Premium Reasons

Journal of Clinical Pharmacology & Pharmacoepidemiology

ISSN (electronic): 1916-694X

2010; Volume 1; Number 2 (May-August): 39-47

The *Journal of Clinical Pharmacology & Pharmacoepidemiology* is an open-access journal published electronically by PremiumReasons®, located in Toronto, Ontario, Canada.

Published manuscripts are peer-reviewed by scientists with proven reputation in their field. Substantial efforts are made to publish only those manuscripts that properly justified the aim of the study, used appropriate methods, adequately summarized the results, and provided a sufficient analysis of the literature in comparison to the findings of the study. However, manuscripts published by the journal represent the sole opinion of the authors. PremiumReasons®, the Editor or the Editorial Board of the *Journal of Clinical Pharmacology & Pharmacoepidemiology* cannot assume any responsibility for the procedures, methods, chemical compounds, drugs, doses, statements of facts, or opinions expressed in the manuscripts, or any involuntary or intentional failure to disclose conflicts of interests. In addition, selected manuscripts may discuss investigational drugs or unlabeled uses of approved medications, or use of devices that had not been yet approved by regulatory agencies.

Finally, in order to promote and encourage environmental awareness, PremiumReasons® invites the readers of the *Journal of Clinical Pharmacology & Pharmacoepidemiology* to use the electronic version of the manuscripts rather than printing hard copies of the documents.

-.-.-.-.-.-.-.-.-

Premium Reasons

Journal of Clinical Pharmacology & Pharmacoepidemiology

ISSN 1916-694X
2010; Volume 1 (Number 2): Pages 39-47

REVIEW ARTICLE

Aspirin resistance: a nebulous concept

Dermot COX

Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin, Ireland

Corresponding author: dcox@rcsi.ie

ABSTRACT

Recently there has been great interest regarding aspirin resistance in both the scientific and lay media, yet it has proven to be a nebulous concept. The lack of an approved assay makes it difficult to agree on a definition of aspirin resistance. Not only can we not define aspirin resistance, we cannot identify the cause. There are three types of aspirin non-response: treatment failure, and pharmacokinetic and pharmacodynamic resistance. Treatment failure is when a clinical event occurs despite aspirin therapy and is due to a COX-independent process. Pharmacokinetic resistance occurs when the patient does not receive adequate exposure to the drug and is due to many factors including non-compliance, inadequate dosing, poor bioavailability of some preparations, and drug interaction with NSAID's. Pharmacodynamic resistance is defined as not being treatment failure or pharmacokinetic resistance and is the only true aspirin resistance. However, it would appear to be rare and to have multiple causes that may be patient-dependent. Whatever its cause or how it is identified, aspirin non-response is associated with poor outcome. This paper reviews the types of aspirin non-response, the methods for detection, the causes of aspirin non-response, and the clinical consequences.

Key words

Acetylsalicylic acid; Mechanism of action; Treatment failure

RÉSUMÉ

Ces derniers temps la communauté scientifique et les média s'intéressent beaucoup à la résistance à l'aspirine, un concept qui reste fort nébuleux. L'absence de méthode d'analyse approuvée fait qu'il est difficile de se mettre d'accord tant sur la définition-même de résistance à l'aspirine que sur son origine. Il y a trois types de non-réponse à l'aspirine : l'échec du traitement, et la résistance soit pharmacocinétique soit pharmacodynamique. L'échec du traitement survient lorsque l'événement clinique est COX-indépendant. La résistance pharmacocinétique survient lorsque l'exposition à l'aspirine est inadéquate, ou faisant suite à une non-compliance, une posologie inadéquate, une mauvaise biodisponibilité de la formulation utilisée ou une interaction avec un AINS. La résistance pharmacodynamique, définie comme n'étant ni un échec du traitement ni une résistance pharmacocinétique, constitue la véritable résistance à l'aspirine. C'est une condition rare dont les causes sont multiples, voire dépendantes du sujet. Quelles qu'en soient la cause et son mode d'identification, la non-réponse à l'aspirine aboutit à un mauvais résultat. Cet article revoit les types de non-réponse à l'aspirine, leurs méthodes de détection, leur origine et les conséquences cliniques.

Mots clés

Acide acétylsalicylique; Mécanisme d'action; Échec du traitement

RESUMEN

Recientemente, la resistencia a la aspirina ha despertado un gran interés tanto en los medios científicos como en el público en general. Sin embargo, continúa siendo un tema poco claro. La falta de una prueba aprobada dificulta la definición de resistencia a la aspirina. No existe hasta el momento una definición clara de este problema, y sus causas permanecen desconocidas. Existen tres tipos de falta de respuesta a la aspirina: falla al tratamiento, resistencia farmacocinética, y resistencia fármacodinámica. La falla

al tratamiento es cuando ocurre un evento clínico a pesar de estar bajo tratamiento con aspirina, debido a un proceso COX-independiente. La resistencia farmacocinética ocurre cuando el paciente no recibe una exposición adecuada al medicamento debido a varios posibles factores: pobre apego al tratamiento, dosificación inadecuada, baja biodisponibilidad de algunas formulaciones, e interacción medicamentosa con AINES. La resistencia farmacodinámica se define cuando las otras dos alternativas han sido descartadas, y se considera como la verdadera resistencia a la aspirina. Sin embargo, parece ser una situación rara y tener múltiples causas dependientes del propio paciente. Cualquiera que sea la causa, o como sea identificada, la falta de respuesta a la aspirina está asociada a un pobre desenlace. Este artículo revisa los tipos de falta de respuesta a la aspirina, los métodos para la detección, las causas, y las consecuencias clínicas.

