

# PROGRESS IN CLINICAL NEUROSCIENCES: The 'Antiplatelet' Agents and the Role of the Endothelium

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**ABSTRACT:** The antiplatelet drugs, commonly used in the prevention and treatment of cerebrovascular disease, possess a number of effects that are independent of direct antiplatelet actions. Beneficial and detrimental effects both occur. The endothelium is an important mediator of these non-antiplatelet effects. We performed a literature search to locate articles related to acetylsalicylic acid (aspirin), clopidogrel, ticlopidine, and dipyridamole and the interactions of these medications with the endothelium. The role of each of the above medications is explored in relation to vasodilation, inflammation, oxidation, platelet-leukocyte interactions, and thrombogenic tendency via platelet-vessel wall interactions.

**RÉSUMÉ: Agents antiplaquettaires et rôle de l'endothélium.** Les antiplaquettaires utilisés couramment dans la prévention et le traitement de la maladie cérébrovasculaire ont certains effets qui sont indépendants de l'activité antiplaquettaire directe. Certains sont bénéfiques, d'autres sont néfastes. L'endothélium est un médiateur important de ces effets non antiplaquettaires. Nous avons fouillé la littérature pour trouver des articles sur l'acide acétylsalicylique, le clopidogrel, la ticlopidine et le dipyridamole et leurs interactions avec l'endothélium. Nous examinons le rôle de chacun de ces médicaments dans la vasodilatation, l'inflammation, l'oxydation, les interactions plaquettes-leucocytes et la thrombogénicité via les interactions plaquettes-paroi vasculaire.

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Acetylsalicylic acid, clopidogrel, and dipyridamole have proven benefit in the secondary prevention of cerebrovascular disease. They work mainly by inhibiting platelet function, but there is emerging evidence to suggest that other important effects occur, particularly through their interactions with the endothelium. These other effects contribute to the clinical benefit of the 'antiplatelet agents' and may further clarify the underlying pathophysiology of cerebrovascular disease.

## METHODS

A literature search (PubMed) was performed to locate articles pertaining to aspirin, thienopyridines, and dipyridamole and their effects mediated through the endothelium. Specific search terms included endothelium, aspirin, ticlopidine, clopidogrel, dipyridamole, inflammation, oxidation, vasodilatation, platelet, leukocyte, and thrombosis.

## The Endothelium

Endothelial cells synthesize many substances that determine blood vessel patency, thrombogenic potential, and atherosclerotic tendency.<sup>1-3</sup> These substances lead to a basal vasodilator and thromboresistant influence on the vascular system.<sup>1</sup> Whereas the healthy endothelium produces antiaggregatory, anticlotting, antiadhesive, and vasodilator mediators,<sup>1,2</sup> dysfunctional endothelium promotes thrombosis, vasospasm, and vascular occlusion in experimental models.<sup>4</sup>

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The endothelium is capable of releasing both constricting agents including endothelin, thromboxane  $A_2$ , prostaglandin  $F_2\alpha$ , and superoxide anion and dilating agents, such as nitric oxide (NO), prostacyclin, and endothelium-derived hyper-polarizing factor.<sup>3</sup> Altered balance in the release of vasodilating and vasoconstricting substances leading to endothelial dysfunction is found in patients with hypercholesterolemia, hypertension, diabetes, smoking, and hypertension<sup>5</sup> and even acute hyperglycemia.<sup>6</sup> Whether this dysfunction results from an impairment in vasodilator production or an increase in vasoconstrictor activity or both is uncertain.<sup>3,7</sup>

Both intracellular and extracellular substances can moderate the endothelial response. Intracellular concentrations of cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), and calcium play a role, and interactions of the endothelium with platelets, white blood cells, and other plasma components also have an effect.<sup>2</sup> Importantly, a variety of external pharmacological and physiological stimuli can cause the release of substances from the endothelium, suggesting that the endothelium may provide an essential link in the connection between infection, inflammation, and vascular disease.<sup>1,4,8,9</sup>

*In vitro* infection of endothelial cells by various respiratory viruses induces procoagulant activity and leads to the expression of tissue factor and cell surface adhesion molecules. Respiratory tract infection, surgical operations, bacteremia, and severe illness requiring intensive care increase the risk of cardiovascular morbidity and mortality,<sup>8</sup> and an immediately preceding febrile respiratory infection increases the risk of stroke, even in young and middle-aged adults.<sup>1,9</sup> According to one case control study, up to 10% of strokes may be associated with previous bacteremic infection.<sup>10</sup> Moreover, damage to the endothelium is thought to be a significant event in the development and progression of atherosclerosis. Prescription medications which, in some cases, act only in disease states may have little measurable effect on the endothelium in healthy subjects.<sup>7</sup>

