

W Aspirin resistance

Graeme J Hankey, John W Eikelboom

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Department of Neurology, Royal Perth Hospital and School of Medicine and Pharmacology, University of Western Australia, Perth, WA, Australia (G J Hankey FRCP); and Department of Medicine, McMaster University, HGH McMaster Clinic, 237 Barton Street East, Hamilton, ON, L9K 1H8, Canada (J W Eikelboom FRACP)

Correspondence to: Dr John Eikelboom eikelbj@mcmaster.ca

Aspirin resistance is the inability of aspirin to reduce platelet production of thromboxane A₂ and thereby platelet activation and aggregation. Increasing degrees of aspirin resistance may correlate independently with increasing risk of cardiovascular events. Aspirin resistance can be detected by laboratory tests of platelet thromboxane A₂ production or platelet function that depend on platelet thromboxane production. Potential causes of aspirin resistance include inadequate dose, drug interactions, genetic polymorphisms of COX-1 and other genes involved in thromboxane biosynthesis, upregulation of non-platelet sources of thromboxane biosynthesis, and increased platelet turnover. Aspirin resistance can be overcome by treating the cause or causes, and reduced by minimising thromboxane production and activity, and blocking other pathways of platelet activation. Future research is aimed at defining aspirin resistance, developing reliable tests for it, and establishing the risk of associated cardiovascular events. Potential mechanisms of aspirin resistance can then be explored and treatments assessed.

In 2004, the *New York Times* reported that up to 40% of aspirin users are resistant to aspirin.¹ Patients prescribed aspirin to prevent atherothrombotic vascular disease are now asking their doctors if they are resistant to aspirin and, if so, what the implications are. The issues to consider include: what is aspirin resistance; how is aspirin resistance diagnosed; is aspirin resistance clinically relevant; what causes aspirin resistance; can aspirin resistance be treated, and if so, how; and are there other forms of antiplatelet drug resistance?

How does aspirin work?

Platelet adhesion, activation, and aggregation

When the intima of a blood vessel is disrupted, as happens after a cut or the rupture of an atherosclerotic plaque, subendothelial collagen and von Willebrand factor are exposed to circulating blood. Platelets in the blood adhere to subendothelial collagen and von Willebrand factor through their glycoprotein Ia/IIa and Ib/V/IX receptors.^{2–4} Platelet adhesion stimulates platelet activation, which leads to a change in their shape and the release of bound calcium within the platelet.

The increased concentration of free ionic calcium within the platelet has several consequences. First, it induces a conformational change in the platelet glycoprotein IIb/IIIa receptors on the surface of platelets, so that they can bind adhesive proteins in the circulation, such as fibrinogen. Second, it catalyses the release of active molecules (eg, ADP) from platelet granules into the circulation where they may bind to receptors (eg, ADP) on the surface of adjacent platelets and trigger their activation. Third, it promotes the action of phospholipase A₂ to produce arachidonic acid.^{2–4}

Arachidonic acid in platelets is converted to thromboxane A₂ in a reaction that is catalysed by the enzymes cyclooxygenase 1 (to form prostaglandin G₂/H₂) and thromboxane synthase (to form thromboxane A₂) (figure 1). Thromboxane A₂ increases the expression of fibrinogen receptors on the platelet's membrane and is released into the circulation where it binds to thromboxane receptors on the surface of adjacent platelets to trigger their activation. Thromboxane A₂ also acts synergistically with other products released by activated platelets (eg, ADP, fibrinogen, factor V) to augment platelet activation. Further, thromboxane A₂ is a potent vasoconstrictor.

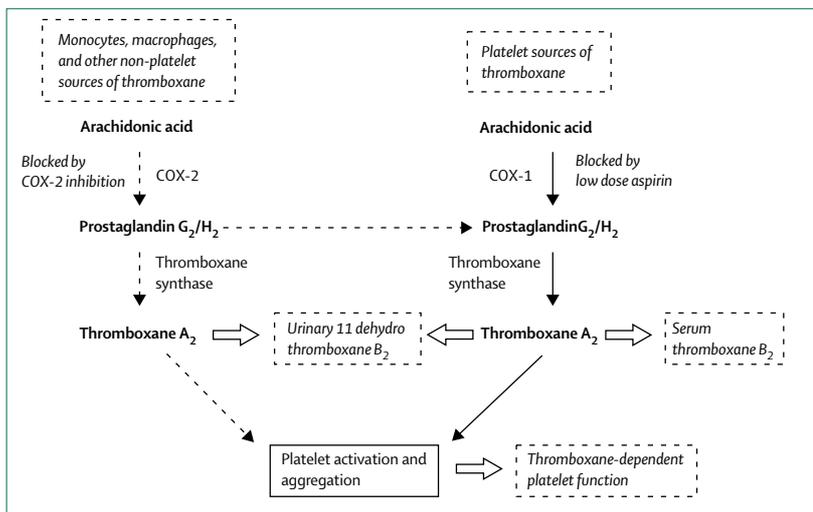


Figure 1: Pathways of thromboxane production and the antiplatelet effects of aspirin. Dotted arrows denote "bypass" of aspirin's inhibition of platelet COX-1.

Search strategy and selection criteria

We searched EMBASE, MEDLINE, and the Cochrane Library to June 30, 2005. We used these search items in the following combinations: "aspirin", "acetylsalicylic acid", "failure", "resistance"; "platelet", "activation", "aggregation", "function"; "atherothrombosis", "atherosclerosis"; "acute coronary syndrome", "myocardial infarction", "angina", "peripheral artery disease", "stroke", "genetics", "COX", "polymorphism". After reviewing the abstracts, we obtained and reviewed the full text of relevant articles, and their reference lists. We also contacted experts in the field for additional data. We included articles that addressed aspirin resistance and non-responsiveness and its diagnosis, prevalence, cause, prognosis, and treatment.