Palabras clave

Ácido acetilsalicílico; Mecanismo de acción; Falla al tratamiento

INTRODUCTION

In recent years there has been a lot of interest in the controversial concept of aspirin resistance [1] [2] [3] [4]. There is much confusion surrounding this topic: What is it? How to detect it? Is it clinically relevant? How should it be managed? Does it exist? The debate has been polarised by commercial interests with a number of key academic supporters for aspirin resistance being funded by manufacturers of devices for detecting aspirin resistance and those opposed being funded by pharmaceutical companies [5]. However, the International Society on Thrombosis and Haemostasis (ISTH) recommendations are that patients should not be screened for aspirin resistance and their treatment should not be altered based on the results of screening [6].

MECHANISM OF ACTION OF ASPIRIN

Before considering the concept of aspirin resistance it is worth briefly reviewing its mechanism of action. Aspirin (or at least aspirin-containing plant extracts) is one of the oldest drugs in use today as its abilities to treat fever and inflammation were known to the ancient Greeks. As a relatively simple molecule (acetylsalicylic acid) it was one of the first modern drugs to be synthesized, in 1853. Aspirin developed a new lease of life when its ability to reduce the risk of heart attacks was realized in the 1980's [7]. This discovery was a combination of two factors: the known ability of aspirin to cause bleeding due to its actions on platelets [8] and the role of platelet-rich clots in causing heart attacks [9].

The elucidation of the molecular basis for the action of aspirin is relatively recent and only occurred after the discovery of prostaglandins. Cyclooxygenase (COX) plays an essential role in the production of prostaglandins (PG) as it converts arachidonic acid to PGH_2. Downstream processing of PGH_2 by a series of cell-specific enzymes, such as thromboxane synthase, convert PGH_2 into a range of prostaglandins and derivatives. Aspirin acts to inhibit COX thus preventing the synthesis of all prostaglandins. Aspirin is an irreversible inhibitor of COX and acts by acetylating Ser^{530} which is an essential residue in the activity of COX [10].

More recently two isoforms of COX have been identified, designated COX 1 and COX 2. Generally COX 1 is constitutive while COX 2 is inducible and aspirin is more potent at inhibiting COX 1 [11]. The cardiovascular effects of aspirin are primarily due to inhibition of COX 1 in platelets. Gastric ulceration is the most significant adverse effect of aspirin and is due to inhibition of COX 1 in the stomach where it is responsible for the secretion of the protective mucosal layer [12].

Low-dose aspirin therapy has become a key strategy to prevent the gastric ulceration due to aspirin use [13]. This utilizes a unique property of platelets – they do not have a nucleus. Thus, a low-dose of aspirin will partially inhibit COX in all cells. However, cells will generate new COX ensuring that there is sufficient COX to maintain activity. However, as platelets have no nucleus they cannot re-generate COX and within a few days all COX is fully inhibited for the life of the platelet (10 days) [14]. This was confirmed in a large meta-analysis which showed that low-dose aspirin was as effective as high-dose aspirin in preventing cardiovascular events [15] [16]. This analysis identified 75 mg aspirin as the lowest effective dose of aspirin. In comparison, aspirin is used at 325 mg as an anti-inflammatory agent. While there is no doubt about the effectiveness of aspirin in the treatment of cardiovascular disease (CVD), there is still only a partial response to aspirin with a 25% reduction in events [15]. However, this is not unique to aspirin as it also true for a number of other treatments and reflects the fact that CVD is a complex multi-factorial disease [17].

ASPIRIN RESISTANCE

The term aspirin resistance has also caused much confusion and its appropriateness in this context has been questioned. The term resistance is usually associated with the treatment of infectious

diseases. It specifically refers to an organism that usually is susceptible to an antibiotic but has acquired, either through genetic manipulation or natural selection, the ability to grow despite the presence of the antibiotic. It is not used when the organism inherently has no sensitivity to an antibiotic. It can be identified either *in vitro* by culturing the organism in the laboratory or the persistence of the infection in a treated patient. The *in vitro* results are directly related to the clinical outcome. Thus, the use of the term aspirin resistance implies that either an event has occurred in a patient treated with aspirin or an *in vitro* test has failed to detect a response to aspirin.