Acetylcholine is a commonly utilized agent for studying the endothelium because its vasodilating effects are endothelium-dependent.<sup>11</sup> The vasodilatory response from acetylcholine is impaired in both conductance and resistance vessels in patients with hypertension, hypercholesterolemia, diabetes, aging, smoking,<sup>3</sup> and congestive heart failure.<sup>12</sup> In contrast, vasodilatory responses to endothelium-independent agents, such as sodium nitroprusside, remain normal when tested in patients with hypertension and hypercholesterolemia.<sup>7</sup> Vascular smooth muscle sensitivity to nitric oxide seems not be the cause of dysfunction. Rather, a dysfunctional response of the endothelium, via decreased release or activity of NO and endothelium-derived hyperpolarizing factor or, in some cases, the production of a cyclooxygenase (COX)-dependent vasoconstricting factor, may be responsible.<sup>3,7,12</sup>

### Aspirin

Although the beneficial effects of *Salix alba* have been known widely for centuries, it was only in the 1960s that aspirin was recognized to have a significant effect on platelet function.<sup>13</sup> Aspirin exerts its antiplatelet effects by irreversibly inhibiting

prostaglandin H synthase (COX), acetylating a specific serine moiety on the enzyme. The isoform COX-1, on which aspirin exerts a 170-fold more potent inhibition than COX-2, is constitutively expressed in the endoplasmic reticulum of most cells, including platelets.<sup>14</sup> Inhibition of COX blocks the biosynthesis of thromboxane- $A_2$  (TXA<sub>2</sub>), a platelet aggregant and potent vasoconstrictor, prostacyclin, and several prostanoids.<sup>15</sup>

### Aspirin and Vessel Wall

In vascular injury, the interaction of blood with the thrombogenic structures of the subendothelium ultimately determines whether thrombosis or hemorrhage will occur.<sup>16</sup> Aspirin has long been recognized to have antithrombotic effects,<sup>17</sup> particularly by reducing the production of thromboxane  $A_2$ . Activation of platelets by low but not high concentrations of adenosine diphosphate (ADP) or thrombin requires the presence of thromboxane  $A_2$ .<sup>18</sup> The same cardioprotection that is afforded by aspirin has not been established for other non-steroidal anti-inflammatory drugs (NSAIDs), which are less selective for COX-1 and the resultant inhibition of TXA<sub>2</sub> formation.<sup>17</sup>

In a randomized, double-blind, placebo-controlled *ex vivo* study of healthy subjects, low-dose aspirin treatment decreased the mean size of all thrombi by approximately 45%. This effect was reduced in the case of very large thrombi.<sup>16</sup> Local aspirin treatment also protects the endothelium in vein grafts used in bypass surgery by reducing the amount of neointimal lesions and thrombosis. This antithrombotic effect provides evidence that thrombus formation occurs even before the development of neointimal lesions in vein grafts.<sup>19</sup>

Platelets are directly involved in the initiation of the inflammatory and thrombotic response of the vessel wall. Platelets express the ligand of the potent immune mediator CD40 (CD40L) during thrombus formation *in vivo* and within seconds of activation *in vitro*. This interaction induces endothelial cells to secrete chemokines and express adhesion molecules.<sup>20</sup> Aspirin inhibits soluble CD40L release from stimulated platelets in response to collagen, likely because TXA<sub>2</sub> production is required for full platelet stimulation.<sup>21</sup> CD40L is of increasing importance because it is prothrombotic, proinflammatory,<sup>21</sup> and over-expressed in atherosclerotic lesions.<sup>22</sup> Elevated levels of soluble CD40L are a risk factor for cardiovascular events in apparently healthy women.<sup>23</sup> Interruption of the interaction between CD40 and CD40L diminishes atheroma formation and leads to changes associated with plaque stability.<sup>24</sup> The beneficial effects of aspirin on CD40L interactions with its receptor may further be enhanced by the GP IIb/IIIa antagonists.<sup>21</sup>

The effect of high-dose aspirin remains controversial. In studies on rats, a 100 mg/kg dose of aspirin led to an increased number of emboli and duration of embolization, eight to ten days after administration.<sup>25</sup> High-dose aspirin may inhibit prostacyclin production (a vasodilatory product of COX-2) by the endothelium, thereby reducing its antiplatelet actions.<sup>26,27</sup> Conversely, aspirin at ultra-low doses may stimulate endothelial cell production of the thrombogenic platelet activating factor.<sup>28</sup>

