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Rutin- potent natural thrombolytic agent

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ABSTRACT

Thrombosis, the formation of blood clots, is a cause not only of heart attacks and strokes, but of deep venous thrombosis (DVT) and pulmonary embolism as well. The number one killer of Americans is a blood clot that blocks blood flow to the heart or to the brain and approximately half of all morbidity and mortality in the United States can be attributed to heart attack or stroke. All the blood clot related conditions are life-threatening, and so there is a need for safe, effective and preventive treatment. A natural substance rutin, also called rutoside, is a citrus flavonoid glycoside found in *Fagopyrum esculentum* (buckwheat), the leaves and petioles of *Rheum* species, and Asparagus. This flavonoid compound has shown effective thrombolytic activity (prevents the formation of blood clots) by blocking the enzyme protein disulfide isomerase (PDI) found in all cells involved in blood clotting. Food and Drug Administration (FDA) has established that rutin is safe and, thus provides a safe and inexpensive drug that could reduce recurrent clots and help save thousands of lives.

Key Words: Fibrinolytics, rutoside, flavonoid, protein disulfide isomerase, clotting, *Fagopyrum esculentum*.

INTRODUCTION

Thrombosis is the process of formation of solid mass or thrombus in circulation from the constituents of flowing blood. A blood clot is the mass of coagulated blood formed in vitro e.g. in a test tube. The extra-vascular accumulation of blood clot e.g. into the tissues is known as Haematoma while the blood clots formed in healthy individuals at the site of bleeding e.g. in injury to the blood vessel are called Haemostatic plugs. In other words, haemostatic plug at the cut end of a blood vessel may be considered the simplest form of thrombosis. Haemostatic plugs are useful as they stop the escape of blood and plasma, whereas thrombi developing in the unruptured cardiovascular system may be life threatening by causing ischaemic injury and Thromboembolism (Mohan, 2006).

Thrombosis or blood clot formation and its consequences remain a leading cause of morbidity and mortality, and recurrent thrombosis is common despite current optimal therapy (Jasuja *et al*., 2012). Clots in arteries are platelet rich where as in veins they are fibrin rich. Rutin presents and treats both types of clots (Hart, 2012).

Thrombolytic drugs rapidly lyse thrombi by catalyzing the formation of plasmin from plasminogen. These drugs create a generalized lytic state when administered intravenously. Thus, both protective hemostatic thrombi and target thromboemboli are broken down (Zehnder, 2009).

Thrombolytics or fibrinolytics can remove established thrombi and emboli. The removing of the products of coagulation when they have served their purposes of stopping a vascular leak is the function of the fibrinolytic system. This system depends on the formation of the fibrinolytic enzyme plasmin from its precursor protein known as plasminogen in the blood. Plasminogen binds to specific sites on fibrin during the coagulation process. Simultaneously, the natural activators of plasminogen i.e. tissue plasminogen activator (tPA) and urokinase are released from endothelial and other tissue cells and act on plasminogen to form plasmin. Since fibrin is the framework of the thrombus its dissolution clears the clot away (Bennett and Brown, 2003).

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MAIN CLASSES OF DRUGS USED IN THROMBOSIS

- *First generation:* Streptokinase, Urokinase, APSAC (Anisoylated plasminogen streptokinase activator complex), Single chain urokinase-type plasminogen activator (Scu-PA, Prourokinase).
- *Second generation:* Recombinant tissue plasminogen activators (rt-PA): Alteplase, Reteplase, Tenecteplase, Lanoteplase, Monteplase, YM866, Staphylokinase (recombinant), recombinant single chain urokinase-type plasminogen activator (r scu-PA).
- *Miscellaneous:* Nattokinase, Rutin.

RUTIN (QUERCETIN-3-RUTINOSIDE)

Source

Rutin is a flavonol abundant in a variety of commonly ingested foods. The name 'rutin' came from a plant known as *Ruta graveolens* that also contains rutin. It is found in high concentrations in teas and fruits (Jasuja *et.al*, 2012). Buckwheat seeds (*Fagopyrum esculantum)* are the richest source (Steal, 2012). It is also found in the leaves and petioles of *Rheum* species and Asparagus, in the fruits and flowers of the pagoda tree, fruits and fruit rinds mainly of citrus fruits (like orange, grapes, lemon, lime) and in ash tree fruits, in berries such as mulberry and cranberries. It is also found in Clingstone peaches as one of the primary flavonols. European Elder (berry), Hawthorn (*Crataegus laevigata*), Horse tail (*Equisetum arvense*), Bilberry (*Viccinium myrtilus*) (Pendleton, 2012).

Rutin was found to inhibit thrombus formation at concentrations that are well tolerated in mice and humans. Inhibition of thrombus formation by rutin in mice was completely reversed by infusion of recombinant Protein Disulfide Isomerase (PDI). Thus, rutin binds to and reversibly inhibits PDI but shows only minimal activity towards other extracellular thiol isomerases present in the vasculature. Evaluation of the effect of flavonol ingestion on cardio-vascular events demonstrated protection from myocardial infarction and stroke with increased intake (Jasuja *et al*., 2012).