The term aspirin resistance is used differently by clinicians and pharmacologists. Clinicians use the term aspirin resistance when an aspirin-treated patient develops a cardiovascular event while pharmacologists consider a patient to be resistant when an *in vitro* test fails to detect the effects of aspirin. However, these two uses of the term 'aspirin resistance' are not synonymous. Unlike the scenario with antibiotics, there is a disconnection between the clinical situation and the *in vitro* tests. Since there is only a 25% reduction in events in patients treated with aspirin, 75% of patients will continue to have events despite aspirin treatment [15] [16]. There are two possible reasons for this: either 75% of the population are aspirin resistant or CVD is a complex disorder and in only 25% of cases is COX-mediated platelet activation a significant factor. Based on evidence of a clinical event alone it is impossible to determine if patient is resistant or has COX-independent pathology. However, from experience with other CVD drugs, it is likely that many patients have complex pathology rather than resistance to aspirin. While it is possible that some patients may be resistant to aspirin, with a background failure rate of 75%, it is difficult to detect these cases. Thus, in the absence of specific evidence that a treated patient is resistant to aspirin they should be designated treatment failures. In contrast, in patients with an infection the pathology is simple — it is caused by the bacterium and if not resistant the antibiotic will work 100% of times.

One important cause of treatment failure is poor compliance [18] [19], with compliance estimated to be around 65% in the CVD population [20] [21]. Aspirin can only work if patients follow their dosing regimen, but it is difficult to confirm compliance as patients will often either not admit to non-compliance or cannot remember taking their medication. There are a number of reasons for poor compliance with aspirin including gastric disturbance and the large number of medications that CVD patients are likely to be taking. A lack of understanding of the importance of aspirin by patients may also be a factor as patients may feel that it is only aspirin and not important to take. Thus, it can be very difficult to distinguish between non-compliance and aspirin resistance.

Pharmacokinetic resistance

One type of aspirin non-response is known as pharmacokinetic resistance [22]. It is due to failure to achieve adequate plasma levels of aspirin often resulting from poor absorption of aspirin. This may be a problem with enteric-coated aspirin where the formulation is designed to prevent release of the drug into the stomach to prevent gastric irritation [23] [24], although other studies have shown no effect of enteric coating [25]. However, as an acid, aspirin is absorbed better at the low pH of the stomach and this can result in poor absorption of aspirin. Equally the use of proton pump inhibitors to prevent gastric irritation may act to reduce aspirin absorption [26]. There is also evidence that the 75 mg dose currently used may be too low for a one-size-fits-all dose as poor response to aspirin has been associated with increased size (both BMI and weight) [23] [24] [27]. This type of resistance is likely to be overcome by a change in formulation [24] or an increase in dose [28]. It is worth noting that this phenomenon occurs with most drugs. The population response to any drug is a bell-shaped curve. Thus, there will always be a small number of patients who show a weak response to a drug and an equal number who show a strong response. This is a particularly noticeable when only a single dose is used and if this dose is low to prevent toxicity.Another form of pharmacokinetic resistance is due to competition with NSAID's. NSAID's are reversible COX inhibitors which presence at the time of taking aspirin prevents aspirin from gaining access to its binding site. Clinical studies of patients on NSAID's have supported this [29] [30].

DIAGNOSTIC TESTS

Aspirin assay

To determine if aspirin non-response is due to treatment failure or aspirin resistance it is necessary to measure its actions on the patient. This distinguishes the failure of aspirin to prevent an event from the lack of response to aspirin. There are two types of assays for aspirin – COX-specific assays and global assays of platelet function, and much of the controversy surrounding aspirin resistance is related to the choice of assay. In

particular the assays do not agree with each other and thus a patient may show aspirin resistance with one assay but not another assay [31]. There is also the issue of what constitutes resistance: is there a threshold in the assay above/below which we can identify resistance or is there a target percentage inhibition that we should aim for and, if so, what is that? In light transmission aggregometry resistance is typically defined as ≥20% aggregation in response to arachidonic acid or ≥70% aggregation in response to low dose ADP [32]. For serum thromboxane, resistance is usually defined as being ≥10 ng mL^{-1} [33] while some studies have chosen not to use a threshold but to treat it as a continuous variable [34]. Another approach is to divide the patient population into quartiles and use the upper quartile of thromboxane to identify the resistant population [35]. However, this is not practical for identifying aspirin resistant patients outside of a clinical trial. Proprietary assays such as PFA-100 and VerifyNow have their own pre-defined threshold values. Other studies have suggested that 95% inhibition of platelet function is required [13] [36] which can only be effective if baseline values have been obtained.

COX-specific assays

These assays focus on the conversion of arachidonic acid to thromboxane by COX and thromboxane synthase. The method that is identified as being the 'gold standard' for detecting aspirin resistance is arachidonic acid-induced platelet aggregation as it was the first assay to be introduced [37], and as a result it is the one that all others must be compared with. The advantages of using arachidonic acid are that it by-passes outside-in signaling and directly provides the substrate for COX and its actions are completely inhibited by aspirin [38]. A version of this assay has been commercialized as the point-of-care device 'VerifyNow' [39] which offers the advantage of rapid assessment of arachidonic acid-induced platelet aggregation in a whole blood setting using a cartridge-based system.The other COX-specific assay is direct measurement of thromboxane B_2 (metabolite of thromboxane A_2) levels as this is a direct reflection of COX activity in platelets [35]. Usually serum thromboxane is measured by ELISA [40]. Though this is a good indicator of platelet COX activity, small amounts of non-platelet COX are also present in the blood (especially COX 2) which will not be inhibited by low-dose aspirin [41]. Urinary thromboxane levels can also be measured [42] [43] [44] and this is available as a commercial assay 'AspirinWorks' [45]. While this has the advantage of providing a direct measure of platelet activation *in vivo*, it is affected by significant non-platelet sources of thromboxane [41] and by the low levels of thromboxane present in the urine. Thromboxane levels can also be measured in the samples post arachidonic acid-induced platelet aggregation [23]. This is also known as supernatant or platelet thromboxane and has the advantage that it is very sensitive as a large excess of substrate is provided and thus the only rate-limiting step is the availability of free COX. Additionally, it is very specific for platelet COX as a pure platelet preparation is used.

