Aspirin and coronary artery disease

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Summary
Coronary atherosclerosis (CAD), a chronic inflammatory disorder, arises when genetic susceptibility, intercurrent conditions such as diabetes and hypertension and environmental factors interact. Although CAD can remain stable for many years, thrombus formation at sites of plaque rupture may lead to unstable angina (UA) or myocardial infarction (MI). Already recognised as the central component of coronary thrombosis, platelets, through their interaction with monocytes and endothelial cells, may also be involved at the earliest stages of atheromatous plaque evolution. Aspirin, the prototype antiplatelet agent, covalently and irreversibly inhibits cyclooxygenase (COX) and thus inhibits platelet thromboxane (TX) A2 biosynthesis. Anti-oxidant properties and the ability to modulate transcription of immunologically important genes have also been attributed to aspirin. Non-selective COX inhibition, however, predisposes to bleeding, predominantly secondary to dose-dependent gastro-intestinal toxicity. The emerging concept of “aspirin resistance” coincides with the development of alternative antiplatelet therapy and point-of-care platelet function assays. Though variable aspirin pharmacokinetics may explain many cases, heritable factors, inducible platelet COX expression and isoprostane formation may also contribute. In future, risk factor screening and point-of-care platelet function assay may identify vulnerable patients who would benefit from additional or alternate antiplatelet therapy.

Keywords
Atherosclerosis, coronary thrombosis, antiplatelet, aspirin resistance, heritable

Chronic vascular inflammation
Once regarded as an inevitable consequence of aging, coronary atherosclerosis, the primary cause of coronary artery disease (CAD), is now understood to be a chronic inflammatory disorder. Coexistence of genetic susceptibility, intercurrent conditions such as dyslipidemia, hypertension and diabetes and persistent exposure to environmental factors such as smoking and poor diet facilitate disease progression.

Occlusive coronary thrombus formation at the site of atherosclerotic plaque rupture is fundamental to the pathophysiology of myocardial infarction (MI) and cardiovascular mortality. Under normal physiological conditions coronary endothelium, a non-thrombogenic surface, generates prostacyclin (PGI2) and nitric oxide (NO), both potent platelet inhibitors. Once disrupted however the sub-endothelial matrix is strongly thrombogenic. Coronary atherosclerosis proceeds insidiously over decades manifest as lipid-rich plaque accumulation in the artery wall. Plaques with a large atheromatous core and thin fibrous cap lacking in smooth muscle cells are most vulnerable to disruption. Proteolytic enzymes released from macrophages within perpetuate a low-grade inflammatory process, which continually remodels the cap matrix. Plaque rupture and endothelial damage expose collagen, von Willebrand factor (vWF) and tissue factor (TF), and platelet-reactive substances, which initiate and accelerate extrinsic coagulation.
Platelet aggregation and activation

Platelet activation is mediated by adhesion and reinforced by soluble agonists such as thrombin. The glycoprotein (GP) Ib/V/IX receptor complex, which binds vWF immobilised on exposed collagen in the vessel wall tethers the platelet. Irreversible adhesion and spreading result from direct collagen binding via platelet GPVI and integrin receptor $\alpha_2\beta_1$. Adhesion of integrins $\alpha_5\beta_1$ and $\alpha_6\beta_1$ to fibronectin and laminin respectively in the extracellular matrix follows. Signals transduced upon vWF and collagen engagement mediate platelet degranulation, thromboxane (TX) $A_2$ generation and serotonin and adenosine diphosphate (ADP) release. These agonists reinforce platelet activation via G protein-coupled receptors and induce vessel wall inflammation and vasoconstriction (1).

Conformational change in the GPIIb/IIIa receptor permits fibrinogen cross bridging and facilitates interaction between circulating and adherent platelets. GAS6, a product of growth arrest specific gene 6, is secreted along with fibrinogen and P-selectin from the platelet $\alpha$-granule and promotes platelet/monocyte aggregate formation (2). CD40L, expressed on the cell surface is processed and released into plasma. Simultaneously on the platelet surface, TF activates extrinsic coagulation, which leads to prothrombinase complex assembly. Locally generated thrombin cleaves fibrinogen to fibrin resulting in clot stabilisation.