Effects of aspirin on platelet activation

Aspirin (acetylsalicylic acid) reduces the activation of platelets by irreversibly acetylating cyclooxygenase-1 (COX-1), and thereby reduces thromboxane A₂ produced by platelets.⁴⁻⁸ The inhibition of COX-1 is rapid, saturable at low doses (ie, dose-independent), irreversible, and permanent for the life of the platelet because platelets lack the biosynthetic machinery to synthesise new protein.

After a single 325-mg dose of aspirin, platelet COX-1 activity recovers by about 10% per day due to new platelet formation.⁸ Once daily, low-dose aspirin (0.45 mg/kg, about 30 mg) suppresses serum thromboxane B₂ formation (a stable metabolite of thromboxane A₂) by at least 95% within about 5 days, and this level of inhibition is maintained with long-term daily administration.⁶⁻⁹ Aspirin also has dose-dependent antithrombotic effects on platelet function and blood coagulation that are unrelated to its ability to inhibit platelet COX-1.⁵ However, these mechanisms have not been correlated with known molecular mechanisms and are believed to be much less important than inhibition of platelet COX-1.^{4,5}

What is aspirin resistance?

Aspirin resistance may be defined as laboratory resistance and clinical resistance.¹⁰ Laboratory aspirin resistance is defined as the failure of aspirin to inhibit platelet thromboxane A₂ production or inhibit tests of platelet function (eg, platelet aggregation) that are dependent on platelet thromboxane production.¹⁰ Clinical aspirin resistance is defined as the failure of aspirin to prevent clinical atherothromboembolic ischaemic events in patients prescribed aspirin.¹⁰ However, this problem is more accurately referred to as aspirin treatment failure.

How is aspirin resistance diagnosed?

Laboratory diagnosis of aspirin resistance

Aspirin resistance can be diagnosed in the laboratory by measurement of platelet thromboxane A₂ production or thromboxane-dependent platelet function (table, figure 1).¹¹⁻¹³

Thromboxane A₂ production

Thromboxane A₂ production can be determined by measuring stable metabolites of thromboxane A₂, such as thromboxane B₂ in the serum (or plasma) and 11-dehydro thromboxane B₂ in the urine (table). Because serum thromboxane B₂ production is largely dependent on platelet COX-1 (aspirin's therapeutic target), it has been used as a measure of the inhibitory effects of low-dose aspirin on platelets.¹⁴

Thromboxane-dependent platelet function

Tests of platelet function that are dependent on platelet thromboxane production include agonist-induced

platelet aggregation measured by light or optical transmission (turbidimetric aggregometry in platelet rich plasma), electrical impedance (whole blood platelet aggregometry), or semi-automated platelet aggregometry (eg, platelet function analyser [PFA]-100, Ultegra rapid platelet function assay [RPFA]; table). The bleeding time is an in-vivo test of platelet function that also is dependent, in part, on platelet thromboxane production but is used rarely because it is highly operator dependent and poorly reproducible.

Light or optical transmission aggregometry measures the increase in light transmission through a platelet suspension when platelets are aggregated (and forms clumps) by an agonist such as thromboxane A₂, ADP, or collagen. This is the historical gold standard test to measure the antiplatelet effects of aspirin and remains the most widely used test for determining platelet function. For studying the effects of aspirin, arachidonic acid (being the precursor of thromboxane A₂) is a more suitable platelet agonist than others (eg, ADP, collagen), which induce platelet aggregation through pathways that are less dependent on thromboxane production.

Impedance aggregometry measures the change in electrical impedance between two electrodes when platelets are aggregated by an agonist. The method is similar to light or optical aggregometry except that it can be done in whole blood, thus obviating the need for preparation of a platelet suspension. Impedance aggregometry can also be done in thrombocytopenic patients.

	Advantages	Limitations
Thromboxane production		
Serum thromboxane B ₂	Directly dependent on aspirin's therapeutic target, COX-1	May not be platelet specific Operator expertise required
Urinary 11-dehydro-thromboxane B ₂	Dependent on aspirin's therapeutic target, COX-1 Correlated with clinical events	Not platelet specific Uncertain sensitivity Uncertain reproducibility Not widely evaluated
Thromboxane-dependent platelet function		
Light or optical aggregation	Traditional gold standard Widely available Correlated with clinical events	Not specific Uncertain sensitivity Limited reproducibility Labour intensive Operator and interpreter dependent
Impedance aggregation	Less sample preparation required	Not specific Uncertain sensitivity Operator and interpreter dependent
PFA-100	Simple Rapid Semi-automated Correlated with clinical events	Dependent on von Willebrand factor and haematocrit Not specific
Ultegra RPFA	Simple Rapid Semi-automated Point-of-care test Correlated with clinical events	Uncertain sensitivity Uncertain specificity Uncertain sensitivity

Adapted in part from Michelson.¹³

Table: Laboratory tests commonly used to measure the antiplatelet effects of aspirin

The PFA-100 could be regarded as an in-vitro bleeding time recorder.¹⁵ It creates an artificial vessel consisting of a sample reservoir, a capillary, and a biologically active membrane with a central aperture, coated with collagen plus ADP or collagen plus epinephrine. The application of a constant negative pressure aspirates the anticoagulated blood of the sample from the reservoir through the capillary (mimicking the resistance of a small artery) and the aperture (mimicking high shear in the injured part of the vessel wall). A platelet plug forms that gradually occludes the aperture. Consequently, the blood flow through the aperture gradually decreases and ultimately stops. The time taken to interrupt blood flow—closure time—is recorded.

The Ultegra RPFA-ASA (Accumetrics, San Diego, CA, USA) is a simple rapid bedside test that measures agglutination of fibrinogen-coated beads in response to propyl gallate or, more recently, arachidonic acid stimulation. If aspirin produces the expected antiplatelet effect, fibrinogen-coated beads will not agglutinate, and light transmission will not increase. The result is expressed as aspirin reaction units.