Global platelet function assays

Low-dose ADP or collagen induces platelet aggregation in a COX-dependent manner, while higher doses are COX-independent [46]. They are often used as agonists in platelet aggregation, however they require the pre-treatment threshold agonist concentration to be determined if they are to be used in detecting aspirin resistance as there is significant variability between individuals. Thus, in many cases where low-dose ADP/collagen have been used as the agonists for platelet aggregation the observation of aspirin resistance could easily have been due to increased sensitivity to the agonists.

One of the first assays used to define aspirin resistance was the PFA-100 [32] which is a cartridge-based device that uses whole blood. A hole is pierced in a membrane that is impregnated with agonists (epinephrine and collagen) and blood is forced through under high shear [47]. This has the advantage of closely reflecting *in vivo* conditions; however, it is not very sensitive to aspirin [48]. PFA-100 is best described as a global platelet function assay. It gives a good picture of platelet function but this is influenced by many factors other than thromboxane production [49] [50], especially von Willebrand factor levels [51].

There are many different assays of platelet function which should make defining aspirin resistance easy. This is not the case, however, as the assays do not agree with each other [31] [32] [42] [43], although the closer they are to being a COX-specific assay the closer the agreement. The concept of a threshold is also problematic as there is a paucity of data to support specific thresholds. Thus, greater than 20% aggregation in response to arachidonic acid is often used as a threshold to define aspirin resistance [32].

However, this means that a 19% response is sensitive and a 21% response is resistant and yet these two readings would be within the variation expected in platelet aggregation. Since the assays do not agree should we add more weight to specific assays and, if so, which ones?

CAUSES OF ASPIRIN RESISTANCE

There are a number of mechanisms implicated in failure to obtain an adequate response to aspirin. The first is lack of compliance which can be difficult to eliminate although there is evidence to suggest that pharmacist-led interventions can greatly improve compliance [20]. In some patients aspirin is not properly absorbed and this has been termed pharmacokinetic resistance [22] and seems to be related to enteric coating [23] [24]. A related phenomenon is under-dosing. Poor response to aspirin has been associated with heavier patients and it is likely that 75 mg aspirin (especially if enteric coated) may be too low to suit all patients [23] [24] [27].

While earlier studies showed this to be an effective dose the weight of the patient population has significantly increased since these studies were performed and the one-dose-fits-all model may need to be revised in light of this.

Aspirin resistance that is not due to poor absorption is often termed pharmacodynamic resistance [22]. This occurs where aspirin is present at a concentration that would be expected to inhibit platelet function but there is incomplete inhibition. However, it is difficult to determine if aspirin is actually present as it is relatively unstable and has a short half-life. While some researchers have measured the levels of salicylate [28], the metabolite of aspirin formed after acetylation of the enzyme, it cannot be assumed that this is a true reflection of the levels of acetylsalicylic acid at the active site of the enzyme as breakdown can occur in the gut and other tissues. In some studies sensitivity to exogenous aspirin added *ex vivo* allowed the determination of the sensitivity to aspirin [33]. A proposed mechanism for aspirin resistance is a potential role for COX 2 in thrombosis. While platelets primarily use COX 1 there is evidence to support the presence of COX 2 in platelets [52]. This appears to primarily occur during periods of rapid platelet turnover such as post-surgery [33]. COX 2 will not be inhibited by aspirin and will produce PGH_2 providing thromboxane synthase with substrate. A related phenomenon is the production of PGH_2 by COX 2 in monocytes. This can be secreted and subsequently taken up by platelets leading to thromboxane production [41]. There are also suggestions that COX 1-independent pathways for eicosanoid synthesis exist [53] [54] [55].Another proposed mechanism of pharmacodynamic resistance is genetic polymorphisms in COX or other associated genes such as COX 1 [56] [57] [58], GPIIb/IIIa [59] [60], and TLR4 [61]. In healthy Japanese volunteers aspirin was found to be less effective for volunteers with SNP's in TXA_2 receptor and GPIbα [62]. However, there is no consensus on which genes, if any, are important.Aspirin resistance has been associated with hyper-reactive platelets [63] and was associated with increased levels of reticulated platelets [64] [65]. Increased oxidant stress has also been implicated [66].

There can also be assay-specific artifacts that may be mistaken for aspirin resistance. Residual arachidonic acid-induced platelet aggregation has been shown to be independent of COX [33] [42] [67] which cannot be aspirin resistance since aspirin is known to act by inhibiting COX.There is evidence that the prevalence of aspirin resistance is influenced by the nature of the patient's illnesses. Thus, aspirin resistance is more prevalent in diabetics [68] and patients with systemic lupus erythematosus [69]. Aspirin resistance was also found to be prevalent in patients following coronary artery by-pass surgery [52]. It was also increased in patients with endothelial dysfunction [70].

