Biological basis and clinical implications of acetylsalicylic acid resistance

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Acetylsalicylic acid (ASA) is effective in preventing strokes, heart attacks and vascular-related events associated with cardiovascular disease (CVD). Notwithstanding, many patients suffer recurrent events while on ASA therapy. During the past decade, a number of investigators have suggested that these patients are unresponsive to ASA or are 'ASA-resistant'. In the past, this view was met with wide skepticism. Although there is mounting evidence that ASA resistance is a real phenomenon, an understanding of its biological basis and how to measure it is still unclear. The complexity of the problem is discussed below in an attempt to stimulate clinicians and CVD researchers to give serious thought to the ASA resistance problem. It is anticipated that a better understanding of ASA resistance will help us to appreciate its relative importance and its implications in the clinical setting.

Key Words: ASA; Acetylsalicylic acid; ASA resistance; Thrombotic risks

BASIC CONTEXT

It is generally accepted that treatment with ASA is an effective antithrombotic therapy for preventing stroke, myocardial infarction (MI) and vascular-related events associated with cardiovascular disease (CVD) (1). However, 8% to 18% of CVD and peripheral vascular disease patients treated with ASA will suffer a recurrent stroke, MI or other (non)fatal thrombotic event within two years of the first event (2-5). ASA is recommended based on our apparent understanding of the mechanism of action of ASA, namely, the effect of the acetyl moiety of ASA on the irreversible acetylation of the platelet enzyme cyclooxygenase (COX) and the subsequent prevention of the metabolism of arachidonic acid to the potent platelet-aggregating agonist thromboxane A_2 (Tx A_2) (Figure 1) (6).

One explanation that has been suggested for the recurrence of thrombotic events in patients with CVD is that not all patients are treated with a sufficient dose of ASA (which ranges from 80 mg/day to 325 mg/day) to inhibit Tx A_2 synthesis and release completely. However, this is unlikely to be the case because these doses (greater than 80 mg/day) achieve

Fondement biologique et portée clinique de la résistance à l'acide acétylsalicylique

L'acide acétylsalicylique (AAS) est efficace pour prévenir les accidents vasculaires cérébraux, les crises cardiaques et les événements vasculaires liés aux maladies cardiovasculaires (MCV). Cependant, beaucoup de patients connaissent des récides d'événements vasculaires malgré la prise d'AAS. Au cours de la dernière décennie, des chercheurs ont émis l'hypothèse que ces patients ne réagissaient pas à l'AAS ou qu'ils étaient « résistants » à l'AAS. Autrefois, le point de vue rencontrait beaucoup de scepticisme. Aujourd'hui, même si de plus en plus de données tendent à montrer que la résistance à l'AAS est un phénomène réel, il est difficile d'en comprendre le fondement biologique et d'en mesurer l'importance. Il sera question, dans le présent article, de la complexité du problème afin de soulever l'intérêt des cliniciens et des chercheurs en MCV à l'égard de la résistance à l'AAS. On croit qu'une meilleure compréhension de la résistance à l'AAS aiderait à apprécier l'importance relative et la portée clinique du problème.

Figure 1 Sites of inhibition of platelet arachidonic acid (AA) synthesis by the acetyl and salicylate moieties of acetylsalicylic acid (ASA). 12HETE 12-Hydroxyeicosatetraenoic acid; 12HPETE 12-Hydroperoxy-5,8,10,14-eicosatetraenoic acid; COX Cyclooxygenase; FGN Fibrinogen; LO Lipoxygenase; PGH $_2$ Prostaglandin H $_2$; PO Peroxidase; Tx A_2 Thromboxane A $_2$

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plasma levels in excess of 50 $\mu\text{mol/L}$, the concentration that completely inhibits COX (Figure 1) (7).

One exception to this argument, however, is related to our improved understanding of the COX pathway in recent years. It is now clear that there are at least two COX enzymes: COX-1, which is constitutive in cells, and COX-2, which is synthesized *de novo* in nucleated cells (which platelets are not) following cell perturbation or injury, such as in monocytes and macrophages during inflammation. Moreover, COX-2 is more than 150-fold less sensitive to ASA inhibition than COX-1 (7). Because inflammation contributes to prothrombotic events, we cannot exclude the possibility that the overall antithrombotic effect of ASA (at the currently recommended doses) is masked, in part, by its inability to attenuate inflammatory events concomitant with the thrombotic response (8).