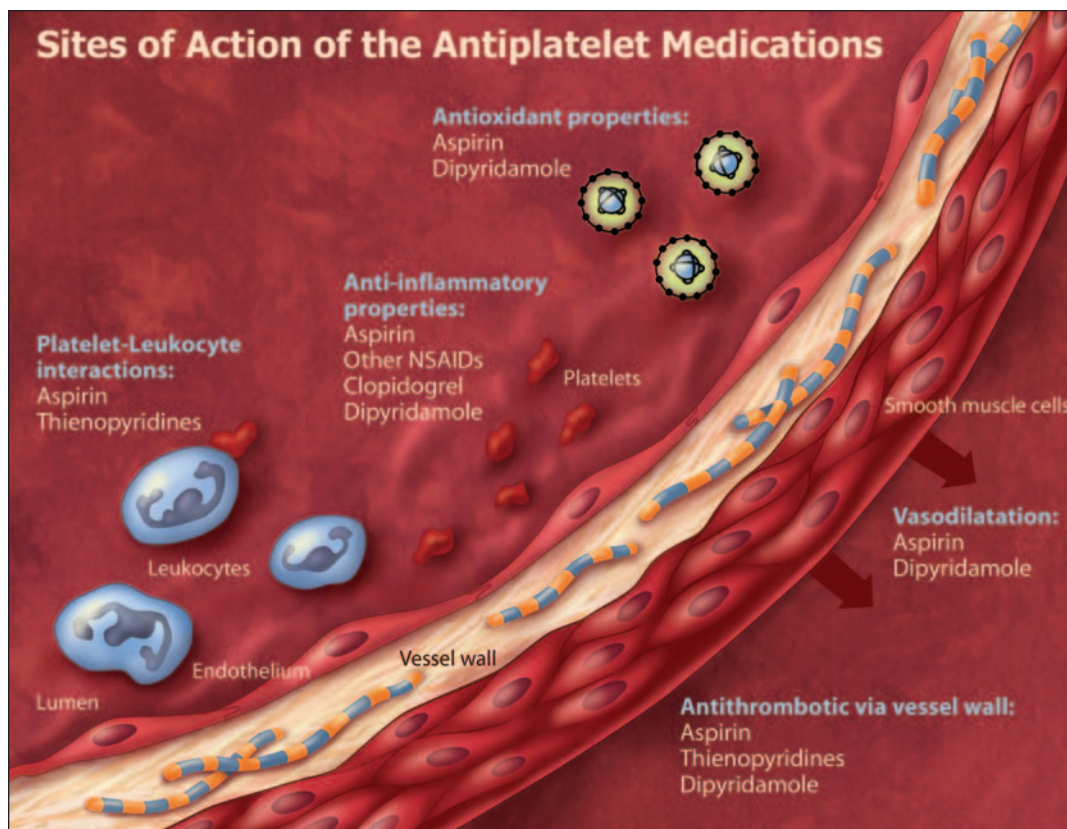


Figure: Sites of action of the antiplatelet medications

### Endothelial Stunning, Vasodilation, and the Role of Aspirin in Endothelial Protection

“Endothelial stunning” is a term used to describe the impairment of endothelium-dependent relaxation following a brief exposure to bacterial endotoxin. This exposure does not morphologically alter the endothelium; however, the impairment in vasodilation lasts for many days.<sup>29</sup> Locally administered aspirin does not reverse endothelial dysfunction after exposure. Pretreatment with anti-inflammatory doses of aspirin, or with glucocorticoids and antioxidants, can prevent dysfunction, suggesting a protective effect on the endothelium.<sup>30</sup> Whether this effect is achieved clinically in unhealthy persons at chronically administered lower doses remains uncertain.

The finding that aspirin prevents the prolonged and profound effects of endothelial stunning has led to the proposal that COX activity contributes to the dysfunction of the endothelium.<sup>4</sup> Impaired relaxation may be due to an endothelium-derived constricting factor which is COX-dependent. The vasoconstrictor prostaglandin  $H_2$  ( $PGH_2$ ) could account for this constricting factor and its upregulation may inhibit the synthesis of the vasodilator prostacyclin ( $PGI_2$ ), an eicosanoid produced from the modification of  $PGH_2$ .<sup>7,15</sup> Clinically, prostacyclin synthesis has been demonstrated to increase in parallel to an increase in the synthesis of thromboxane that occurs during chest

pain in patients with unstable angina.<sup>18</sup> This may be a local compensatory effect of the vascular endothelium<sup>17</sup> and may protect the integrity of the vascular wall itself.<sup>31</sup>

### Other NSAIDs and Vasodilation

It is uncertain whether other non-steroidal anti-inflammatory drugs that act as competitive and reversible inhibitors of cyclooxygenases-1 and -2 share the protective effect of aspirin. Naproxen mimics aspirin’s suppression of thromboxane  $A_2$  via COX-1, but unlike low-dose aspirin, naproxen decreases the synthesis of  $PGI_2$  *in vivo*.<sup>32</sup>

### Aspirin and Inflammation

Aspirin is a potent anti-inflammatory drug.<sup>14</sup> Both an anti-inflammatory dose (1 g) and a cardioprotective dose (75 mg) of aspirin abrogate arachidonic acid-induced platelet aggregation, but only the anti-inflammatory dose significantly reduces the effects of proinflammatory cytokines on endothelial function. Aspirin protects against this endothelial dysfunction experimentally<sup>4</sup> and even mild inflammatory reactions can disrupt endothelial function.<sup>8</sup>

The primary mediator of inflammation in endothelial dysfunction is not clear. The proinflammatory cytokines tumor

necrosis factor- $\alpha$  (TNF $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ) impair the endothelium-dependent vasorelaxant response to bradykinin and arachidonic acid;<sup>4</sup> however, aspirin increases the level of TNF- $\alpha$ , even in the absence of endothelial dysfunction, either via a lipopolysaccharide-induced release or the loss of PGE<sub>2</sub>-mediated inhibition of TNF- $\alpha$  production.<sup>30</sup>