CLINICAL IMPLICATIONS

The incidence of aspirin resistance has been estimated to be as high as 25% depending on the definition used [71] [72]. Aspirin resistance has been shown to be associated with worse outcome than with responders [35] [73] [74]. Recent meta-analyses found a three-fold increase in adverse events for patients with residual platelet activity on aspirin and this was independent of the assay used [74] [75].

Screening for aspirin resistance

There are a number requirements for performing any screening — a reliable assay, alternative treatment options based on the results of the assay, a significant incidence and impact on public health. Even if there is lack of agreement between them, there is no shortage of assays for aspirin resistance. There is also alternative treatment, primarily clopidogrel [76]. The incidence of resistance is also relatively high and is probably at least 10% depending on the assay. The impact on public health is significant as well due to the high

incidence of death and MI in aspirin resistant patients [35] [73] [74]. So why have the ISTH recommended that screening for aspirin resistance should not happen [6]?

One of the reasons given for not screening for aspirin resistance is the lack of evidence to support a particular assay. This is seen as a problem due to the lack of agreement between assays and the commercial interests behind the assays. However, serum thromboxane is an assay that could easily be adopted as it is a direct measurement of COX activity and it is a non-proprietary assay. In our hands, 75 mg plain aspirin causes at least 98% inhibition of serum thromboxane production in healthy volunteers [24]. An aspirin-treated patient with less than this level of inhibition should be further investigated. Some assays do not agree with the serum thromboxane result but that does not mean that they are wrong as in many cases they are measuring different things. Thus, lack of inhibition in the PFA-100 may not necessarily be due to aspirin resistance but could reflect enhanced platelet reactivity which may have significant clinical implications itself. Screening is also beneficial as it will identify patients who are non-compliant. This provides an opportunity for doctors to interact with their patients and discuss their medication and the importance of taking all of their tablets. This type of intervention has been shown to be effective at improving compliance in patients [21]. Since the patient is to be prescribed aspirin for life it is not unreasonable that at some point it is confirmed that the patient is compliant and that the aspirin is achieving its expected pharmacological effect.

CONCLUSIONS

Is aspirin resistance a real, clinically significant phenomenon? The answer depends on how we define aspirin resistance. Clinically, treatment failure is usually due to non-COX-dependent processes and not aspirin resistance. Aspirin resistance can only be defined by an appropriate laboratory assay but it is essential to rule out non-compliance, interaction with NSAIDs, under-dosing and non-COX-1 mediated processes. It is likely that when all of these factors are taken into account few patients will be aspirin resistant. Whatever its cause, aspirin non-response is clearly a significant problem with poor outcome for patients. Screening patients for non-response can identify problems such as compliance and dosing, which can easily be addressed, or identify patients that may be candidates for alternative therapy such as clopidogrel.

ACKNOWLEDGEMENTS

This work was funded by a grant from The Irish Heart Foundation.

CONFLICT OF INTERESTS/DISCLAIMERS

The author is member of the Editorial Board of the journal.

REFERENCES

[1] Hankey GJ, Eikelboom JW. Aspirin resistance. Lancet 2006; 367: 606-617.
[2] Patrono C, Rocca B. Drug insight: aspirin resistance--fact or fashion? Nat Clin Pract Cardiovasc Med 2007; 4: 42-50.
[3] Undas A, Brummel-Ziedins KE, Mann KG. Antithrombotic properties of aspirin and resistance to aspirin: beyond strictly antiplatelet actions. Blood 2007; 109: 2285-2292.
[4] Cattaneo M. Laboratory detection of 'aspirin resistance': what test should we use (if any)? Eur Heart J 2007; 28: 1673-1675.
[5] Armstrong D. Aspirin dispute is fueled by funds of industry rivals. Wall Street Journal 2006; April 24.
[6] Michelson AD, Cattaneo M, Eikelboom JW, Gurbel P, Kottke-Marchant K, Kunicki TJ, et al. Aspirin resistance: position paper of the Working Group on Aspirin Resistance. J Thrombos Haemost 2005; 3: 1309-1311.
[7] Cairns JA, Gent M, Singer J, Finnie KJ, Froggatt GM, Holder DA, et al. Aspirin, sulfinpyrazone, or both in unstable angina. Results of a Canadian multicenter trial. N Engl J Med 1985; 313: 1369-1375.
[8] Weiss HJ, Aledort LM, Kochwa S. The effect of salicylates on the hemostatic properties of platelets in man. J Clin Invest 1968; 47: 2169-2180.
[9] Fitzgerald D, Roy L, Catella F, FitzGerald G. Platelet activation in unstable coronary disease. N Engl J Med 1986; 315: 983-989.
[10] Vane JR, Botting RM. The mechanism of action of aspirin. Thromb Res 2003; 110: 255.
[11] Smith WL, Langenbach R. Why there are two cyclooxygenase isozymes. J Clin Invest 2001; 107: 1491-1495.
[12] Derry S, Loke YK. Risk of gastrointestinal haemorrhage with long term use of aspirin: meta-analysis. Br Med J 2000; 321: 1183-1187.
[13] Patrono C, Ciabattoni G, Patrignani P, Pugliese F, Filabozzi P, Catella F, et al. Clinical pharmacology of platelet