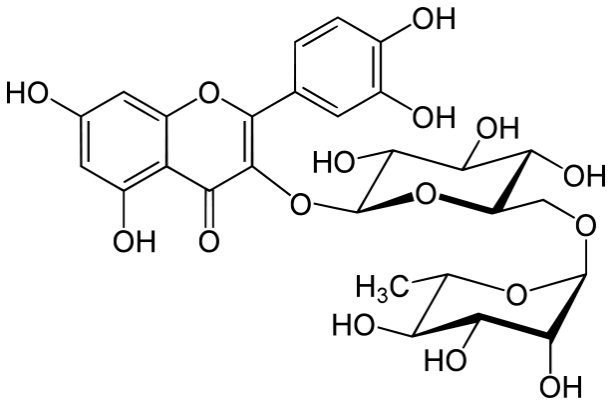


Figure 1: Structure of Rutin (Jasuja *et al.*, 2012).

Two flavonoids, rutin and hesperidin, were investigated *in vitro* for anticoagulant activity through coagulation tests: activated partial thromboplastin time (aPTT), prothrombin time (PT) and thrombin time (TT). Only an ethanolic solution of rutin at the concentration of 830µM prolonged aPTT, while TT and PT were unaffected. Rutin could thus also be used as an anticoagulant (Kuntic *et al*., 2011).

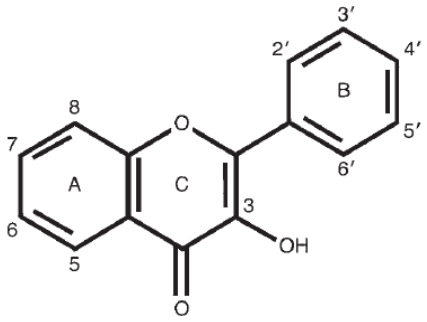
Chemistry

Rutin is the glycoside between the flavonol quercetin and the disaccharide rutinose (α-L-Rhamnopyranosyl-(1→6)-β-D-glucopyranose) as shown in figure 1.

PROTEIN DISULFIDE ISOMERASE (PDI)

Protein disulfide isomerase (PDI) is the prototypical member of an extended family of oxidoreductases (endoplasmic reticulum-resident enzymes). These enzymes catalyze posttranslational disulfide bond formation and exchange and serve as chaperones during protein folding (Hatahet *et al.,* 2009). Although having a C-terminal endoplasmic reticulum retention sequence, PDI has been identified at many diverse subcellular locations outside the endoplasmic reticulum. It has biological functions on the cell surfaces of lymphocytes, hepatocytes, platelets, and endothelial cells (Manickam *et al.,* 2008; Hotchkiss *et al.,* 1998; Essex, Li, 1999; Burgess *et al.,* 2000; Bennett *et al.,* 2000).

Platelets are a rich source of extracellular PDI, expressing this protein on their surface and also secreting PDI in response to thrombin stimulation (Burgess *et al.,* 2000; Cho *et al.,* 2008). Endothelial cells also express PDI upon agonist stimulation or



Name	2′	3′	4′	5′	3	IC_{50} (μM) (95% confidence interval)
Quercetin	H	OH	OH	H	OH	>100
Tamarixetin	H	OH	OCH_3	H	OH	>100
Isorhamnetin	H	OCH_3	OH	H	OH	>100
Diosmetin	H	OH	OCH_3	H	H	>100
Hyperoside	H	OH	OH	H	Galactose	5.9 (2.8–12.5)
Isoquercetin	H	OH	OH	H	Glucose	7.1 (4.3–12.0)
Quercetin-3-glucuronide	H	OH	OH	H	Glucuronic acid	5.9 (3.5–10.1)
Quercetin-3-rutinoside	H	OH	OH	H	Rutinose	6.1 (1.1–10.7)
Datiscin	OH	H	H	H	Rutinose	8.8 (3.2–24.3)

Figure 2: Structure activity relationship of the flavonols and their potency (IC_{50}) of PDI inhibition. Numbers in the structure correspond with those in the column headings (Jasuja *et al.*, 2012).

when challenged by a vascular injury (Hotchkiss *et al.*, 1998; Jasuja *et al.*, 2010).

PDI has recently been shown to participate in thrombus formation (Jasuja *et.al*, 2012). PDI is found in all cells and is rapidly secreted from both platelets and endothelial cells during thrombosis. It is of two types: Extra-cellular and Intra-cellular.

Intra-cellular PDI is necessary for the proper synthesis of proteins. It is the extra-cellular PDI which is involved in thrombus formation. A high through put screening of a wide array of compounds (more than 5,000) resulted in the emergence of a potent flavonoid compound called Rutin which selectively blocked the extra-cellular PDI (Hart, 2012).

MECHANISM OF THROMBOLYTIC ACTION

The currently available anti-thrombotic agents inhibit either platelet aggregation or fibrin generation where as the inhibition of secreted PDI blocks the earliest stages of thrombus formation and, therefore, suppress both the pathways. Cellular assays have shown that Rutin inhibits aggregation of human and mouse platelets and endothelial cell mediated fibrin generation in human endothelial cells.

