infused exogenous recombinant PDI can overcome the inhibitory effect of rutin on thrombus formation. These results indicate that PDI is the relevant antithrombotic target of rutin *in vivo*. Rutin is well tolerated at concentrations higher than that required to inhibit PDI activity *in vivo*. Thus, targeting extracellular PDI for antiplatelet and anticoagulant therapy may be a viable approach to prevent thrombosis in a setting of coronary artery disease, stroke and venous thromboembolism.

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Footnotes

* Asterisk with author names denotes non-ASH members.