cyclooxygenase inhibition. Circulation 1985; 72: 1177-1184.
[14] Giuliani DC, Ford EH, Morse BS. A Rapid Method for Estimating Mean Platelet Survival Time. J Nucl Med 1989; 30: 1550-1553.
[15] Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. Br Med J 2002; 324: 71-86.
[16] Campbell CL, Smyth S, Montalescot G, Steinhubl SR. Aspirin dose for the prevention of cardiovascular disease: a systematic review. J Am Med Assoc 2007; 297: 2018-2024.
[17] Patrono C. Aspirin resistance: definition, mechanisms and clinical read-outs. J Thromb Haemost 2003; 1: 1710-1713.
[18] De Schryver EL, van Gijn J, Kappelle LJ, Koudstaal PJ, Algra A. Non-adherence to aspirin or oral anticoagulants in secondary prevention after ischaemic stroke. J Neurol 2005; 252: 1316-1321.
[19] Schwartz KA, Schwartz DE, Ghosheh K, Reeves MJ, Barber K, DeFranco A. Compliance as a critical consideration in patients who appear to be resistant to aspirin after healing of myocardial infarction. Am J Cardiol 2005; 95: 973-975.
[20] Murray MD, Young J, Hoke S, Tu W, Weiner M, Morrow D, et al. Pharmacist Intervention to Improve Medication Adherence in Heart Failure: A Randomized Trial. Ann Intern Med 2007; 146: 714-725.
[21] Lee JK, Grace KA, Taylor AJ. Effect of a pharmacy care program on medication adherence and persistence, blood pressure, and low-density lipoprotein cholesterol: a randomized controlled trial. J Am Med Assoc 2006; 296: 2563-2571.
[22] Weber AA, Przytulski B, Schanz A, Hohlfeld T, Schrör K. Towards a definition of aspirin resistance: a typological approach. Platelets 2002; 13: 37-40.
[23] Maree AO, Curtin RJ, Dooley M, Conroy RM, Crean P, Cox D, et al. Platelet response to low-dose enteric-coated aspirin in patients with stable cardiovascular disease. J Am Coll Cardiol 2005; 46: 1258-1263.
[24] Cox D, Maree AO, Dooley M, Conroy R, Byrne MF, Fitzgerald DJ. Effect of enteric coating on antiplatelet activity of low-dose aspirin in healthy volunteers. Stroke 2006; 37: 2153-2158.
[25] Karha J, Rajagopal V, Kottke-Marchant K, Bhatt DL. Lack of effect of enteric coating on aspirin-induced inhibition of platelet aggregation in healthy volunteers. Am Heart J 2006; 151: 976e7-11.
[26] Fernandez-Fernandez FJ. Might proton pump inhibitors prevent the antiplatelet effects of low- or very low-dose aspirin? Arch Intern Med 2002; 162: 2248.
[27] Cohen HW, Crandall JP, Hailpern SM, Billett HH. Aspirin resistance associated with HbA1c and obesity in diabetic patients. J Diabetes Complications 2008; 22: 224-228.
[28] Cerletti C, Dell'Elba G, Manarini S, Pecce R, Di Castelnuovo A, Scorpiglione N, et al. Pharmacokinetic and pharmacodynamic differences between two low dosages of aspirin may affect therapeutic outcomes. Clin Pharmacokinet 2003; 42: 1059-1070.
[29] Catella-Lawson F, Reilly MP, Kapoor SC, Cucchiara AJ, DeMarco S, Tournier B, et al. Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. N Engl J Med 2001; 345: 1809-1817.
[30] Kurth T, Glynn RJ, Walker AM, Chan KA, Buring JE, Hennekens CH, et al. Inhibition of clinical benefits of aspirin on first myocardial infarction by nonsteroidal antiinflammatory drugs. Circulation 2003; 108: 1191-1195.
[31] Harrison P, Segal H, Silver L, Syed A, Cuthbertson FC, Rothwell PM. Lack of reproducibility of assessment of aspirin responsiveness by optical aggregometry and two platelet function tests. Platelets 2008; 19: 119-124.
[32] Gum PA, Kottke-Marchant K, Poggio ED, Gurm H, Welsh PA, Brooks L, et al. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. Am J Cardiol 2001; 88: 230-235.
[33] Frelinger AL, III, Furman MI, Linden MD, Li Y, Fox ML, Barnard MR, et al. Residual arachidonic acid-induced platelet activation via an adenosine diphosphate-dependent but cyclooxygenase-1- and cyclooxygenase-2-independent pathway: a 700-patient study of aspirin resistance. Circulation 2006; 113: 2888-2896.
[34] Muir AR, Patterson C, McMullin MF, McKeown PP. Assessment of aspirin resistance varies on a temporal basis in patients with ischaemic heart disease. Heart 2008: hrt.2008.150631.
[35] Eikelboom JW, Hirsh J, Weitz JI, Johnston M, Yi Q, Yusuf S. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. Circulation 2002; 105: 1650-1655.
[36] Reilly IA, FitzGerald GA. Inhibition of thromboxane formation in vivo and ex vivo:

implications for therapy with platelet inhibitory drugs. Blood 1987; 69: 180-186.
[37] Born G. The aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 1962; 194: 927-929.
[38] Willis AL. An Enzymatic mechanism for the antithrombotic and antihemostatic actions of aspirin. Science 1974; 183: 325-327.
[39] Malinin A, Spergling M, Muhlestein B, Steinhubl S, Serebruany V. Assessing aspirin responsiveness in subjects with multiple risk factors for vascular disease with a rapid platelet function analyzer. Blood Coagul Fibrinolysis 2004; 15: 295-301.
[40] Ciabattoni G, Maclouf J, Catella F, FitzGerald G, Patrono C. Radioimmunoassay of 11-dehydrothromboxane B2 in human plasma and urine. Biochimica Biophysic Acta 1987; 918: 293-297.
[41] Cipollone F, Patrignani P, Greco A, Panara MR, Padovano R, Cuccurullo F, et al. Differential suppression of thromboxane biosynthesis by indobufen and aspirin in patients with unstable angina. Circulation 1997; 96: 1109-1116.
[42] Gurbel PA, Bliden KP, DiChiara J, Newcomer J, Weng W, Neerchal NK, et al. Evaluation of dose-related effects of aspirin on platelet function: results from the Aspirin-Induced Platelet Effect (ASPECT) study. Circulation 2007; 115: 3156-3164.
[43] Lordkipanidze M, Pharand C, Schampaert E, Turgeon J, Palisaitis DA, Diodati JG. A comparison of six major platelet function tests to determine the prevalence of aspirin resistance in patients with stable coronary artery disease. Eur Heart J 2007; 28: 1702-1708.
[44] Bruno A, McConnell JP, Cohen SN, Tietjen GE, Wallis RA, Gorelick PB, et al. Serial urinary 11-dehydrothromboxane B2, aspirin dose, and vascular events in blacks after recent cerebral infarction. Stroke 2004; 35: 727-730.
[45] Geske FJ, Guyer KE, Ens G. AspirinWorks: a new immunologic diagnostic test for monitoring aspirin effect. Mol Diagn Ther 2008; 12: 51-54.
[46] Armstrong P, Truss N, Ali F, Dhanji A, Vojnovic I, Zain Z, et al. Aspirin and the in vitro linear relationship between thromboxane A2 mediated platelet aggregation and platelet production of thromboxane A2. J Thromb Haemost 2008; DOI 10.1111/j.1538-7836.2008.03133.x.
[47] Kundu S, Heilmann E, Sio R, Garcia C, Ostgaard R. Characterization of an in vitro platelet function analyzer, PFA-100TM. Clinical Applications Thromb Hemost. 1996; 2: 241-249.
[48] Andersen K, Hurlen M, Arnesen H, Seljeflot I. Aspirin non-responsiveness as measured by PFA-100 in patients with coronary artery disease. Thromb Res 2002; 108: 37-42.
[49] Crescente M, Di Castelnuovo A, Iacoviello L, de Gaetano G, Cerletti C. PFA-100 closure time to predict cardiovascular events in aspirin-treated cardiovascular patients: a meta-analysis of 19 studies comprising 3,003 patients. Thromb Haemost 2008; 99: 1129-1131.
[50] Crescente M, Di Castelnuovo A, Iacoviello L, Vermylen J, Cerletti C, de Gaetano G. Response variability to aspirin as assessed by the platelet function analyzer (PFA)-100. A systematic review. Thromb Haemost 2008; 99: 14-26.
[51] Chakroun T, Gerotziafas G, Robert F, Lecrubier C, Samama MM, Hatmi M, et al. In vitro aspirin resistance detected by PFA-100 closure time: pivotal role of plasma von Willebrand factor. Br J Haematol 2004; 124: 80-85.
[52] Zimmermann N, Wenk A, Kim U, Kienzle P, Weber A-A, Gams E, et al. Functional and biochemical evaluation of platelet aspirin resistance after coronary artery bypass surgery. Circulation 2003; 108: 542-547.
[53] Patrignani P. Aspirin insensitive eicosanoid biosynthesis in cardiovascular disease. Thromb Res 2003; 110: 281-286.
[54] Belton O, Byrne D, Kearney D, Leahy A, Fitzgerald DJ. Cyclooxygenase-1 and -2-dependent prostacyclin formation in patients with atherosclerosis. Circulation 2000; 102: 840-845.
[55] Csiszar A, Stef G, Pacher P, Ungvari Z. Oxidative stress-induced isoprostane formation may contribute to aspirin resistance in platelets. Prostaglandins Leukot Essent Fatty Acids 2002; 66: 557-558.
[56] Maree AO, Curtin RJ, Chubb A, Dolan C, Cox D, O'Brien J, et al. Cyclooxygenase-1 haplotype modulates platelet response to aspirin. J Thromb Haemost 2005; 3: 2340-2345.
[57] Takahashi S, Ushida M, Komine R, Shimodaira A, Uchida T, Ishihara H, et al. Platelet responsiveness to in vitro aspirin is independent of COX-1 and COX-2 protein levels and polymorphisms. Thromb Res 2008; 121: 509-517.
[58] Hillarp A, Palmqvist B, Lethagen S, Villoutreix BO, Mattiasson I. Mutations within the cyclooxygenase-1 gene in aspirin non-responders with recurrence of stroke. Thromb Res 2003; 112: 275-283.