Cyclooxygenase and prostanoid biosynthesis

Platelets produce the cyclic prostanoid TXA$_2$, a short-lived agonist, in response to stimuli such as thrombin, collagen, ADP and epinephrine. Platelet activation promotes cytosolic free calcium and protein kinase C elevation, which induce phospholipase A$_2$ (PLA$_2$) translocation and activation. PLA$_2$ catalyses cleavage of membrane phospholipids and liberates arachidonic acid (AA), the main substrate for the membrane-associated platelet enzyme prostaglandin (PG) H$_2$-synthase, often referred to as cyclooxygenase (COX). COX exists as a dimer of identical monomers, each containing a tightly bound heme cofactor necessary for catalytic activity. AA is first metabolised to PGG$_2$ and then PGH$_2$ by the cyclooxygenase and peroxidase activities of the enzyme respectively in the committed step of prostaglandin biosynthesis. PGH$_2$ in turn is converted to TXA$_2$ by thromboxane synthase (Fig. 1).

Two COX isozymes, COX-1 and COX-2, have been identified (3). COX-1, is constitutively expressed and particularly

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**Figure 1:** The Eicosanoid synthesis pathway is depicted and the main action of the prostaglandin products on platelets and the vasculature is detailed. Aspirin’s inhibition of COX-1 dependent steps in the pathway is shown.
abundant in platelets, the kidney and gastrointestinal tract where it produces cytoprotective PGs. Pro-inflammatory cytokines and growth factors induce COX-2 expression, hence its role in inflammation and regulation of cell growth (4). The primary amino acid sequences of these membrane-bound glycosylated homodimers are 67% identical and both metabolise AA, albeit at different rates.

AA oxidation by COX occurs at the apex of a long hydrophobic pocket in the region of a tyrosyl radical (tyrosine 385) in what is referred to as the enzyme’s active site. COX-1 has a 25% smaller substrate-binding pocket; the increased volume in COX-2 results from a side-pocket extension of the hydrophobic channel (5). A third COX isozyme initially described in canine brain was subsequently detected in human brain and cardiac tissue. This protein is derived from a splice variant of the COX-1 gene with persistence of the first intron (6).

### The platelet as a therapeutic target

The platelet’s central role in coronary thrombosis makes it an attractive therapeutic target. This is offset, however, by its haemostatic role at sites of vascular injury. Salicin, a natural analgesic and antipyretic derived from white willow bark was modified with an acetyl group in 1853 and broadly prescribed as acetyl salicylic acid (Aspirin) from 1915. Four decades later an American General Practitioner discovered its antiplatelet potential (7). The first prospective randomised trial of aspirin administration post MI was published in 1974 and though inconclusive prompted further evaluation (8). The US Food and Drug Administration (FDA) licensed aspirin use after MI in 1985 and for suspected MI in 1996. A recent meta-analysis concluded that in a population at high risk of occlusive vascular events, antiplatelet therapy reduces the combined endpoint of serious vascular events by one quarter, non-fatal myocardial infarction by one third, non-fatal stroke by one quarter and serious vascular events by one quarter, non-fatal myocardial infarction by one third, non-fatal stroke by one quarter and vascular mortality by one sixth (9). Therefore, both European and American guidelines recommend antiplatelet therapy in patients with established CAD (10, 11).

### Aspirin

Aspirin, the prototype antiplatelet agent covalently and irreversibly modifies COX-1 and COX-2 by acetylating serine 530 and serine 516 in their respective active sites. However, affinity for COX-1 is 50 to 100 times that for COX-2. COX-1 acetylation results in steric inhibition of AA binding and thus blocks TXA<sub>2</sub> biosynthesis. In the case of COX-2, however, enzyme activity persists. It acquires 15-lipoxygenase activity and produces 15(R) hydroxy-eicosatetraenoic acid (HETE), a novel product (12). Thus, although aspirin inhibits COX non-selectively its antiplatelet properties are attributed to COX-1 inhibition.