Other laboratory tests that have been proposed for diagnosing aspirin resistance include plateletworks (Helena Laboratories, Beaumont, TX, USA); the IMPACT cone and plate analyser (Diamed, Cressier, Switzerland); and flow cytometry to measure activation dependent changes in platelet surface P-selectin or glycoprotein expression.¹³ However, these tests are less well studied than those listed in the table.

Limitations of the laboratory diagnosis of aspirin resistance

Thromboxane A₂ production

Measurement of serum (or plasma) thromboxane B₂ is labour intensive, not readily available, and might not be specific for platelet function. Urinary 11-dehydrothromboxane B₂ reflects in-vivo thromboxane production but it is not specific (renal as well as other, non-platelet, sources of thromboxane A₂ affect urinary 11-dehydrothromboxane B₂ concentrations; table, figure 1).

Thromboxane A₂ can also be produced in circulating monocytes and macrophages within atherosclerotic plaque (figure 1). Arachidonic acid is converted to prostaglandin G₂/H₂ by COX-2, which is upregulated by proinflammatory mediators of cardiovascular disease.¹⁶ Prostaglandin G₂/H₂ is converted to thromboxane A₂ by thromboxane synthase, which is present in large quantities in monocytes and macrophages.¹⁷ Prostaglandin H₂ produced by monocytes or macrophages and endothelial cells can also be converted to thromboxane A₂ by platelet thromboxane synthase by means of transcellular metabolism, thus bypassing the platelet COX-1 blocked by aspirin.¹⁸ Further, thromboxane A₂ can be generated from platelets by pathways not catalysed by COX-1, but rather COX-2, which may also be present in platelets and megakaryocytes.^{19–21} COX-2 expression can be particularly

elevated in high platelet turnover states when there are increased numbers of immature platelets (eg, recent surgery, infection, inflammation—perhaps active atherosclerosis).^{19,20} Thus, thromboxane A₂ can be generated from monocytes, macrophages, endothelial cells, and perhaps platelets, through the action of COX-2.

Urinary 11-dehydrothromboxane B₂ is also substantially affected by the dose of aspirin.^{22–24} Higher doses of aspirin result in greater inhibition of COX-2, and substantially lower concentrations of urinary 11-dehydrothromboxane B₂.^{22–24} Because this finding is at odds with clinical trial evidence showing that aspirin is effective in preventing cardiovascular events independent of its dose, urinary 11-dehydrothromboxane B₂ might not be a valid laboratory measure of the antiplatelet effect of aspirin.

Thromboxane-dependent platelet function

Tests of thromboxane-dependent platelet function (eg, platelet aggregation induced by arachidonic acid) may not be specific because platelets are activated not only by stimulation of the thromboxane A₂ receptor but also by stimulation of other pathways of platelet activation, such as the platelet glycoprotein receptors for collagen (Ia/IIa), von Willebrand factor (Ib/V/IX), ADP, thrombin, and epinephrine, as well as by shear stress on platelets.^{2–4}

The specific limitations of light or optical transmission and impedance aggregometry are that results can vary by age, sex, race, diet, and haematocrit level,¹⁴ and that the accuracy and reproducibility of these techniques are poor even after controlling for the many variables that could affect the results of platelet aggregation. These tests are also poorly standardised, so results from one laboratory might not be comparable with those from another. Platelet aggregometry is not ideal for testing platelet sensitivity to aspirin because it does not reproduce the high shear conditions that occur in vivo and, depending on the agonist used and its concentration, the aggregation response is only partly modulated by thromboxane A₂. Furthermore, light or optical aggregometry does not take into account interactions between cells that arise in vivo.

The limitations of the PFA-100 system are that it is sensitive to many variables (other than thromboxane A₂ production) including platelet count, platelet function, red blood cells, and plasma von Willebrand factor.²⁵ For example, because the system studies platelet function under flow conditions that are characterised by high shear, plasma von Willebrand factor is a major determinant of closure time. As a result, the effect of aspirin on closure time of the collagen-epinephrine cartridge²⁶ can be outweighed by other variables, such as von Willebrand factor, that are not affected by aspirin. This could be one explanation for the high proportion of patients with short closure time, despite being treated with aspirin, and the poor agreement between the PFA-100 and light or optical aggregation ($\kappa=0.1$, 95% CI 0.04–0.25).²⁷

The limitation of the Ultegra RPFA is its diagnostic criterion for aspirin resistance is based on a cut-off that was established by comparison with light or optical platelet aggregation in response to adrenaline after a single 325-mg dose of aspirin.^{28,29} Although adrenaline-induced platelet aggregation can provide a sensitive measure of aspirin's antiplatelet effect, it is not specific and it is unclear how well it correlates with the antiplatelet effects of low dose aspirin.

In summary, the historical gold standard test of platelet function (light or optical aggregation) has well established limitations for measuring the antiplatelet effects of aspirin. Other tests for aspirin resistance might overcome some of these limitations but they have not been standardised and they do not correlate well with each other.³⁰ The laboratory diagnosis of aspirin resistance is therefore highly test-specific.

Irrespective of which laboratory test of aspirin resistance is used, the antiplatelet effects of aspirin, as measured in the laboratory, seem to be variable in different individuals, and probably have a continuous (and broad) distribution,^{31,32} as do blood pressure,³³ blood cholesterol concentrations³⁴ and the antiplatelet effects of clopidogrel.³⁵ As a result there is probably no clear cut-off between the presence and absence of aspirin resistance, just as there is no clear cut off between the presence and absence of hypertension,^{33,36,37} and so the sensitivity and specificity of the laboratory tests for the diagnosis of laboratory aspirin resistance are also uncertain (table).

