Protein Disulfide Isomerase

Protein disulfide isomerase (PDI) is the archetypal member of a family of thiol isomerases originally identified for their role in modifying disulfide bond formation during protein synthesis and folding (for more detailed information on the biochemistry and cell biology of thiol isomerases please refer to recent reviews1,2). It is a 57-kDa protein that possesses an a-b-b‘-x-a‘-c domain structure (Figure 1). The a and the a‘ domains contain the active CGHC motifs, which face each other in the crystal structure of PDI (Figure 1).3 These motifs catalyze oxidoreductive activities. The b and b‘ domains are substrate binding and the x domain consists of a short linker that connects the b‘ and a‘ domains. The C-terminal c domain functions in chaperone activity4 and terminates with a KDEL sequence. These domains are attached in an U-shaped structure that is open in the oxidized state and closed in the reduced state (Figure 1).3

PDI is capable of several different distinct activities. It can act as a reductase or an oxidase depending on the redox potential of its substrate (Figure 1). Such reactions facilitate the isomerase activity of PDI, which is essential for proper folding of nascent proteins as they are synthesized in the endoplasmic reticulum (ER). PDI also acts as a chaperone and its binding can promote proper folding even in proteins that lack disulfide bonds.5 The vicinal cysteines in the CGHC motif can undergo S-nitrosylation or glutathionylation, regulating their activity.8,9 Likewise, PDI can act as a denitrosylase, removing nitric oxide from a substrate protein, or as a transnitrosylase, transferring nitric oxide into cells.10,11 These varied activities are influenced by the redox environment, pH, allosteric modulators, and substrate characteristics.

The subcellular localization of PDI also influences its activity. PDI is primarily sequestered in the ER of nucleated cells where it is reported to be concentrated to ≈200 μmol/L.12 In platelets, it has been identified within the dense tubular system. Yet in both nucleated cells and platelets, a population of PDI exists in storage granules and on the extracellular surface.13 The mechanism by which PDI is transported to the extracellular is not well understood. KDEL sequences usually serve as an ER retention signal. However, more recent studies suggest that it may, in some instances, facilitate expression of PDI on the extracellular surface.14 In platelets, PDI colocalizes with toll-like receptor 9 in a novel organelle termed the T-granule (Figure 2).15 In endothelial cells, PDI colocalizes
with chemokines, including growth-related oncogene-α and monocyte chemoattractant protein-1, but not with von Willebrand factor (Figure 2).

PDI stores can be released from platelet and endothelial cell granules in an activation-dependent manner. Newly released PDI binds cTlrβ3 on the platelet surface and αvβ3 on the endothelial cell surface. Activation-dependent release of PDI is critical for thrombus formation.

PDI in Platelet Function

Chen et al13 first described the release of PDI from platelets >2 decades ago. Initial efforts to understand its role in activation were directed at identifying potential substrates of surface-exposed platelet PDI. Thrombospondin 1,14,19 GPIβ2α2, α2β1,21 and cTlrβ322 have all been identified as potential substrates. Platelet PDI has also been implicated in the formation of disulfide linkages between proteins, such as the formation of complexes of vitronectin or thrombospondin with thrombin–antithrombin.23,24 However, which of these putative substrates is important for PDI function in platelet activation and thrombus formation is not clear.

Several studies have implicated PDI in platelet activation. Earlier studies demonstrated that the cyclic polypeptide, bacitracin, a nonselective PDI inhibitor or antibodies directed against PDI block platelet aggregation and secretion.22,25 Although the mechanisms by which PDI participates in platelet activation are not entirely understood, there is evidence that PDI participates in the transformation of cTlrβ3 to an active conformation (Figure 2).26 Recently, mice with a megakaryocyte/platelet-specific defect in PDI have been generated. These mice demonstrate decreased, but not absent, platelet aggregation in response to multiple agonists.27 PDI-deficient mouse platelets also demonstrate a defect in platelet dense granule secretion in response to low-to-intermediate concentrations of agonists. All activation defects in PDI-deficient platelets were overcome by either high concentrations of strong agonists or by addition of recombinant PDI. Thrombus formation on collagen at high shear rates was inhibited in PDI-deficient platelets compared with wild-type platelets.27 These results provide strong support for earlier studies implicating PDI in platelet activation.