Aspirin is rapidly absorbed from the stomach and acetylates platelet COX-1 in the pre-systemic circulation (13). Administration of plain aspirin at a dose of 325 mg achieves full platelet inhibition (>95% TX inhibition) in approximately 3 hours, however, at doses of 40 to 80 mg the effect is cumulative and maximum inhibition occurs after 4 days. Being anucleate, inhibition through COX-1 acetylation persists for the lifespan of the platelet (8-10 days). Only 10% of the platelet pool is replenished daily so doses as low as 30 mg of plain aspirin can fully inactivate COX-1 upon repeat daily dosing (14). Thus, although aspirin has a short half life (15-20 min) and is rapidly converted to the inactive salicylic acid (15), its pharmacodynamic half-life is 5 days.

Aspirin’s role in secondary prevention of cardiovascular events is well established. Increased TXA<sub>2</sub> biosynthesis during ischaemic episodes may account for its particular benefit in high-risk individuals with established cardiovascular disease (16). Its role in the primary preventative setting is less clear. Several placebo-controlled studies have addressed this issue with mixed results (Table 1) (17-22). Thus, neither European nor American guidelines recommend antiplatelet therapy for primary prevention of cardiovascular disease. The recent Antithrombotic Trialists Collaborative Meta-analysis, however, proposed that diabetic patients with substantial risk of a first vascular event and no specific risk of bleeding are likely to benefit from anti-platelet therapy.

### Platelets – beyond thrombus formation

Thrombosis, atherosclerosis and inflammation coexist in most acute coronary syndromes (ACS). The platelet, previously regarded as a component of thrombosis, appears to play a pivotal role in all three processes. A source of cytokines, chemokines and TF, platelets may contribute to both local and systemic vascular inflammation.

Surface-expressed P-selectin (CD62P), released from platelet α-granules during activation, acts as a receptor for monocytes and neutrophils in thrombin-activated platelets and may thus facilitate their recruitment to sites of thrombosis (23). Moreover, platelets are known to induce monocyte chemokine synthesis and TF expression in a P-selectin dependent manner (24, 25). Monocytes and endothelial cells also express CD40, a membrane-bound glycoprotein originally described in B-lymphocytes. Its ligand CD40L, expressed predominantly by activated T-helper cells, also exists in its functional form on macrophages, smooth muscle cells and activated platelets. Recent studies show that activated platelets trigger an inflammatory response and procoagulant activity in endothelial cells and monocytes via a CD40/CD40L dependent pathway (26, 27).

Clinically silent mural platelet aggregates are also thought to mediate CAD progression. Platelets incorporated into vascu...
Aspirin or salicylate may influence gene expression. An effect on nuclear factor (NF)-κB-mediated gene transcription is evident at supra-pharmacological concentrations (>5mM); levels not attained in humans (35). At pharmacological levels however aspirin may modulate COX-2 transcription through selective inhibition of a CCAAT/enhancer (C/EBPβ) binding protein (36). C/EBPβ mediates transcription of immunologically important genes. Consistent with this, salicylate mediated suppression of interleukin-4 expression has recently been described (37).

Epidemiological studies show that aspirin use reduces risk of colon cancer, breast cancer and lymphoma.\(^3\) This may be a consequence of coincident COX-2 inhibition, which reduces occurrence of large bowel carcinoma and also decreases the size of pre-cancerous polyps. In addition to direct inhibition, effects on COX gene transcription may be involved (39). Moreover, COX-1 has been linked to tumour formation (40). Indeed, tumorigenesis is markedly reduced in both COX-1 and COX-2 knockout mice. This may reflect differential effects of the two isozymes on tumour-induced angiogenesis and tumour growth. Similar effects on cell growth within an evolving atherosclerotic plaque may influence CAD progression.