Clinical diagnosis of aspirin resistance

Aspirin resistance can be diagnosed clinically by the occurrence of an atherothrombotic ischaemic event in a patient taking a therapeutic dose of aspirin.¹⁰ The clinical diagnosis of aspirin resistance is limited because it is retrospective (made after the event) and non-specific. Aspirin only prevents up to about 25% of all ischaemic vascular events³⁸ and, for the other 75% of vascular events, there are many causes (panel 1), including all the causes of (laboratory) aspirin resistance (panel 2). It is more appropriate to classify these patients as having a failure of (response to) therapy, rather than clinical resistance to therapy.

Recommendation for the diagnosis of aspirin resistance

Aspirin resistance should be defined and diagnosed according to the results of laboratory tests, when proven to be reliable, valid, standardised, and clinically relevant (ie, they correlate independently with risk of ischaemic events).

Is aspirin resistance clinically relevant?

Prognostic significance

One of the first studies to show an association between a laboratory measure of aspirin resistance and the risk of serious vascular events was a cohort study of 181 patients

with a previous stroke who were treated with a very high dose of aspirin (500 mg three times a day) and who underwent a laboratory test of platelet reactivity at baseline.³⁹ This test measured the extent of platelet activation induced by blood collection, with more platelet activation reflecting less platelet inhibition by aspirin. A third (n=60) of patients were diagnosed with aspirin resistance. After two years of follow-up, 40% of patients with aspirin resistance experienced a serious vascular event compared with only 4.4% who did not have aspirin resistance (relative risk 9.1, 95% CI 3.7–22.7).

In 2002, a substudy of the HOPE trial measured urinary 11-dehydro thromboxane B₂ (a marker of in-vivo thromboxane generation) in 976 high vascular risk patients taking aspirin 75–325 mg/day at enrolment

Panel 1: Possible causes of aspirin failure

Reduced bioavailability of aspirin

- Inadequate intake of aspirin (poor compliance)
- Inadequate dose of aspirin
- Reduced absorption or increased metabolism of aspirin

Altered binding to COX-1

- Concurrent intake of certain non-steroidal anti-inflammatory drugs (for example ibuprofen, indometacin), possibly preventing the access of aspirin to COX-1 binding site

Other sources of thromboxane production

- Biosynthesis of thromboxane by pathways that are not blocked by aspirin (for example, by COX-2 in monocytes and macrophages, and vascular endothelial cells)

Alternative pathways of platelet activation

- Platelet activation by pathways that are not blocked by aspirin (for example, red-cell induced platelet activation; stimulation of collagen, adenosine diphosphate, epinephrine, and thrombin receptors on platelets)
- Increased platelet sensitivity to collagen and ADP

Increased turnover of platelets

- Increased production of platelets by the bone marrow in response to stress (for example, after coronary artery bypass surgery), introducing into bloodstream newly formed platelets unexposed to aspirin during the 24-h dose interval (aspirin is given once daily and has only a 20-min half-life)

Genetic polymorphisms

- Polymorphisms of COX-1, COX-2, thromboxane A₂-synthase or other arachidonate metabolism enzymes
- Polymorphisms involving platelet glycoprotein Ia/IIa, Ib/V/IX, and IIb/IIIa receptors, and collagen and von Willebrand factor receptors
- Factor XIII Val34Leu polymorphism, leading to variable inhibition of factor XIII activation by low dose aspirin

Loss of the antiplatelet effect of aspirin with prolonged administration

- Tachyphylaxis

Non-atherothrombotic causes of vascular events

- Embolism from the heart (red, fibrin thrombi; vegetations; calcium; tumour; prostheses)
- Arteritis

Panel 2: Possible mechanisms of reduced suppression of thromboxane production (laboratory aspirin resistance)

Impaired suppression of platelet cyclooxygenase-1

Inadequate dose of aspirin

Drug interactions

- Concurrent intake of NSAIDs, preventing access of aspirin to the COX-1 binding site

Increased turnover of platelets

- Immature platelets unexposed to aspirin during the 24-h dose interval

Genetic polymorphisms

- Polymorphisms of COX-1

Bypass of aspirin's inhibition of platelet cyclooxygenase-1 (aspirin bypass)

Alternative sources of thromboxane production

- Non-platelet sources of thromboxane biosynthesis (eg, monocyte COX-2)

Increased turnover of platelets

- Immature platelets containing measurable levels of COX-2

Genetic polymorphisms

- Polymorphisms of COX-2

with a nested case-control design.⁴⁰ Compared with patients in the lowest quartile of urinary 11-dehydrothromboxane B₂ concentration (<15.1 µg/mol creatinine, indicating suppression of thromboxane production by aspirin), those in the highest quartile (>33.8 µg/mol creatinine) had an adjusted increased odds of a serious vascular event (stroke, myocardial infarction or death from vascular causes) of 1.8 (1.2–2.7) during a median of 4.5 years' follow up (figure 2). Furthermore, the association between increasing urinary 11-dehydrothromboxane B₂ concentrations and increasing risk of serious vascular events was continuous, rather than categorical. These data suggest that the aspirin resistance is a continuum, and the association between increasing aspirin resistance and increasing risk of vascular events is linear or log-linear, similar to the association between other risk factors, such as increasing blood pressure and blood cholesterol, and risk of serious vascular events.^{33,34}

In 2003, Gum and colleagues⁴¹ reported a cohort of 326 patients with coronary or cerebrovascular disease who were treated with aspirin 325 mg/day and who were followed up for the next 2 years.⁴¹ At baseline, blood was taken for light or optical platelet aggregometry and aspirin resistance was defined as a failure to inhibit arachidonic acid and ADP-induced platelet aggregation. The cut-off point for diagnosing aspirin resistance was derived from 40 in-house controls and constituted a mean aggregation of ≥70%