Rutin blocks thrombus formation in vivo by inhibiting PDI in a dose dependent manner using intra vital microscopy in mice. Intra-venous infusion of Rutin resulted in a dose dependent inhibition of platelet accumulation with 71% reduction at 0.1 mg/kg dose. Fibrin generation was inhibited after Rutin infusion with 0.3 mg/kg dose. Both platelet accumulation and fibrin generation were nearly absent after infusion of 0.5 mg/kg dose of Rutin. Thus, PDI inhibition is a viable target for small molecule inhibition of thrombus formation and its inhibition can prove to be a useful adjunct in refractoty thrombotic diseases that are not controlled with conventional anti-thrombotic agents (Jasuja *et al.*, 2012).

USES OF RUTIN THERAPY

Rutin therapy can be used for prevention and treatment of heart attacks and stroke, as well as in deep vein thrombosis (DVT) and pulmonary embolism (Hart, 2012).

PHARMACOKINETICS OF RUTIN

Rutin is incompletely absorbed and extensively metabolized after ingestion. Plasma levels of rutin decrease rapidly after either intra-venous or oral administration (Jasuja *et al.*, 2012). Ingested rutin is hydrolyzed to quercetin in the intestine and further changed to other conjugated metabolites of quercetin (Gee *et al.*, 2000).

Rutin results in the generation of more than 60 metabolites (Olthof *et al.*, 2003). Many major metabolites, such as quercetin-3-glucuronide, possess a 3-O-glycosidic linkage and are active against PDI, as demonstrated by structure activity relationships (Figure 2).

Quercetin-3-glucuronide is one of the abundant metabolites of rutin found in plasma, demonstrated on IC_{50} of 5.9 μM. Isoquercetin, hyperoside, and datiscin – all of which have a 3-D-glycosidic linkage – also inhibit PDI reductase activity. The inhibitory activity of these

compounds has been found to be similar irrespective of the nature of glycoside in the 3 position on ring C or the substituents on ring B. Orally administered rutin blocks platelet accumulation with an IC_{50} of about 10 mg/kg and fibrin formation with an IC_{50} of about 15 mg/kg (Jasuja *et al.*, 2012).

ADVANTAGES OF RUTIN

- Rutin is anti-thrombotic at flavonol concentrations that are well tolerated based on extensive animal and human clinical literature.
- Rutin has demonstrated no toxicity in cultured endothelial cells for at least 72 hours at concentrations as high as 100 μM.
- Rutin lacks toxicity because the same glycosidic linkage that is required for inhibition of PDI activity impairs cell permeability (Jasuja *et al.*, 2012).
- Agents like Juniferdin or Bacitracin which also inhibit PDI function and thus inhibit thrombus formation *in vivo* (Khan *et al.*, 2011; Dickerhof *et al.*, 2011; Cho *et al.*, 2008) and are either cytotoxic or non-selective (Karala and Ruddock, 2010; Khan *et al.*, 2011). When compared with these agents, rutin demonstrated selectivity towards extra-cellular PDI and is relatively non-toxic.
- In addition, rutinosides are known to bind to the blood vessel wall (Neumann *et al.*, 1992; Patwardhan *et al.*, 1995) where they may maintain antithrombotic activity but are not detected in plasma.

CONTRA-INDICATION OF RUTIN

Concurrent rutin administration is likely to reduce the anti-coagulant effect of racemic warfarin as reflected by a significant decrease in the elimination half life of the more potent S-enantiomer (Chan *et al.*, 2009). Rutin supplements can cause miscarriage so should not be used during pregnancy. Its use should be avoided during lactation period (Pasillas, 2012).

AVAILABLE PREPARATIONS OF RUTIN

- Rutin has been sold as a herbal supplement approved by US FDA (Hart, 2012).
- It is used in many countries and is ingredient of numerous multi-vitamin and herbal preparations.
- It is usually sold in 500 mg caplets, but dosage can be anywhere from 200-600 mg once or twice per day (Pasillas, 2012).

SIDE EFFECTS OF RUTIN

- Rutin supplements can cause dizziness, headache, increase in heart rate, stiffness, diarrhoea, upset stomach and fatigue (Pasillas, 2012).
- Allergic reactions are rare but skin rashes, facial swelling and breathing problems can occur sometimes (Moore, 2012).
- Fatigue, vomiting, hair loss are also observed (Hart, 2012).

CONCLUSION

Rutin is an antagonist of PDI and an inhibitor of thrombus formation. This also validates PDI as a drug target for anti-thrombotic therapy. The small molecule inhibition of PDI could be used to control thrombus formation in vivo, particularly given the advantage that both platelet accumulation and fibrin generation are blocked following inhibition of PDI. The anti-thrombotic activity of rutin is entirely reversed after infusion of recombinant PDI. The dominant effect of rutin in thrombus formation is to inhibit extra-cellular PDI function, thereby preventing thrombi formation after vascular injury. It is a safe and inexpensive drug that could reduce clots and thus help save thousands of lives.

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