Tumor necrosis factor- $\alpha$  initiates a proinflammatory cascade, not usually found in healthy persons,<sup>33</sup> and perpetuates a prothrombotic endothelial phenotype, enhancing tissue factor expression, especially in the presence of procoagulant proteases like thrombin.<sup>34</sup> Increased circulating levels of TNF- $\alpha$  are found in disease states such as diabetes, sepsis, and coronary artery disease.<sup>34</sup> In congestive heart failure, elevated levels identify the patients who are most severely affected and least likely to survive.<sup>35</sup> Tumor necrosis factor- $\alpha$  further stimulates monocyte adhesion in stimulated endothelial cells by mobilizing the transcription factor nuclear factor kappa B. Aspirin inhibits the mobilization of NF kappa-B *in vitro*.<sup>36</sup>

Interleukin-1 $\beta$  stimulates endothelial cells to produce prostaglandin E<sub>2</sub> by inducing phospholipid A<sub>2</sub> and COX-2 gene expression. Healthy subjects experience headache, fever, arthralgia, and myalgia when injected with IL-1; this can be reduced by coadministration of COX inhibitors.<sup>33</sup>

Proinflammatory cytokine interleukin-6 (IL-6) levels are also elevated during endothelial stunning.<sup>30</sup> Elevated levels of IL-6 are associated with an increased risk of future myocardial infarction in apparently healthy men<sup>37</sup> and a complicated course after hospital admission for unstable angina.<sup>38</sup> Aspirin protects endothelial function without modulation of IL-6, which has no independent effect on endothelial dysfunction.<sup>4,30</sup> Experimental studies, however, are limited, because circulating levels of cytokines may fail to demonstrate local tissue or cellular cytokine generation.<sup>8</sup>

### **Aspirin and Platelet-Leukocyte Interactions**

Aspirin has many effects on leukocyte function, and interactions between leukocytes, platelets, and the endothelium are crucial in the development of a thrombus.<sup>39</sup> In addition to inhibiting monocyte adhesion in stimulated human endothelial cells,<sup>36</sup> aspirin may attenuate the expression of adhesion molecules by other leukocytes. Aspirin attenuates N-formyl-methionyl-leucyl-phenylalanine (fMLP)-induced neutrophil activation *in vitro* following exhausting exercise.<sup>40</sup> Increased neutrophil-platelet adhesion recruits neutrophils to sites of thrombus formation, via a P-selectin mechanism,<sup>41</sup> and may contribute to neutrophil activation in unstable angina.<sup>42</sup>

Aspirin increases NO production by neutrophils via a NO/cGMP-dependent mechanism. Nitric oxide may have an anti-aggregatory and antiadhesive effect on platelets which can be overturned in states of endothelial damage and in the presence of the vasoconstricting peptide endothelin-1.<sup>39</sup> By inhibiting PGI<sub>2</sub> synthesis in endothelial cells, aspirin further enhances NO production.<sup>14</sup> In addition, salicylate interferes with NO-modulated leukocyte adhesion.<sup>43,44</sup>

Aspirin does not inhibit the activation of single platelets. Approximately one percent of circulating platelets in healthy volunteers are activated, as marked by P-selectin positivity; a dose of either 75 or 500 mg of aspirin does not diminish this percentage.<sup>45</sup> Leukocyte-platelet aggregates, a more accurate

marker of platelet activation<sup>46</sup> and an early marker of acute myocardial infarction<sup>47</sup> are also not affected by aspirin,<sup>45</sup> which may be less effective than chimeric 7E3 Fab (ReoPro) in the setting of coronary angioplasty.<sup>48</sup> The addition of clopidogrel to aspirin, however, results in a statistically significant reduction in platelet-leukocyte microparticles in *ex vivo* studies of subjects one to three months post-ischemic stroke.<sup>49</sup>

### **Aspirin and Antioxidation**

All major conditions predisposing to atherosclerosis are associated with increased vascular production of superoxide.<sup>5</sup> Oxygen-derived free radicals cause tissue injury and cell death by attacking proteins, lipids, and nucleic acids.<sup>50</sup> *In vitro* and *in vivo* studies demonstrate the efficacy of aspirin in reducing such oxidative stress.<sup>51</sup> The metabolism of arachidonic acid by cyclooxygenase produces superoxide anion radicals,<sup>52,53</sup> and aspirin prevents this production by irreversibly inhibiting cyclooxygenase.