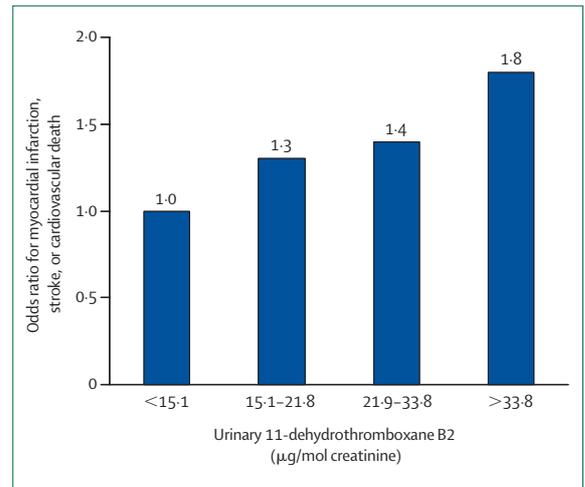


Figure 2: Association between elevated urinary 11-dehydrothromboxane B₂ and incidence of myocardial infarction, stroke, or cardiovascular death in the HOPE study⁴⁰

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with 10 µmol/L ADP as an agonist, and ≥20% with 0.5 g/L arachidonic acid as an agonist. Of the 17 (5%, 3–8%) patients who were diagnosed as having aspirin resistance, five (29% 10–57%) experienced a serious vascular event over the next 2 years, compared with 30 (10%, 7–14%) of the 309 patients diagnosed as non aspirin-resistant (figure 3). The adjusted hazard ratio of a serious vascular event was 4.1 (1.4–12.1) for patients with aspirin resistance. About 10% (0–20%) of recurrent vascular events could be attributed to aspirin resistance. Other studies that used impedance platelet aggregometry,⁴² the PFA-100, or RPFA^{43–45} confirm that laboratory aspirin resistance predicts cardiovascular risk and that functional aggregometry identifies at-risk individuals.

Limitations of the prognostic studies

Although the direction of the results of all of these studies is consistent (ie, positive), thus supporting their

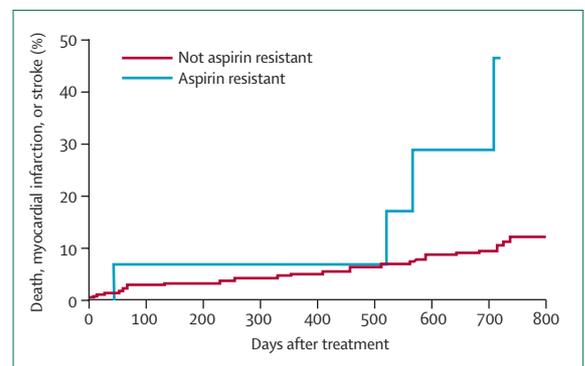


Figure 3: Association between reduced inhibition of agonist induced platelet aggregation and incidence of death, myocardial infarction, or stroke

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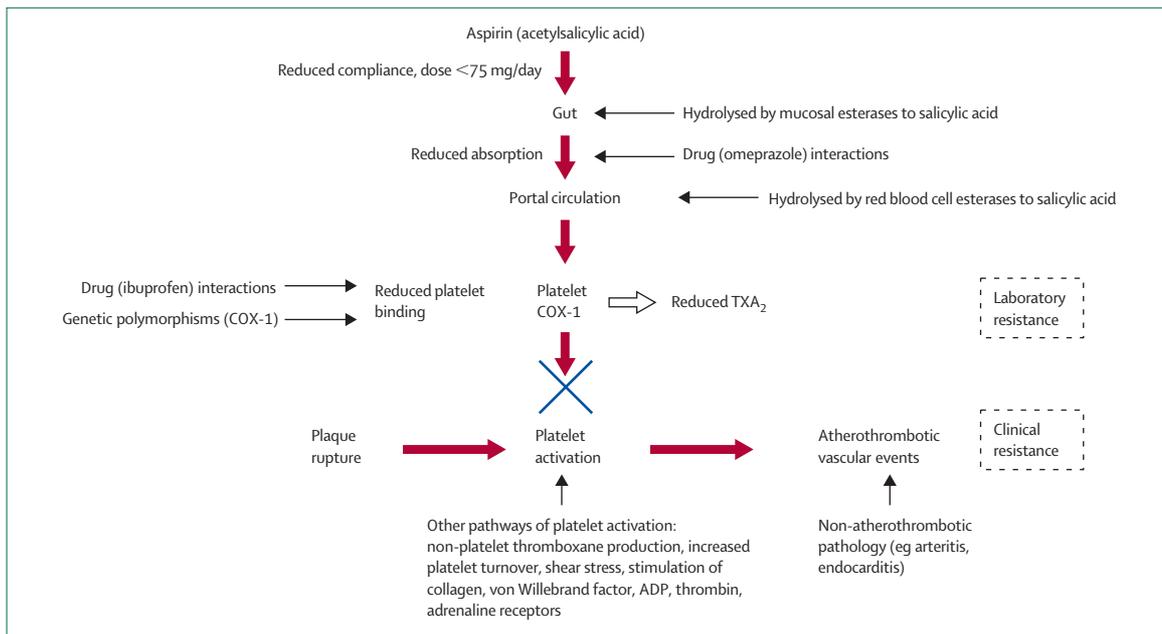