Observations that PDI contributes to the activation of isolated platelets raises the question of whether PDI is important for platelet activation in vivo. Several lines of evidence demonstrate that PDI mediates platelet accumulation during thrombus formation. Cho et al28 demonstrated that infusion of anti-PDI antibody totally inhibited accumulation of platelets after laser injury of mouse cremaster arterioles. Inhibition of PDI was subsequently shown to block platelet accumulation in other models of thrombosis, including FeCl3-induced thrombosis.29 Thrombus formation has also been evaluated in mice with megakaryocyte/platelet-specific
deficiency of PDI. These mice demonstrated normal initial adhesion of platelets after laser injury but impaired platelet accumulation.27 Thus, consistent with its role in the activation of isolated platelets, PDI also functions in the activation of platelets in vivo, affecting primarily platelet aggregation and not primary adhesion.

PDI in Coagulation

After the demonstration that PDI modulates platelet functions such as α, β-mediated platelet aggregation, attention turned to the potential regulatory role of PDI in the generation of fibrin. The generation of fibrin by tissue factor in vivo is mediated through the binding of factor VIIa and X to form the tenase complex resulting in a thrombin burst and ultimately fibrin deposition. However, tissue factor in the quiescent vasculature is postulated to be present in an inactive or cryptic conformation.30,31 The description of a Cys186-Cys209 disulfide bond in tissue factor that is susceptible to thiol-dependent changes in procoagulant activity sparked interest in the possibility that PDI serves as the master switch regulating thiol-dependent generation of fibrin and platelet accumulation (Figure 2).32,33 However, this hypothesis has not been proven and remains highly controversial 8 years after its proposal.

A series of experiments conducted using the laser-induced vascular injury model demonstrated that inhibition of PDI by a blocking monoclonal antibody reduces fibrin generation in vivo.28,34 The generation of fibrin was independent of platelet accumulation as fibrin deposition was normal even in the absence of platelet accumulation in mice that lacked the G protein–coupled platelet receptor Par4. Accordingly, the initial delivery of PDI into a developing thrombus is primarily through its release from endothelial cells rather than accumulating platelets.40,42 Although animal models clearly demonstrate that PDI inhibition diminishes fibrin generation, dissecting these observations performed under a tightly regulated redox environment in vivo is challenging. As such, the specific targets and mechanism by which PDI regulates fibrin generation are actively debated. Inhibition of PDI decreases tissue factor activity in vitro and mutagenesis of either Cys186 or Cys209 impairs tissue factor-VIIa-mediated factor X activation.32-34 Conversely, other groups have failed to confirm that tissue factor activity expressed in certain cell lines is regulated by thiol exchange.35,36 It remains unclear whether the interaction between PDI and tissue factor is mediated directly through disulfide bond exchange or via downstream effectors of other PDI-regulated targets.37 For instance, Furlan-Fregua et al.38 demonstrated that inhibition of PDI prevented the release of procoagulant tissue factor–bearing microparticles via activation of the purigene P2X7 receptor on macrophages and smooth muscle cells. There is also evidence that inhibition of PDI alters the binding of coagulation factors on a negatively charged phospholipid surface and reduces thrombin generation in a tissue factor–independent manner.39 The clinical development of PDI inhibitors as antithrombotics will be facilitated by an improved understanding of the mechanisms and targets by which PDI regulates coagulation.

PDI Inhibitors as Antithrombotics

The discovery that PDI serves a critical role in thrombus formation in vivo raises the question of whether inhibitors of PDI could serve as a new class of antithrombotics. Although there are an increasing number of antithrombotic agents with demonstrated clinical efficacy, thrombosis remains the leading cause of mortality in developed countries. Although most antithrombotics target either platelet or coagulation activation, PDI inhibitors have the potential to prevent thrombosis in conditions with pathological activation of both pathways as implicated in complex thrombotic disorders such as myocardial infarction and cancer-associated thrombosis.

It is not intuitive that PDI would be a tractable target for antithrombotic therapy. It is ubiquitously expressed and serves an important function in protein folding, as evidenced by the fact that knockdown of the protein is lethal in yeast and mammalian cell lines.40 However, extracellular PDI, not the ER pool that mediates protein folding, functions in thrombus formation. Selective inhibition of extracellular PDI is therefore a viable strategy. Furthermore, extracellular PDI may be more amenable to small molecule inhibition than PDI that is highly concentrated in the ER. Still the strategy of targeting extracellular PDI to block thrombus formation did not seem appealing until the discovery that commonly ingested quercetin flavonoids found in fruits, vegetables, teas, and grains inhibit PDI.