Aspirin can scavenge hydroxyl radicals to form 2,3- and 2,5-dihydroxybenzoate derivatives, markers of oxidative stress.<sup>14</sup> The active metabolite of aspirin, salicylic acid, is a scavenger of both hydroxyl and oxygen free radicals in activated granulocytes<sup>54</sup> and penetrates ischemic brain tissue as well as the damaged blood brain barrier.<sup>51</sup>

Prevention of oxidation also occurs. Aspirin acetylates the  $\epsilon$ -amino groups of protein lysine residues, preventing their oxidation and further enhancing fibrinolysis.<sup>14</sup> Experimentally, the antioxidant properties of aspirin may reduce ischemia-induced alterations in cellular metabolism, including enzymatic auto-oxidation of neurotransmitters, nitrous oxide production, membrane depolarization, and cellular calcium and glutamate overload.<sup>51</sup>

Oxidized low density lipoproteins (LDLs) have a key role in atherogenesis.<sup>55</sup> *In vivo* and *in vitro*, aspirin modifies LDL to resist endogenous oxidation and oxidation induced by ultraviolet light.<sup>56</sup> An antioxidative mechanism is also responsible for inhibiting monocyte adhesion to LDL-stimulated endothelium.<sup>57</sup> Antioxidants such as vitamin E and  $\beta$ -carotene have been disappointing in similarly preventing LDL oxidation.<sup>55</sup>

Superoxide radicals avidly metabolize NO to biologically inactive nitrogen oxides and reduce its bioavailability of NO.<sup>3</sup> Nitric oxide is vital to the normal homeostasis of the endothelium and plays an important role in development of atherosclerotic lesions.<sup>58</sup> Aspirin enhances NO production in platelets.<sup>59</sup> Therapeutically relevant concentrations of aspirin may elicit NO release from the endothelium due to the direct acetylation of endothelial nitric oxide synthase.<sup>60</sup> This effect has not been found in all studies.<sup>59</sup>

Oxidative stress that occurs as a result of hyperglycemia is responsible for the expression of redox-sensitive genes and activation of transcription factors. These convert the endothelium to a prothrombotic, vasoconstrictive, adhesive state conducive to platelet activation, adhesion, and aggregation. Hyperglycemia blocks endothelial nitric oxide synthase activation and increases the production of free radicals. Thus, aspirin may provide an enhanced benefit in diabetic patients.<sup>61</sup>

### **The Thienopyridines: Ticlopidine and Clopidogrel**

Ticlopidine was first used in 1978.<sup>62</sup> It is an effective antiplatelet medication in cerebrovascular, cardiovascular and peripheral arterial presentations of atherosclerotic disease and is at least equivalent to aspirin in preventing events in cerebrovascular disease.<sup>63,64</sup>

Ticlopidine antagonizes the P2Y<sub>12</sub> adenosine diphosphate membrane platelet receptor,<sup>65</sup> likely via selective blockade by an unknown and unstable metabolite, ultimately preserving cAMP in platelets.<sup>66</sup> This antagonism is detected *ex vivo* only hours after oral intake and cannot be demonstrated *in vitro*<sup>62</sup> since ticlopidine must undergo hepatic metabolism via the cytochrome P450-1A enzyme system.<sup>63</sup> Upon oral administration in humans, ticlopidine is rapidly metabolized to 13 metabolites.<sup>67</sup>

Clopidogrel has been available since the mid-1990s<sup>62,64</sup> and has been found to have a modest but statistically significant benefit over aspirin in patients with atherothrombotic disease.<sup>64,68</sup> It has largely replaced ticlopidine in clinical practice because it has accelerated antiplatelet activity after first administration,<sup>69</sup> is more potent as an antiplatelet drug, has fewer side effects, and is more conveniently administered, usually once rather than twice daily.<sup>70</sup> Clopidogrel is also inactive in its native form but forms an active thio derivative which selectively and irreversibly binds *in vivo* to the P2Y<sub>12</sub> ADP receptor.<sup>49</sup> Clopidogrel acts similarly to ticlopidine, via an unknown active metabolite, but exists as two enantiomers. This property has been helpful experimentally: the *R*-enantiomer demonstrates no antiplatelet effects.<sup>62</sup>

### **The Thienopyridines and Vasodilation**

*In vitro*, ticlopidine and both enantiomers of clopidogrel release NO and PGI<sub>2</sub> from cultured human and bovine endothelial cells.<sup>71</sup> Experimentally, clopidogrel is at least equivalent to aspirin at increasing endothelial NO production and is more efficient than aspirin at increasing leukocyte NO production. Unlike aspirin, the thienopyridines do not appear to inhibit the synthesis of the vasodilator PGI<sub>2</sub>.<sup>70</sup> Clinically, a randomized, double-blind study of heart-transplant recipients found that ticlopidine increases systemic generation of NO and does not reduce PGI<sub>2</sub> in these patients.<sup>72</sup>

The thienopyridines inhibit the production of thromboxane A<sub>2</sub>, a known vasoconstrictor; however, the effect on COX is likely weak because prostacyclin synthesis is not modified. Like aspirin, clopidogrel has a methylcarboxy group which is thought to be responsible for COX inhibition.<sup>70</sup>