### Side effects of aspirin therapy

Platelet inhibition through suppression of TXA\(_2\) generation predisposes to bleeding. This may explain the increased incidence of haemorrhagic stroke observed in both primary and secondary
prevention cardiovascular trials with aspirin which offset benefit (18).

PGE₂ activates the G-protein coupled prostaglandin receptor EP₂ in gastric mucosa, inhibits acid secretion and increases mucous formation. Hence aspirin, which antagonises COX-1 dependent PGE₂ synthesis, results in dose-dependent gastrointestinal toxicity (41). Even at a dose as low as 75 mg aspirin may still cause gastrointestinal bleeding (42). Buffered and enteric-coated aspirin preparations developed to attenuate local gastric erosion and minimise this side effect appear to fall short of their goal (43).

In the kidney prostaglandins support renal perfusion, diminish vascular resistance and facilitate natriuresis. Therefore chronic aspirin use may reduce renal blood flow and glomerular filtration therein impairing renal function (44). These effects, which are due to PGI₂ depletion, occur at high aspirin doses and most frequently in elderly patients and those with established renal disease.

High dose aspirin may also attenuate morbidity and mortality benefit of angiotensin-converting enzyme (ACE) inhibitors in hypertensive and congestive heart failure patients (45). Renin-angiotensin-aldosterone system activation as a consequence of cardiovascular disease, causes ACE dependent bradykinin degradation. ACE inhibition, however, promotes bradykinin dependent NO, PGE₂ and PGH₂ synthesis, an effect which may be attenuated by aspirin.

**Aspirin resistance**

Recent studies have demonstrated heterogeneity in the way individuals respond to aspirin and have given rise to the concept of “aspirin resistance.” This term has been broadly applied although the definition often varies from study to study. It is noteworthy that ESC and ACC Guidelines for aspirin use to treat cardiovascular disease have been interpreted to embrace a variety of aspirin formulations. Thus before addressing the issue of “aspirin resistance” it is worth reappraising what the term “aspirin” currently incorporates.

Transformed from powder to tablet in 1915, acetyl salicylic acid has since evolved considerably. Now available as a calcium salt, combined with citrate, glycine, bicarbonate-bound, polymer-coated in rapid-release (compressed, soluble), buffered and enteric-coated preparations, pharmacokinetic diversity mirrors formulation variety. The modern epidemic of obesity is also relevant to drug pharmacokinetics. Our rapidly evolving body habitus may be challenging treatment thresholds established in earlier studies. Daily dosing with 30 mg plain aspirin achieves maximum TX suppression in healthy subjects (14), however, is that evidence adequate to support use of 75 mg enteric-coated aspirin once daily in a 110 kg patient with CAD? (46).

Many patients who take aspirin for secondary prevention will have further vascular events. Indeed, treatment with aspirin prior to presentation with an ACS indicates poor prognosis (47). However this may reflect complex pathology rather than resistance to therapy. To define what constitutes “resistance” or “non-response” it is important to clarify what response is expected and with what assay. In vivo, erythrocytes and leukocytes modulate platelet reactivity (48), while collagen, ADP, thrombin, epinephrine, serotonin and shear stress act synergistically on the platelet. Clearly, ex-vivo replication of this environment is impractical. Assays of TXB₂ generation and arachidonic acid-induced platelet aggregation depend on COX-1 function and thus reflect aspirin’s pharmacological effect on platelets. Aggregation to agonists such as epinephrine, ADP, collagen and thrombin receptor activating peptide (TRAP), however, is less TX dependent. Thus, although they reveal the heterogeneous nature of platelet agonism, these assays may be less appropriate determinants of aspirin efficacy.