The discovery that quercetin flavonoids block PDI function occurred during a high throughput screen to identify novel inhibitors of PDI. For this screen, an insulin-based turbidimetric assay was used to identify compounds that inhibited PDI reductase activity.28 This assay is based on the observation that insulin aggregates after cleavage of disulfide bonds in the insulin β-chain. Cleavage of these bonds by PDI results in increased turbidity of the reaction mixture, which can be detected at 650 nm. Quercetin-3-rutinoside was identified as a PDI inhibitor on screening an annotated small molecule library consisting of 5000 compounds with known biological function.29 Quercetin-3-rutinoside demonstrated the most potent inhibitory activity of all compounds identified. Structure activity relationship assays in which several analogs of quercetin-3-rutinoside were tested showed that only those analogs that possessed carbohydrate at position 3′ of the C ring inhibited PDI activity. All compounds with this moiety had similar activity against PDI. When tested in mice, quercetin-3-rutinoside inhibited thrombus formation when infused at concentrations as low as 0.1 mg/kg and with full inhibition of both platelet accumulation and fibrin formation at 0.5 mg/kg. Quercetin-3-rutinoside was also inhibitory when given orally, although ≈100× as much compound was required to achieve inhibition.28 Nonetheless, quercetin-3-rutinoside was antithrombotic at concentrations that are safely ingested in humans. These findings provided preliminary proof of principle that inhibition of PDI to block thrombus formation could be accomplished safely.

Although quercetin-3-rutinoside is a viable drug for inhibiting PDI in the clinical setting, it is by no means the only...
PDI inhibitor to which human subjects have been exposed. Bacitracin is a topical antibiotic that has been used to inhibit PDI activity in cell-based studies for >2 decades.\(^{41}\) Despite its widespread use as a PDI antagonist, it is a poor inhibitor of PDI, with an estimated IC\(_{50}\) of \(\approx 100 \mu\text{mol/L}\). It is also nonselective, blocking the activity of several other thiol isomerases as well as interacting with a large number of proteins. Furthermore, its ingestion is associated with nephrotoxicity. Thus, bacitracin is not useful as either a PDI antagonist, with an estimated IC\(_{50}\) of \(\approx 100 \mu\text{mol/L}\). It is estimated that it does not become covalently linked to PDI. As a consequence of its widespread use as a PDI antagonist, it is a poor inhibitor\(^{41}\) of PDI and demonstrates irreversible inhibition. Although such inhibitors may be useful as antineoplastic agents, their mechanism of action is a liability with regard to antithrombotic therapy.

Several synthetic PDI antagonists have been developed in recent years (Table 1; comprehensive review of PDI inhibitors\(^{45}\)). All the new PDI inhibitors have better potency and selectivity than bacitracin and should replace its use as reagents to probe PDI function in biological systems. Whether any of the synthetic PDI inhibitors are potential antithrombotics is worth considering. Based on our experience with quercetin-3-rutinoside as an antithrombotic PDI inhibitor, we performed a much larger high throughput screen to identify more potent and selective PDI antagonists. An insulin-based turbidimetric assay was used to screen 345,505 compounds from the Molecular Libraries Small Molecule Repository. This screen identified several novel inhibitors of PDI, some of which blocked PDI with submicromolar potency. ML359 was the most potent and selective inhibitor identified (Figure 3). It inhibited PDI in the insulin reductase assay with an IC\(_{50}\) of \(\approx 250 \mu\text{mol/L}\). ML359 also demonstrated superior selectivity. It was tested in hundreds of other assays referenced in the PubChem database and was not active at <10 \(\mu\text{mol/L}\) in any other assay. When tested against other thiol isomerases, ML359 did not inhibit ERp5, thioredoxins, or thioredoxin reductase. ML359 was not cytotoxic when incubated with HEK293, HepG2, or HeLa cells for 48 hours. It demonstrated reversible inhibition of platelet aggregation, indicating that it does not become covalently linked to PDI. Further studies are required to confirm that ML359 is antithrombotic in vivo.