### **The Thienopyridines and Thrombosis**

Clopidogrel reduces the tendency towards thrombosis in various animal models. As an adjunct to tissue plasminogen activator, clopidogrel delays and prevents coronary artery reocclusion after thrombolysis in dogs and does so more effectively than aspirin.<sup>73</sup> Models of venous thrombus formation that are unaffected by oral administration of aspirin show a significant reduction with clopidogrel.<sup>74</sup> One recent study found that the endothelium did not modify the antiplatelet action of ticlopidine or clopidogrel, whereas the presence of an endothelium reduced the antiplatelet action of aspirin by 33%, possibly due to aspirin's inhibition of prostacyclin synthesis by the endothelium.<sup>70</sup>

Beyond their mild antiaggregatory effects on platelets, the thienopyridines have a direct effect on vascular mediators, notably ADP, released by cells as they are lysed in a developing thrombus.<sup>75</sup> Clopidogrel inhibits platelet activation and aggregation stimulated by ADP, thromboxane A<sub>2</sub>, and 5-hydroxytryptamine.<sup>76</sup> These substances also possess mitogenic effects, stimulating medial smooth muscle cells to proliferate and amplify their response to growth factors.<sup>77</sup> Neointimal proliferation is particularly important in causing restenosis in diabetic patients<sup>78</sup> and in patients with coronary stents.<sup>79</sup> Clopidogrel may inhibit neointima formation both by reducing the number of platelets deposited and by blocking amplification signals released via ADP, TXA<sub>2</sub>, and 5-HT<sub>3</sub> receptors.<sup>77</sup>

Cyclic flow variation is a model of platelet accumulation at sites of endothelial injury and coronary stenosis. In this model, there is repetitive aggregation and dislodgement of platelets, along with dynamic vasoconstriction, correlating with the potential clinical consequences of infarction, malignant arrhythmias, thrombotic occlusion, and death.<sup>77</sup> Clopidogrel eliminates cyclic flow variation in dogs<sup>76</sup> and baboons<sup>80</sup> and protects against epinephrine-stimulated cyclic flow variation in these animals. In comparison, aspirin is not protective against epinephrine-induced cyclic flow variation.<sup>81</sup>

The response to clopidogrel varies between individuals, and patients with the greatest pretreatment platelet reactivity to ADP may have the least protection against thrombosis after coronary stenting.<sup>82</sup> This "resistance" may be a result of the production of an active metabolite, polymorphism in the P2Y<sub>12</sub> receptor, or intracellular signaling mechanisms via P-selectin.<sup>75,82</sup> One study, which lacked a control group, found that 30% of patients scheduled for cardiac catheterization exhibited <10% platelet inhibition while on clopidogrel therapy.<sup>83</sup> Persistent platelet reactivity and activation during post-stenting clopidogrel treatment may indicate a subpopulation in which platelet inhibition is insufficient.<sup>84</sup> Although clopidogrel is used in combination with aspirin to prevent stent thrombosis,<sup>82,85</sup> experiments indicate that a combination of aspirin and clopidogrel, in standard doses (325 mg aspirin, 300 mg load/75 mg daily clopidogrel) does not attenuate post-drug platelet reactivity, even 30 days post-coronary stenting.<sup>84</sup> The amount of clopidogrel necessary to inhibit platelets *in vivo* may therefore vary interindividually, and targeting post-operative dosing of clopidogrel after carotid endarterectomy has been suggested to address this concern.<sup>85</sup>

Ticlopidine is a potent inhibitor of thrombus formation in flowing blood. Although conflicting reports exist, ticlopidine has been found to reduce platelet adhesion to the subendothelium when the vessel wall is damaged<sup>86</sup> and decrease thrombus formation *in vitro*.<sup>66</sup> Thrombolysis by ticlopidine is immediate and transient and related to stimulation of the endothelium. Thrombolysis is likely mediated by endothelial prostacyclin and tissue plasminogen activator.<sup>66</sup> At low doses, aspirin also potentiates endothelium-mediated thrombolysis by ticlopidine.<sup>71</sup>

### **Clopidogrel and Anti-inflammation**

Patients treated with clopidogrel before a percutaneous coronary intervention show an attenuated increase in C-reactive protein (CRP) around the time of the procedure.<sup>87</sup> Elevated baseline CRP before coronary stenting is associated with an

increased rate of acute myocardial infarction and death within 30 days as well as an increased rate of repeat target artery vascularization interventions over the longer term.<sup>88</sup> Patients with the highest levels of CRP may benefit the most from clopidogrel treatment, and attenuation in risk is partly related to the reduced inflammatory state.<sup>87</sup>

Different experiments have demonstrated a 32% attenuation in CRP by abciximab, compared to placebo,<sup>87</sup> and a 65% attenuation in periprocedural CRP in those pretreated with clopidogrel.<sup>89</sup> The Chimeric c7E3 AntiPlatelet Therapy in Unstable angina Refractory to standard treatment trial showed that abciximab provided similar efficacy in patients with and without elevated CRP levels.<sup>88</sup> Thus, a reduced inflammatory state may not be due simply to reduced platelet activation.