Platelet function assays are moving from laboratory to bedside. Automated aspirin-sensitive point-of-care assays may detect sub-optimal platelet inhibition and make individualised antiplatelet therapy possible. Whatever the approach, clinical studies are needed to determine the usefulness of assays developed to detect aspirin resistance (49). Two recent studies support the significance of “aspirin resistance” determined by persistent TX biosynthesis and persistent platelet aggregation. A Heart Outcomes and Prevention Evaluation (HOPE) sub-study showed that high levels of urinary 11-dehydrothromboxane B₂, an enzymatic metabolite of TX, predicted myocardial infarction and cardiovascular death in CAD patients treated with aspirin (50). Similarly, Gum et al. documented a greater than threefold increased risk of major adverse events associated with persistent platelet aggregation to arachidonic acid and ADP despite aspirin therapy (51).

When contemplating causes of “aspirin resistance” poor patient compliance, inadequate dosing and drug interaction must be considered at the outset. In the Aggrenox versus Aspirin Treatment Evaluation (AGATE) Trial, almost 10% of patients with recent ischaemic stroke discontinued aspirin therapy despite medical advice (52). Under dosing due to sub-optimal bioavailability of low dose enteric-coated aspirin could also be a factor (53, 54). Delayed absorption associated with some aspirin preparations may facilitate esterase deactivation or chemical deactivation at the high pH of the upper small intestine (55). In addition, some non-steroidal anti-inflammatory drugs (NSAIDS), specifically demonstrated for ibuprofen, may obstruct aspirin’s access to the COX-1 active site and prevent acetylation of the target serine residue. Ibuprofen is a relatively short-acting and reversible inhibitor of COX-1, which fails to provide 24-hour antiplatelet activity and prevents aspirin from inactivating the enzyme. Thus, easy access to popular over the counter preparations may predispose to aspirin resistance (56).

Individuals who take high dose plain aspirin also show resistance therefore not all cases can be explained by subopti-
nal pharmacokinetics. Interindividual pharmacodynamic variability occurs with most drugs and aspirin is no different, thus aspirin resistance assays may reflect, at least in part, pharmacodynamic heterogeneity. Patients who have undergone coro-nary artery bypass grafting demonstrate enhanced platelet aggregation despite aspirin therapy (57). Increased platelet turnover and generation of isoprostanes, which mimic the activity of TX are potential mechanisms (58). Isoprostanes are generated non-enzymatically by direct arachidonic acid oxidation and are insensitive to aspirin (59).

Heritable factors are greater determinants of platelet reactiv-
ty than environmental factors in patients with cardiovascular disease (60, 61). Consistent with this finding, women from fami-
lies with premature atherosclerosis demonstrate platelet hyper-reactivity (62). Indeed, when genetic risk of MI is examined by gender, risk to women is greater than to men (63). Enhanced platelet sensitivity to ADP, collagen and epinephrine or genetic variation in glycoproteins which modulate platelet reactivity may predispose to thrombotic complications (64-67). There is some evidence that variation in genes that encode for proteins of the eicosanoid synthesis pathway may also determine platelet sensitivity to aspirin. For example, polymorphism of the COX-1 gene has been linked with a variable response to aspirin (68-70).

In conclusion, aspirin remains the cornerstone of antiplate-
let therapy in patients with coronary artery disease. However, evidence of heterogeneity in the way individuals respond has led to the concept of “aspirin resistance.” While pharmacokinetics may explain many cases, heritable factors, non-cyclooxygenase pathways of platelet activation, inducible platelet COX expression and isoprostane formation may also be important. In future, at risk patients detected by risk factor screening and point-of-care platelet function assay may benefit from additional or alternative antiplatelet therapy.

References

4. Vane JR, Bakhle YS, Botting RM. Cyclo-
6. Chandrasekharan NV, Dai H, Roos KL, et al. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesics/antipy-
7. Craven LL. Prevention of coronary and cere-
platelet agents. The task force on the use of antiplatelet agents in patients with atheroscle-
13. Pedersen AK, FitzGerald GA. Dose-related kinetics of aspirin. Presystemic acetylation of platelet cyclooxyge-

tension: principal results of the Hyperten-
23. Norden A. Human Platelet Glycoproteins. In: Bloom AF, CD, ed. Haemostasis and Throm-