### Identifying PDI Inhibitors for Clinical Use

Several properties merit consideration in determining which PDI inhibitors should advance to clinical trials. One property that could be important for an antithrombotic PDI antagonist is reversibility. Reversible inhibition is important for an antithrombotic because reversal of inhibition in the setting of bleeding is a significant and practical advantage. PAMCA-31, 16F16, RB-11-ca, and a novel phenyl vinyl sulfonate contain (Table 1) all target vicinal cysteines within PDI and demonstrate irreversible inhibition. Although such inhibitors may be useful as antineoplastic agents, their mechanism of action is a liability with regard to antithrombotic therapy.

A second consideration in developing an antithrombotic PDI antagonist is cell permeability. Although enhanced cell

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**Table 1. Protein Disulfide Isomerase Inhibitors With Therapeutic Potential**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Reversibility</th>
<th>IC(_{50}), (\mu\text{mol/L})</th>
<th>Cell-Based and Preclinical Studies Comments</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML359</td>
<td>Reversible</td>
<td>0.3–0.6</td>
<td>Inhibits platelet aggregation. Does not inhibit other thiol isomerases tested</td>
<td>49</td>
</tr>
<tr>
<td>Quercetin-3-rutinoside</td>
<td>Reversible</td>
<td>6</td>
<td>Inhibits platelet aggregation and fibrin formation on endothelial cells; blocks thrombus formation in vivo.</td>
<td>29</td>
</tr>
<tr>
<td>Juniferdin</td>
<td>Reversible</td>
<td>0.16–3</td>
<td>Inhibits reduction of HIV-1 gp120. Cytotoxic in several cell lines</td>
<td>43</td>
</tr>
<tr>
<td>RB-11-ca</td>
<td>Irreversible</td>
<td>30–50</td>
<td>Inhibits proliferation of HeLa cells. Shows selectivity for Cys53 of the a domain</td>
<td>44</td>
</tr>
<tr>
<td>PACMA-31</td>
<td>Irreversible</td>
<td>10</td>
<td>Cytotoxic in cancer cell lines. Suppresses tumor growth in mouse xenograft model of ovarian cancer</td>
<td>45</td>
</tr>
<tr>
<td>P1 (phenyl vinyl sulfonate)</td>
<td>Irreversible</td>
<td>1.7</td>
<td>Cytotoxic in multiple cancer cell lines at 4 mmol/L</td>
<td>46</td>
</tr>
<tr>
<td>Adenanthin</td>
<td>Irreversible</td>
<td>7.3</td>
<td>Cytotoxic in lymphoma leukemic cell lines. Tolerated in vivo at &gt;20 mg/kg</td>
<td>47</td>
</tr>
</tbody>
</table>

PDI indicates protein disulfide isomerase.
Table 2. Risk of Cardiovascular Mortality in Population Cohort Studies Based on Dietary Flavonoid Consumption

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>Sex</th>
<th>Total, n</th>
<th>Flavonoid Intake, mg/d (High vs Low Flavonoid)</th>
<th>Adjusted RR (High vs Low, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hertog et al51</td>
<td>1993</td>
<td>Holland</td>
<td>M</td>
<td>535</td>
<td>≥29.9 vs ≤19</td>
<td>0.32 (0.15–0.71)</td>
</tr>
<tr>
<td>Knkst et al52</td>
<td>2002</td>
<td>Finland</td>
<td>M+F</td>
<td>4565</td>
<td>≥26.9* vs 4.3</td>
<td>0.92 (0.80–1.04)</td>
</tr>
<tr>
<td>McCullough et al53</td>
<td>2012</td>
<td>US</td>
<td>M+F</td>
<td>39,387</td>
<td>≥360 vs ≤121</td>
<td>0.82 (0.73–0.92)</td>
</tr>
<tr>
<td>Mink et al57</td>
<td>2007</td>
<td>US</td>
<td>F</td>
<td>13,796</td>
<td>&gt;425 vs &lt;133</td>
<td>0.93 (0.81–1.07)</td>
</tr>
<tr>
<td>Hirvonen et al58</td>
<td>2001</td>
<td>Finland</td>
<td>M</td>
<td>58,181 (person-years)</td>
<td>17.8 vs 3.94</td>
<td>0.89 (0.71–1.11)</td>
</tr>
</tbody>
</table>

CI indicates confidence interval.