It remains unclear whether clopidogrel has an independent anti-inflammatory effect beyond its ability to act on the P2<sub>Y12</sub> receptor.<sup>90</sup> Receptor blockade itself may fully account for the prevention of a heightened inflammatory state. Further, the precise role of CRP in directing management with antiplatelet agents has yet to be fully elucidated.<sup>88</sup>

### ***Clopidogrel and Leukocyte-Platelet Interactions***

Circulating platelet-leukocyte aggregates are a sensitive marker of the link between inflammation and thrombosis.<sup>90</sup> Platelet-leukocyte aggregate formation can be stimulated by ADP-receptor agonists including ADP and the more potent thrombin receptor activating peptide.<sup>69,90</sup> One study found that in spite of a 300 mg loading dose of clopidogrel before coronary stenting, along with a 75 mg daily dose for 30 days, there remained a modest but significant increase in platelet-leukocyte aggregates.<sup>91</sup> Other studies have found a significant decrease in platelet-leukocyte aggregates as compared to other antiplatelet drugs. Clopidogrel, not compared to any other antiplatelets, reduced *ex vivo* formation of platelet-leukocyte aggregates by 55% to 75% of baseline values in one study.<sup>92</sup> When used as pretreatment, clopidogrel also prevents abciximab-induced increase in platelet-leukocyte aggregates.<sup>92</sup>

### ***Clopidogrel and Antioxidation***

Ticlopidine inhibits lipid autooxidation in an experimental model but is a rather weak scavenger of superoxide anions.<sup>66</sup> The role of clopidogrel as an antioxidant has received little emphasis in the current literature.

### ***Dipyridamole***

Dipyridamole was introduced in 1959 as an antianginal medication and was used for its coronary vasodilator effects. It was later found to suppress platelet activation and consumption in animal models.<sup>93</sup>

Dipyridamole inhibits platelet phosphodiesterase E5, preventing the breakdown of cAMP.<sup>15</sup> Consequently, the antithrombotic effects of endogenous prostacyclin are potentiated.<sup>94</sup> Further, dipyridamole increases the concentration of adenosine at the platelet-vascular interface by inhibiting its cellular uptake and metabolism, including its metabolism by endothelial and red blood cells.<sup>93,95</sup>

### ***Dipyridamole and Vasodilation***

Dipyridamole is a well-known vasodilator and pharmacologic stressor, used to measure maximal coronary blood flow. Maximal cerebral blood flow increases shear stress and depends on the release of vasodilators from the endothelium. Although dipyridamole's direct action on adenosine is mediated through the vascular smooth muscle and not the endothelium, the resultant increase in blood flow from shear stress is dependent upon the endothelium.<sup>96</sup>

The vasodilatory effect of dipyridamole on resistance vessels is best explained by potentiation of adenosine mechanisms as opposed to potentiation of cGMP-related NO pathways.<sup>97</sup> However, dipyridamole is not an ideal vasodilator in the clinical setting. It has the potential for promoting ischemia by dilating normal vessels more than diseased vessels, the so-called coronary "steal" phenomenon.<sup>98</sup>

### ***Dipyridamole and Thrombosis***

The combination of extended-release dipyridamole and low-dose aspirin is twice as effective as either drug alone for the prevention of transient ischemic attack and stroke.<sup>99</sup> The antithrombotic action of dipyridamole, however, is not limited to its vasodilatory actions, as evidenced by its antithrombotic effects in a rigid tube.<sup>100</sup> An antiproliferative effect occurs on mesangial cells and endothelial cells *ex vivo*<sup>101</sup> but the inability of investigators to demonstrate an antiplatelet effect of dipyridamole at therapeutic doses has led to the conclusion that dipyridamole is only a weak antiplatelet agent.<sup>100</sup> High-dose dipyridamole is thought to inhibit platelet aggregation and may prevent platelet adhesion to exposed vascular sub-endothelium,<sup>93,102</sup> although this has not been confirmed by all studies.<sup>103</sup>

Although several biochemical effects have been proposed to account for the antithrombotic benefits of dipyridamole, the contribution of these effects to clinical outcome remains uncertain because of the very low (0.8 to 3.6  $\mu\text{mol/L}$ ) plasma concentrations of dipyridamole achieved. A recent *ex vivo* study of platelets, however, suggests that therapeutically relevant concentrations (1.6  $\mu\text{mol/L}$  = IC 50) of dipyridamole selectively inhibit phosphodiesterase type V, verifying amplification of the endothelium-dependent NO/cGMP-mediated pathway.<sup>104</sup>

Dipyridamole also reduces the size of formed platelet aggregates on a subendothelial matrix, including both large and very large-sized aggregates. This near-field antithrombotic enhancement effect requires the presence of endothelial cells.<sup>105</sup> Finally, decreased levels of platelet-derived growth factor occur during clotting when dipyridamole has been administered, but not with other antiplatelet drugs, including aspirin, ticlopidine, and trapidil.<sup>101</sup>