Figure 4: Possible mechanisms of aspirin resistance

validity, this pattern could reflect publication bias to some extent. Furthermore, inherent weaknesses in all the studies might have biased the magnitude of the estimates. For example, these studies did not consistently adjust for potential confounders (such as age, sex, ethnic and clinical conditions, haemoglobin concentration, platelet count, and hyperlipidaemia) or compliance. In one study,³⁹ 20% of the sample stopped treatment because of adverse effects from the very high dose of aspirin, and these patients were not followed up or included in the analysis. In a subgroup analysis from a randomised trial designed for other purposes⁴⁰ aspirin resistance was measured at one time (and might not be stable over time), and compliance with aspirin was not objectively measured. In another study,⁴¹ aspirin resistance was also measured at only one time, compliance with aspirin was not assessed, 11 patients were lost to follow-up (3.1%; 5.1% in aspirin resistant group), and there were only a few outcome events (making the estimates unreliable). It has also been argued that by use of ADP at 10 $\mu\text{mol/L}$, full platelet aggregation would have been induced largely independent of thromboxane A_2 production. It is only at intermediate concentrations of 2–4 $\mu\text{mol/L}$ that thromboxane A_2 production causes amplification of the aggregation response to ADP.²⁵

Prevalence

Estimates of the prevalence of laboratory aspirin resistance range from 5.5% to 61%.^{25,46–50} However, these estimates are unreliable because of the small sample sizes, different types of patients studied (different prevalence of potential confounders such as age, sex, ethnic origin, and

clinical conditions), uncertainty about compliance, different definitions of aspirin resistance, lack of agreement between different tests of platelet function, and uncertainty about measurement stability over time.

What causes aspirin resistance?

There are many reasons why aspirin might not suppress production of thromboxane A_2 and activation and aggregation of platelets, and might cause laboratory aspirin resistance (panel 2, figure 4), and even more reasons why aspirin may fail to prevent clinical atherothrombotic vascular events and cause aspirin treatment failure (panel 1, figure 4).

Weber and colleagues⁵¹ have proposed a classification of laboratory aspirin resistance that distinguishes pharmacokinetic resistance (in-vitro but not oral aspirin completely blocks collagen-induced platelet aggregation and thromboxane formation) from pharmacodynamic resistance (neither in-vitro nor oral aspirin completely blocks collagen induced platelet aggregation and thromboxane formation) and pseudo-resistance (low dose collagen, which is not dependent on thromboxane production, induces platelet aggregation despite complete block of thromboxane production). Although it provides a useful framework for considering the mechanisms of aspirin resistance, the reproducibility and clinical utility of this classification system remain to be proven.

Compliance

Up to 40% of patients with cardiovascular disease do not comply with aspirin.^{52–54} Poor compliance with aspirin is a common, yet often neglected, reason why aspirin is ineffective in the laboratory and clinically.

Dose

Laboratory studies indicate that low-dose aspirin (as low as 30 mg daily) uniformly suppresses platelet COX-1 in healthy controls^{6–9,22} and in patients recovering from myocardial infarction.^{55,56} Moreover, systematic reviews of randomised controlled trials of antiplatelet treatment showed no significant difference in effectiveness of different aspirin doses within 75–1300 mg compared with placebo, and an increased risk of adverse effects (eg, upper gastrointestinal symptoms and bleeding) with higher doses of aspirin.^{5,38,57} However, the estimates of the magnitude of the effectiveness of each dose are not precise (the 95% CIs are reasonably wide) and the comparisons are indirect. Direct comparisons of different doses are more reliable but the estimates are also imprecise (wide 95% CIs) and cannot exclude the possibility that higher doses might be more effective in some patients.⁵⁸ Indeed, the laboratory response to aspirin can be improved by increasing the dose from 100 mg/day or less to 300 mg/day or more,^{22–24,59–62} but this benefit has not been correlated with a reduction in clinical events.

Intestinal absorption and metabolism

Aspirin is a weakly acidic drug that crosses the mucosa of the stomach and upper intestine in its lipophilic state and achieves peak blood concentrations within 30–40 min after ingestion of soluble aspirin and within 3–4 h with enteric-coated formulations.⁶³ As aspirin is being absorbed across the gastric and upper intestinal mucosa, some of it is hydrolysed by abundant mucosal esterases to salicylic acid, its inactive form.⁶⁴ Differences in aspirin absorption or pharmacokinetics, or other unrecognised factors, can lead to a lack of effect of low dose aspirin in some people.⁶⁵ Acid suppression with proton pump inhibitors can increase the potential for mucosal esterases to hydrolyse aspirin to its inactive form, and thereby reduce enteral absorption of (active) acetylsalicylic acid.⁶⁶ This mechanism could be relevant in patients taking low doses of aspirin, but the data are conflicting.⁶⁷

Metabolism in the presystemic portal circulation

Acetylsalicylic acid absorbed from the gut is rapidly hydrolysed to salicylic acid by esterases in the erythrocytes of the portal circulation and the liver. The plasma half-life of aspirin is about 15 min.⁵ Because most of the effects of aspirin in acetylating platelet COX-1 are in the presystemic circulation, the antiplatelet effect of aspirin is not affected by systemic bioavailability.

Binding of aspirin to COX-1

Ibuprofen, indometacin, naproxen, and probably some other non-steroidal inflammatory drugs (NSAIDs), prevent access of aspirin to the COX-1 substrate binding site, and can thereby reduce the antiplatelet action of aspirin.^{68,69} Supporting evidence for this mechanism

comes from an observational study of patients with cardiovascular disease in whom the risk of all-cause mortality over 9 years of follow-up was highest in those taking aspirin plus ibuprofen, followed by aspirin plus other NSAIDs, aspirin alone, and aspirin combined with diclofenac.⁷⁰ However, studies have been conflicting and this issue remains to be clarified.⁷¹

Before coronary artery bypass graft surgery, thromboxane formation is completely inhibited by aspirin but postoperatively this inhibition is temporarily prevented or attenuated,^{72,73} even after the in-vitro addition of 100 $\mu\text{mol/L}$ aspirin.⁷² Temporary failure of inhibition of arachidonic-acid-induced platelet aggregation by aspirin has also been recorded in patients undergoing carotid surgery.⁷⁴ Lack of response to in-vitro aspirin⁷² suggests that this is not simply caused by enhanced platelet turnover (see below) but that there is true pharmacokinetic resistance, which is currently unexplained.