*Mean flavonoid for highest quartile for women was 39.5 mg.

permeability may be an asset for developing an antineoplastic PDI inhibitor, cell permeability is not necessary for inhibiting PDI during thrombus formation and could be a liability for an antithrombotic PDI inhibitor. Antibodies that target PDI are potently antithrombotic in vivo, indicating that inhibition of extracellular PDI is sufficient for thrombus formation and infusion of recombinant PDI into mice lacking platelet PDI reverses the defect in thrombus formation, indicating that extracellular PDI is necessary for thrombus formation. In this regard, it is worth noting that the same glycosidic linkage in quercetin flavonoids that is necessary for their anti-PDI activity also impairs their cell permeability.

The mechanism of inhibitor activity is another parameter to consider in deciding which compounds should advance to clinical trials. Allosteric inhibitors that act outside the catalytic domain of PDI could act either by preventing enzyme-substrate interactions or by inducing conformational changes in the enzyme that interfere with its oxidoreductase activity. For example, quercetin-3-rutinoside not only blocks the ability of PDI to reduce disulfide bonds in insulin, which interacts with PDI substrate binding domains, but also inhibits the ability of PDI to reduce the small molecule di-eosin-GSSG, which does not interact with binding domains. This observation suggests that quercetin-3-rutinoside has allosteric effects that interfere with the function of the catalytic domain. Allosteric control of PDI using drug-like molecules is in its nascency. However, allosteric modulators of PDI are likely to represent the best strategy for inhibition of PDI in the setting of thrombus formation.

**Clinical Trials of PDI Inhibition**

The identification of quercetin-3-rutinoside and related flavonoids as small molecule inhibitors of PDI was fortuitous as this discovery accelerated the clinical evaluation of PDI inhibitors as a target for antithrombotics in humans. The flavonol quercetin and its derivatives are ubiquitously present in fruits and vegetables including in onions, tea, berries, and apples. In Western diets the daily consumption of quercetin is >10 to 20 mg. The structure of the related quercetin compounds differ based on type and location of sugar moieties with quercetin as an aglycone present in only small quantities in food. In contrast, quercetin-3-rutinoside (rutin) with a glycoside group at the 3 position of the pyrone ring is found in higher concentrations in food sources but is poorly absorbed. Although the absorption of the related quercetin compounds differ, all quercetin glycosides, such as rutin or quercetin-3-O-β-D-glucoside (isoquercetin), are metabolized in the liver after oral ingestion. Metabolism of orally ingested flavonoid quercetins includes deglycosylation locally at the enterocyte and subsequent additions of glucuronate, methyl or sulfonyl groups, with glucuronlated conjugates being a dominant form. The glucuronidated conjugate of quercetin has been shown to have similar PDI inhibitory activity to quercetin-3-rutinoside. Thus, regardless of which quercetin flavonoids are ingested, glucuronlated flavonoids can be found in the circulation. Although safety of newer PDI inhibitors is not yet known, the flavonoid quercetin has been studied extensively in several clinical settings without significant toxicity. For instance, in a randomized clinical trial exploring the antiviral activity of quercetin, there was no reported toxicity in >600 individuals consuming 500 or 1000 mg daily.

The efficacy of antithrombotic targeting of PDI using flavonoid quercetins has yet to be established in clinical trials. However, evidence from population-based cohorts suggests that quercetin and related flavonoids significantly reduce cardiovascular mortality. In a longitudinal investigation of elderly men in the Netherlands, the relative risk of death from coronary artery disease was ≈70% lower in those who consumed diets with high amounts of flavonoid (highest tercile) compared with lower amounts (lowest tercile) even when adjusted for age, diet, and other cardiovascular risk factors.

**Figure 4.** Randomized, phase II/III clinical trial to evaluate the efficacy of oral isoquercetin to prevent thrombosis in patients with advanced cancer. Phase II studies will be completed as 2 sequential dosing cohorts A and B. Based on evidence of safety in cohort A, patients will then enroll into cohort B using a higher dose of daily isoquercetin. D-dimer will be measured before and after 2 months of daily isoquercetin administration. Based on safety, efficacy in reducing D-dimer levels as well as preliminary data on venous thromboembolic events (VTEs), the dose will be selected to proceed to phase III. The phase III trial will involve randomization between placebo and isoquercetin with a primary end point of venous thromboembolic events.
factors (adjusted RR, 0.32; 95% confidence interval, 0.15–0.71; \( P=0.003 \)). Similarly, in a large prospective cohort of \( \approx 100000 \) adults in the United States, the highest quintile versus lowest quintile flavonoid consumption was associated with a significant 18% reduction in cardiovascular deaths adjusted for age, weight, smoking, activity, hormone use, and alcohol (adjusted RR, 0.82; 95% confidence interval, 0.73–0.92; \( P=0.01 \)). The trend for reduced cardiovascular death has been consistent across several large epidemiological studies (Table 2) \(^{16} \) and flavonoid consumption has also been linked with a decreased incidence of nonfatal and fatal stroke. \(^{62,63} \) The ascribed cardiovascular bioactivity of quercetin is unlikely to be mediated entirely through PDI inhibition as flavonoids are also known antioxidants and can inhibit collagen-stimulated platelet activation through PDI-independent pathways. \(^{64,65} \)