However, it should be noted that ASA – in particular, the salicylate moiety of ASA – also modulates platelet function. Specifically, the salicylate moiety of ASA inhibits the peroxidase step in the lipoxygenase pathway, thereby inhibiting 12-hydroxyeicosatetraenoic acid (12-HETE) synthesis (Figure 1) (9). Platelet 12-HETE and related monohydroxides, such as 5-HETE and 15-HETE, facilitate integrin expression in and cell adhesivity of inflammatory, metastatic and vascular cells or, as in this case, the glycoprotein Ib and glycoprotein IIb/IIIa receptors in platelets (10). 12-HETE is not released from platelets, but rather remains associated with the lipophilic domain of the membrane-spanning region of the adhesion receptors. In addition, unlike the acetylation of COX-1 and the subsequent inhibition of TxA_2 in platelets, markedly higher doses of ASA (or salicylate) are required to achieve the salicylate-dependent inhibition of 12-HETE (9,11). Finally, there is evidence to suggest that platelet 12-HETE synthesis and platelet adhesivity are increased following COX-1 inhibition (11), perhaps due to a reshunting of arachidonic acid through the lipoxygenase pathway when the COX pathway is inhibited. Thus, the biological relevance of an enhanced activity of the lipoxygenase pathway following ASA ingestion raises the possibility that certain patients not only may not benefit from ASA therapy, but also may be at an increased risk of thrombosis (3,11).

In addition, we cannot exclude the possibility that ASA resistance is exacerbated by drug-drug interactions, such as those seen between ASA and COX-2-specific inhibitors (eg, rofecoxib or celecoxib) (12) or between ASA and a thienopyridine (eg, clopidogrel) (13), or by other factors that affect ASA metabolism and CVD outcome.

Thus, ASA affects platelet function by at least two, not one, mechanisms: irreversible inhibition of TxA_2 synthesis and release, and inhibition of 12-HETE. The former mechanism is well understood, relates to the inhibition of platelet aggregation, and is relatively easy to assess simply by measuring plasma or urinary TxA_2 metabolites. The latter mechanism is not as well understood, but it appears to be related to platelet adhesivity (11). Platelet 12-HETE is not released from 'activated' platelets. Thus, 12-HETE must be extracted from platelets, making it more difficult to measure. Moreover, the platelet response to ASA (ie, the salicylate moiety) is more complex and variable. Finally, these effects of ASA appear to be modified by concomitant drug therapy.

CLINICAL CONTEXT

One major stumbling block for recognizing ASA resistance as being both a real phenomenon and clinically relevant has been

the lack of agreement on how to measure ASA resistance. Conceptually, one might argue that the most logical approach would be based on our rationale for giving ASA in the first place. Specifically, because ASA is given on the assumption that it impairs platelet function, thereby attenuating thrombotic events in the arteries, all patients should be hemostatically defective (ie, they should have prolonged bleeding times), but not all patients are. That was clearly shown many years ago by Mielke et al (14).

There is mounting evidence that a significant proportion of CVD patients do not benefit from ASA treatment and, in fact, may be at an increased risk of thrombotic events, regardless of the method used to classify patients as ASA resistant (2-5). For example, Gum et al (4) compared the risks of major adverse events in 326 CVD patients classified prospectively as ASA resistant with those in CVD patients classified as being ASA responsive (patients were classified as ASA resistant if platelet aggregation in response to ADP and arachidonic acid exceeded 70% and 20%, respectively). The mean follow-up was 679 days and the primary outcome was a composite of MI, cerebrovascular event and death. All patients were treated medically, with no invasive procedures. The authors found that ASA resistance was an independent predictor of long-term adverse effects (hazard ratio 4.14, 95% confidence interval 1.42 to 12.06; $P < 0.009$) (4). Similarly, Grottemeyer et al (2) found that in 180 patients with a history of stroke, ASA resistance was associated with a 10-fold increased risk of a recurrent event. In that study, ASA resistance was defined as little or no impairment in platelet adhesivity with ASA treatment. The authors also reported that 15% of patients who initially responded to ASA became ASA resistant over time (2).