Other (non-platelet) sources of thromboxane A₂ production

Thromboxane A₂ can be produced in monocytes and macrophages by conversion of arachidonic acid to thromboxane A₂ in a reaction that is catalysed by the enzymes COX-2 (to form prostaglandin G₂/H₂) and thromboxane synthase (to form thromboxane A₂; figure 1).^{17–20} This reaction might be particularly likely in inflammatory states, such as active atherosclerosis, when COX-2 production is upregulated.⁷⁵

Aspirin-insensitive thromboxane biosynthesis is associated with increased F₂-isoprostanes (prostaglandin F₂-like compounds), which are produced by lipid peroxidation of arachidonic acid in a non-COX reaction that is catalysed by oxygen free radicals.^{14,76,77} Isoprostane production is augmented by smoking, diabetes, hyperlipidaemia, and unstable angina, and is associated with resistance to the effect of aspirin on platelet activation and altered response of platelets to other agonists.^{77–80} This effect could in part explain the mechanism of the association between conventional risk factors for cardiovascular disease and enhanced platelet activation.^{81–84}

Altered thromboxane metabolism

Smokers have increased urinary concentrations of thromboxane metabolites, which might result from enhanced platelet activation (eg by isoprostanes) or from altered thromboxane metabolism.^{83,85}

Other pathways of platelet activation

Pathways of platelet activation, besides stimulation of the thromboxane A₂ receptor, include stimulation of the platelet glycoprotein receptors for collagen (Ia/IIa), von Willebrand factor (Ib/V/IX), ADP, thrombin, and epinephrine; and shear stress on platelets.^{2–4} The in-vitro response of platelets to ADP and collagen is enhanced in patients with aspirin resistance,^{86,87} and amplified by

F₂-isoprostanes.^{14,76} Enhanced sensitivity to ADP suggests a particular role for ADP receptor antagonists (eg, clopidogrel) in patients who are aspirin resistant.⁸⁸

Increased platelet turnover

Increased platelet turnover, which occurs during coronary artery bypass graft surgery, infection, and inflammation, can result in an increased proportion of non-aspirinated platelets during the 24-h dosing interval (because aspirin has a very short half-life), manifesting as impaired suppression of platelet COX-1.⁷²

Genetic polymorphisms

Single nucleotide polymorphisms involving COX-1, COX-2, and other platelet genes can modify the antiplatelet effect of aspirin.⁸⁹⁻⁹⁶ Indeed, epidemiological studies suggest that a third of the variation in laboratory response to antiplatelet drugs is genetically determined.⁹⁷ Hundreds of single nucleotide polymorphisms have been identified in genes involved in the thromboxane biosynthetic pathway but the effect of these polymorphisms on laboratory aspirin resistance is unclear.

Aspirin resistance has been associated with genetic variation in platelet glycoprotein receptors⁹²⁻⁹⁵ as well as the ADP receptor gene *P2Y1*.⁹⁶ Corresponding functional changes in the P2Y1 receptor can play a part in altering the function of ADP signalling and lead to prothrombotic changes and a decreased responsiveness to aspirin (and other antiplatelet agents, including P2Y12 inhibitors such as clopidogrel).⁹⁶

Non-atherothromboembolic pathology

Ischaemic vascular events of the heart, brain, limbs, eyes, kidneys, and other organs are not always caused by atherothromboembolism. For example, only about 50% of all recurrent ischaemic strokes are due to atherothrombotic disease of the large extracranial and intracranial arteries; about 20% arise from emboli from the heart, about 25% are due to occlusion of one of the small, deep, perforating cerebral arteries by lipohyalinosis or microatheroma, and about 5-10% are due to various much rarer causes, such as arterial dissection, vasculitis, and infective endocarditis.⁹⁸ It is not certain whether aspirin is effective in preventing ischaemic events as a result of non-atherothrombotic pathologies. Furthermore, the pathophysiology of atherothrombotic ischaemic stroke, ischaemic heart disease, and peripheral arterial disease is complex, involving inflammation, thrombosis, vascular biology, and haemodynamics. Clearly, aspirin cannot be a single magic bullet to prevent all ischaemic events.

Loss of the antiplatelet effect of aspirin with prolonged administration (tachyphylaxis)

Although complete suppression of platelet COX-1 by aspirin is maintained in healthy controls during the first month,²² loss of suppression of agonist-induced

platelet aggregation has been reported during long-term (months or years) treatment.^{99,100} This observation is consistent with an increased incidence of adverse cardiovascular outcomes in prior aspirin users.^{101,102} The mechanism by which aspirin treatment could lose some of its antiplatelet effect during long-term administration is unknown but might be explained by progression of atherosclerosis or progressive reduction in compliance over time.

Can aspirin resistance be treated, and if so, how?

The most logical way to treat aspirin resistance and aspirin failure, and thereby improve the effectiveness of aspirin to prevent clinical atherothrombotic vascular events, is to identify and treat the underlying cause(s) of aspirin resistance and other causes of aspirin failure. Potential effective treatments include identification and treatment of non-atherothrombotic causes of the qualifying vascular event that are not likely to respond to aspirin (eg, antibiotics for infective endocarditis, and steroids for arteritis, causing stroke), improvement of patient compliance with aspirin, avoidance of the use of drugs that might adversely interact with the effectiveness of aspirin (eg, ibuprofen), stopping smoking, increasing the frequency of aspirin administration, and replacement of aspirin with (or adding aspirin to) antiplatelet drugs which inhibit other upstream pathways of platelet activation (eg, ADP receptor blockers, thromboxane receptor antagonists) or the final common downstream pathway of platelet aggregation (ie, intravenous glycoprotein IIb/IIIa receptor blockers).