Based on the in vitro, animal and epidemiological data supporting antithrombotic efficacy of PDI as a therapeutic target, we initiated several proof-of-concept clinical trials in humans with isoequercetin. Quercetin and isoquercetin are attractive small molecule PDI inhibitors because of their established safety profile and improved absorption compared with quercetin-3-rutinoside. These studies serve to lay the groundwork for future clinical studies with more specific PDI inhibitors that are currently under development. An initial clinical study was conducted in healthy individuals to evaluate pharmacokinetic profiles as well as pharmacodynamic inhibition of PDI with isoquercetin. Preliminary data demonstrated that significant and sustained inhibition of PDI is achieved after oral ingestion of isoquercetin in healthy individuals. \(^{66} \)

The mechanisms underlying pathological thrombosis of certain disorders such as malignancy are complex, including increased platelet activation and circulating tissue factor. Based on the potential for a PDI Inhibitor to mediate antithrombotic activity through inhibition of both platelet activation and fibrin generation, we are currently conducting a phase II/III randomized clinical trial to evaluate whether daily isoquercetin prevents thrombosis in high-risk patients with cancer (Figure 4). Despite the high incidence of vascular events in patients with cancer, the benefit of routine thromboprophylaxis has not been established. The phase II trial will confirm the safety of isoquercetin in patients with cancer and evaluate whether there is a significant reduction in thrombotic biomarkers (eg, D-dimer) at 2 months. The phase III study will compare the effectiveness of isoquercetin versus placebo in reducing the cumulative incidence of venous thromboembolic events at 2 months.

**Perspective**

The molecular targets of PDI in thrombosis continue to be explored and will be further facilitated by specific PDI small molecule inhibitors under development. The holy grail of antithrombotic therapeutics remains a drug that prevents pathological thrombosis without increasing the risk of hemorrhage. Although it is too soon to know whether oral PDI inhibitors representing a novel class of antithrombotics fit that description, the clinical studies underway will hopefully lead to new therapeutic options for individuals where current standard approaches to thrombosis are either inadequate or ineffective.

**Acknowledgments**

We thank past and present members of the Flaumenhaft, Furie, and Zwicker laboratories for their contributions to the research described in this review and the Broad Institute for work related to synthetic protein disulfide isomerase inhibitors.

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**Disclosures**

Dr Flaumenhaft is named as an inventor in a patent describing ML359. Jl Zwicker is PI for clinical trials with isoquercetin, which are funded in part by a research grant from Quercegen Pharma. The other authors report no conflicts.

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Vascular thrombosis, including coronary artery disease, stroke, and venous thromboembolic disease, remains the most common cause of morbidity and mortality in the United States. Limitations of current therapies for thrombotic disorders are evidenced by the high incidence of recurrent thrombosis. There is a need for novel therapies targeting alternative components of the blood clotting mechanism based on new knowledge of thrombus formation. Protein disulfide isomerase is now known to serve an essential role in thrombus formation after vascular injury and inhibition of protein disulfide isomerase represents a new approach for the development of antithrombotics. The feasibility of this strategy is underscored by the observation that the widely consumed quercetin flavonoid, rutin, inhibits thrombus formation in preclinical studies at concentrations that are safely ingested by individuals. Inhibition of protein disulfide isomerase either by quercetin flavonoids or more potent and selective small molecule inhibitors of protein disulfide isomerase is presently being developed as a new modality for anti-thrombotic therapy.

**Significance**

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Therapeutic Implications of Protein Disulfide Isomerase Inhibition in Thrombotic Disease
Robert Flaumenhaft, Bruce Furie and Jeffrey I. Zwicker

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