In a third study (3), the Benefits and Risks of ASA on Thrombosis (BRAT) investigators found that among 294 patients undergoing coronary artery bypass grafting, the risk of a nonfatal MI, stroke or vascular-related event was increased in ASA-resistant patients. Patients were classified as ASA resistant if their bleeding time was not prolonged by more than 30% while on ASA.

All of these studies can be easily faulted for their small sample size, the generalizability (or lack thereof) of the patient populations, and the semiquantitative and quantitative nature of their measures of ASA resistance. However, the pattern is consistently clear. The risk of adverse thrombotic events is increased in ASA-resistant patients, regardless of the methodology used to classify the defect or the specific patient population studied. Similarly, there are other studies, too many to mention here, that also suggest that ASA-resistant patients with CVD have a higher risk of thrombotic events than CVD patients who are responsive to ASA treatment.

The three studies cited above show that thrombotic risks are increased in ASA-resistant patients, be they patients who are relatively stable and deemed not to require invasive procedures, or patients requiring surgical intervention or with other comorbidities. This latter point is highlighted further by the recent studies of Poston et al (15) and Eikelboom et al (16). Poston et al (15) reported that ASA resistance in 125 patients undergoing off-pump coronary artery bypass grafting provoked early graft failure in veins that also had poor endothelial cell integrity. Thus, the combination of ASA resistance and vessel wall injury significantly increased the risk of early graft failure. Similarly, Eikelboom et al (16) reported that ASA resistance increased the risk of MI, stroke and cardiovascular death in CVD and diabetic patients who had one other comorbid risk

TABLE 1
A possible impact of acetylsalicylic acid (ASA) resistance on the overall benefits of ASA in the Aspirin Trialists' Collaboration study

	Adverse events, n (%)	Change	Expected adverse events increase threefold, n (%)	Change	Expected adverse events increase 10-fold, n (%)	Change
'Adjusted' control subjects (n=22,548)	3422 (15.2)					
All ASA patients (n=22,471)	2675 (11.9)	25%↓				
ASA-responsive (n=15,730)			1161 (7)	49%↓	506 (4)	75%↓
ASA-resistant (n=6741)			1514 (22)	44%↑	2169 (32)	112%↑

↑ Increase; ↓ Decrease. Data from reference 1

factor for CVD. These observations are reminiscent of Virchow's triad of thrombosis – hypercoagulation, stasis and injury – specifically, the need to have two of the three conditions present to manifest a prothrombotic event, such as (hyper)functional platelets (ASA-resistant) and vessel wall injury.

CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

It is becoming increasingly more difficult to deny the existence and clinical relevance of ASA resistance. The potential clinical significance of ASA resistance can be illustrated by applying the relative risks and incidence of ASA resistance found in studies, such as those of Mueller et al (5) or Grottemeyer et al (2), to the Aspirin Trialists' Collaboration study (1). For example, if we assume a 30% incidence of ASA resistance and a three- to 10-fold increase in thrombotic risk (2,5), then the benefits and risks of ASA therapy change markedly. The Aspirin Trialists reported that ASA therapy reduced thrombotic risks by 25% overall (Table 1). This is based on the observation that there were 2675 MIs, strokes or vascular-related deaths in 22,471 ASA-treated patients (11.9%)

compared with 3422 adverse effects in 22,548 'adjusted' control subjects (15.2%). If, however, we assume that 30% of the 2675 events among the ASA-treated patients were, in fact, related to ASA resistance, and there is, indeed, a three- to 10-fold increase in thrombotic risk, we would expect markedly different results. Specifically, 6741 patients would be ASA-resistant and account for 1514 to 2169 of the adverse events (Table 1). Thus, the risk of adverse events in patients who responded to ASA therapy would be decreased by approximately 50% to 75%, not by a modest 25%, and the risk of adverse events in ASA-resistant patients would be increased at least two- to threefold. ASA resistance was not taken into consideration in the Aspirin Trialists' Collaboration study (1).

In summary, there is increasing evidence that ASA resistance is a real, clinically relevant entity. There are a sufficient number of studies in various patient populations that classify approximately 30% of the patients as ASA-resistant who not only may not benefit from ASA therapy, but also may be at an increased risk of thrombosis. Does this not behoove us to pursue this issue further to generate better 'evidence-based' data to refute or confirm these data, and to optimize current clinical practice?

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