### ***Dipyridamole and Preconditioning***

Dipyridamole has been shown to affect therapeutic preconditioning, in which a brief period of ischemia protects the tissue from a subsequent, prolonged ischemic episode.<sup>98</sup> Clinically, preconditioning may have a role in preparation for vascular intervention, notably angioplasty, and may also prevent myocardial ischemia during high-dose dipyridamole echo-stress tests in patients with coronary artery disease.<sup>106</sup> Pharma-

cologically, preconditioning is accomplished by increasing the concentration of adenosine available before, and not after, sustained ischemia.<sup>107</sup>

The half-life of adenosine in human blood has been estimated to be ten seconds<sup>108</sup> because of the avid uptake of adenosine by high-affinity nucleoside transporters expressed by the endothelium and vascular smooth muscle cells.<sup>109</sup> The endothelial expression of the nucleoside transporter varies among species<sup>110</sup> and blockade of these transporters is achieved by dipyridamole.<sup>98</sup>

Physiologically, the endothelium acts as an impermeable metabolic barrier to the accumulation of intravascular and interstitial adenosine, suggesting that the vasodilatory action of adenosine must be, at least in part, mediated by the endothelium.<sup>111</sup> The adenosine is preferentially sent to nucleotide sinks, leaving little adenosine to reach the underlying myocardium to directly protect it against ischemia.<sup>98</sup> Recently, dipyridamole and intraluminally applied adenosine have been postulated to each act on different types of cells in the vascular wall.<sup>109</sup>

### *Dipyridamole and Inflammation*

Dipyridamole has selective anti-inflammatory properties not found with aspirin, such as differential blockade of gene expression in stimulated leukocytes. Modulation of inflammatory gene expression in platelet-leukocyte aggregates by dipyridamole may be critical to its therapeutic benefit.<sup>112</sup>

### *Dipyridamole and Platelet-leukocyte Interactions*

Preconditioning with dipyridamole attenuates endothelial cell-leukocyte interactions, preventing end-organ damage.<sup>113</sup> Adenosine has been demonstrated to interfere with leukocyte adhesion, activated neutrophil function, neutrophil-endothelial interactions, production and release of inflammatory mediators, and the expression of adhesion molecules.<sup>114</sup> In ischemia-reperfusion studies on feline endothelial cells, adenosine reduced the number of leukocytes adherent to the endothelium by 25% while leukocyte extravasation was reduced by 65-70% at 10 and 60 minutes after reperfusion of ischemic tissue. There was no difference in leukocyte rolling velocity, leukocyte adherence, or venular blood flow.<sup>115</sup>

### *Dipyridamole and Antioxidation*

Studies comparing dipyridamole to Vitamin E, a reference antioxidant in biological systems, demonstrate dipyridamole is a potent antioxidant *in vitro* at pharmacologically relevant doses.<sup>116</sup> Low density lipoprotein is protected from oxidation by dipyridamole, at concentrations as low as 2.5  $\mu\text{mol/L}$ . This protects endothelial cells from toxic damage.<sup>101</sup> Dipyridamole also scavenges superoxide anion and hydroxyl radicals as demonstrated by electron spin resonance spectroscopy.<sup>116,117</sup>

Nitric oxide reacts with superoxide anion to form the oxidant species peroxynitrite<sup>118</sup> and the vasodilating effects of nitric oxide may therefore be preserved when dipyridamole is administered. Similarly, free radical  $\text{PGI}_2$  inactivation and platelet activation may be prevented with dipyridamole.<sup>117</sup> Antioxidant activity compensates for trophic or chemically mediated insults to cultured rat cortical neurons *ex vivo*, such that dipyridamole may have a further role in neuroprotection.<sup>119</sup>

### *Dipyridamole and Tissue Plasminogen Activator*

Dipyridamole has recently been discovered to have profibrinolytic effects by enhancing the release of tissue plasminogen activator (tPA) from brain capillary endothelial cells. This occurs in a dose-dependent manner, through posttranscriptional release of tPA, in part through a protein kinase G-mediated pathway.<sup>120</sup>

### CONCLUSIONS

This review has summarized the non-antiplatelet effects of three classes of antiplatelet agents. While most of the work is preliminary, it is clear that such agents inhibit inflammation and have positive effects on the endothelium, allowing for additional mechanisms to prevent thrombosis.

Cardiovascular research has set the groundwork for this understanding, and, in many instances, work specific to the cerebrovascular system has lagged behind. Some of the studies above, as mentioned throughout this review, have focussed on the coronary vessels and cardiovascular system with a paucity of comparable literature in the cerebrovascular system. Debate has ensued on whether the results of laboratory and clinical studies related to the cardiovascular system are consistently applicable to patients with stroke. In many cases, there appears to be a similar effect on the vessels of the heart and brain, whereas in other studies, the results may be surprisingly different, leading to different clinical outcomes.<sup>121,122</sup> Therein is an opportunity for comparative work, both in the laboratory and in patient care, looking at the role of the endothelium in different vascular events. Moreover, it may permit for a better selection of antiplatelet therapies in patients with different stroke subtypes.

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