However, although these things seem logical, they are not necessarily effective and safe. For example, although increasing the dose of aspirin to improve suppression of platelet COX-1 seems logical, there is a reliable (albeit imprecise) body of evidence from randomised trials that low-dose aspirin is as effective as higher doses for preventing cardiovascular events. Indeed, there are several examples in medicine where experiments, by means of randomised controlled trials, have exposed the fallacies and pitfalls of logical reasoning (eg, hormone replacement therapy causes, rather than prevents, coronary heart disease; β carotene causes, rather than prevents, lung cancer).¹⁰³

Nevertheless, there is evidence from randomised trials that the addition of clopidogrel to aspirin in patients with acute coronary syndromes and undergoing percutaneous coronary interventions improves outcomes without excessive hazard.¹⁰⁴⁻¹⁰⁶ Whether this effect is mediated to some extent by overcoming aspirin resistance⁸⁸ is uncertain. Further research is needed to determine the effectiveness and safety of other potential treatments, and identify which factors (eg, genetic) are associated with a favourable (and unfavourable) response.^{107,108}

Are there other forms of antiplatelet drug resistance?

The mechanisms of aspirin resistance are probably not particular to aspirin but apply to other antiplatelet drugs (eg, clopidogrel^{109,110}).

Implications for clinical practice

New concepts about aspirin resistance

We add to previous reviews^{10,14,25,46–48,50} by suggesting that aspirin resistance is a laboratory occurrence which might be continuous (quantitative), rather than categorical (qualitative). Individuals vary in their platelet responsiveness to aspirin, and the range of responses might follow a normal distribution, as do other biological variables such as blood pressure and blood cholesterol. Further, the magnitude of an individual's aspirin resistance might be dynamic, varying over time during long-term aspirin treatment, in the same way that blood pressure and blood cholesterol can vary over time. The burning, and still unanswered, question for clinicians is whether there is an association between the degree of aspirin resistance (as measured in the laboratory) and the risk of future vascular events, as with blood pressure and blood cholesterol, and if so, whether the degree of aspirin resistance independently and significantly improves prediction of vascular risk, over and above other prognostic factors (such as blood pressure and blood cholesterol).¹¹¹

In this Review we also distinguish aspirin resistance as a subset of aspirin failure, and emphasise that although aspirin failure is a retrospective and non-specific entity, it could have clinical utility if it is not regarded as a dustbin diagnosis for the horse that has bolted, but rather as a window of opportunity to diagnose and treat one of more of its many causes to prevent a further vascular event.

Screening for aspirin resistance with laboratory tests

Patients treated with aspirin to prevent atherothrombosis do not need to have platelet function measured to see whether they have laboratory evidence of aspirin resistance.¹¹² Laboratory results are not likely to be clinically meaningful until the tests are standardised, the results accurately predict risk of future vascular events, and the risk can be reduced by effective and safe treatments that are targeted to the cause in patients likely to respond.

Management of patients with aspirin treatment failure (clinical aspirin resistance)

Patients who have experienced a recurrent vascular event while taking aspirin require reassessment to find the cause of the initial and recurrent events, and to establish if the cause(s) is likely to respond to aspirin (eg, atherothromboembolism). Compliance with aspirin should be optimised, drugs that might adversely interact

with the effectiveness of aspirin (eg, ibuprofen) should be avoided, and consideration should be given to replacing aspirin with (or adding aspirin to) another antiplatelet drug that inhibits other pathways of platelet activation and aggregation (eg, an ADP-receptor blocker such as clopidogrel or a phosphodiesterase inhibitor such as dipyridamole). Despite treatment failures, aspirin remains the single most cost-effective and widely used drug for the secondary prevention of atherothrombotic ischaemic events.

Implications for research

Further research is required to develop and evaluate measures of platelet function that are valid, reliable (reproducible), specific, easy to do, affordable, and standardised. These measures, and their development over time, need to be correlated with the occurrence of subsequent ischaemic events by means of analytical epidemiological studies. Such studies must include large number of patients (to reduce random error); record and adjust for factors such as sex, ethnic origin, smoking, compliance with aspirin and other NSAIDs, platelet count, haemoglobin concentration, and lipid concentration (to reduce confounding); and be repeated (to ensure consistency of the results).

Various blood and urine measures of platelet function are currently being correlated with major clinical outcome events in a large substudy of the Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance trial,¹¹³ which is comparing the effect of clopidogrel plus aspirin with aspirin alone for high-risk primary prevention and secondary prevention of cardiovascular events. These types of studies should allow identification of which measure(s) of platelet function, and respective results, is associated independently, significantly, and consistently with clinical outcomes. An appropriate definition and measure of aspirin resistance can then be established.

It will then be possible to establish the population attributable risk of cardiovascular disease associated with aspirin resistance, evaluate the effectiveness of interventions to prevent cardiovascular events attributable to aspirin resistance in randomised studies, and assess the clinical utility and cost-effectiveness of testing for treating aspirin resistance. Concurrently, research should continue to identify and assess other non-platelet markers of antiplatelet drug resistance (eg, genetic polymorphisms) so that patients who are unlikely to respond to aspirin can be identified before antiplatelet treatment is initiated rather than after it has been declared by a disabling or fatal ischaemic event.

Conflict of interest statement

G J Hankey has received honoraria from Bayer, Sanofi-Aventis, Bristol-Myers-Squibb, and Boehringer-Ingelheim for speaking at sponsored scientific symposia and serving on advisory boards. J W Eikelboom has received honoraria from Sanofi-Aventis, Bristol-Myers-Squibb, and McNeil Pharmaceuticals for speaking at sponsored scientific symposia and/or serving on advisory boards. J W Eikelboom is named on a patent

application for a method for measuring aspirin resistance (US Patent Application No. 20040115735); although no royalties are anticipated from this source, any such accruing to J W Eikelboom will be donated to the Population Health Research Institute, McMaster University.

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