[59] Macchi L, Christiaens L, Brabant S, Sorel N, Ragot S, Allal J, et al. Resistance in vitro to low-dose aspirin is associated with platelet PlA1 (GP IIIa) polymorphism but not with C807T(GP Ia/IIa) and C-5T kozak (GP Ib[alpha]) polymorphisms. J Am Coll Cardiol 2003; 42: 1115-1119.

[60] Goodman T, Ferro A, Sharma P. Pharmacogenetics of aspirin resistance: a comprehensive systematic review. Br J Clin Pharmacol 2008; 66: 222-232.

[61] Patrignani P, Di Febbo C, Tacconelli S, Moretta V, Baccante G, Sciulli MG, et al. Reduced thromboxane biosynthesis in carriers of toll-like receptor 4 polymorphisms in vivo. Blood 2006; 107: 3572-3574.

[62] Fujiwara T, Ikeda M, Esumi K, Fujita TD, Kono M, Tokushige H, et al. Exploratory aspirin resistance trial in healthy Japanese volunteers (J-ART) using platelet aggregation as a measure of thrombogenicity. Pharmacogenomics J 2007; 7: 395-403.

[63] Guthikonda S, Mangalpally K, Vaduganathan M, Patel R, Delao T, Bergeron AL, et al. Increased platelet sensitivity among individuals with aspirin resistance - platelet aggregation to submaximal concentration of arachidonic acid predicts response to antiplatelet therapy. Thromb Haemost 2008; 100: 83-89.

[64] Guthikonda S, Alviar CL, Vaduganathan M, Arikan M, Tellez A, DeLao T, et al. Role of reticulated platelets and platelet size heterogeneity on platelet activity after dual antiplatelet therapy with aspirin and clopidogrel in patients with stable coronary artery disease. J Am Coll Cardiol 2008; 52: 743-749.

[65] Guthikonda S, Lev EI, Patel R, Delao T, Bergeron A, Dong J, et al. Reticulated platelets and uninhibited COX-1 and COX-2 decrease the antiplatelet effects of aspirin. J Thromb Haemost 2007; 5: 490-496.

[66] Cipollone F, Ciabattoni G, Patrignani P, Pasquale M, Di Gregorio D, Bucciarelli T, et al. Oxidant stress and aspirin-insensitive thromboxane biosynthesis in severe unstable angina. Circulation 2000; 102: 1007-1013.

[67] Ohmori T, Yatomi Y, Nonaka T, Kobayashi Y, Madoiwa S, Mimuro J, et al. Aspirin resistance detected with aggregometry cannot be explained by cyclooxygenase activity: involvement of other signaling pathway(s) in cardiovascular events of aspirin-treated patients. J Thromb Haemost 2006; 4: 1271-1278.

[68] DiChiara J, Bliden KP, Tantry US, Hamed MS, Antonino MJ, Suarez TA, et al. The Effect of Aspirin Dosing on Platelet Function in Diabetic and Nondiabetic Patients: An Analysis From the Aspirin-Induced Platelet Effect (ASPECT) Study. Diabetes 2007; 56: 3014-3019.

[69] Avalos I, Chung CP, Oeser A, Milne GL, Borntrager H, Morrow JD, et al. Aspirin therapy and thromboxane biosynthesis in systemic lupus erythematosus. Lupus. 2007; 16:981-986.

[70] Cheng G, Shan J, Xu G, Liu P, Zhou Y, Zhu Y, et al. Relationship between endothelial dysfunction, oxidant stress and aspirin resistance in patients with stable coronary heart disease. J Clin Pharmacy Therapeut 2007; 32: 287-292.

[71] Hovens MMC, Snoep JD, Eikenboom JCJ, van der Bom JG, Mertens BJA, Huisman MV. Prevalence of persistent platelet reactivity despite use of aspirin: A systematic review. Am Heart J 2007; 153: 175-181.

[72] Poulsen TS, Jorgensen B, Korsholm L, Bjorn Licht P, Haghfelt T, Mickley H. Prevalence of aspirin resistance in patients with an evolving acute myocardial infarction. Thromb Res 2007; 119: 555-562.

[73] Pamukcu B, Oflaz H, Oncul A, Umman B, Mercanoglu F, Ozcan M, et al. The role of aspirin resistance on outcome in patients with acute coronary syndrome and the effect of clopidogrel therapy in the prevention of major cardiovascular events. J Thromb Thrombolysis 2006; 22: 103-110.

[74] Krasopoulos G, Brister SJ, Beattie WS, Buchanan MR. Aspirin "resistance" and risk of cardiovascular morbidity: systematic review and meta-analysis. Br Med J 2008; 336: 195-198.

[75] Sofi F, Marcucci R, Gori AM, Abbate R, Gensini GF. Residual platelet reactivity on aspirin therapy and recurrent cardiovascular events -- A meta-analysis. Int J Cardiol 2008; 128: 166-171.

[76] Dropinski J, Jakiela B, Sanak M, Wegrzyn W, Biernat M, Dziedzina S, et al. The additive antiplatelet action of clopidogrel in patients with coronary artery disease treated with aspirin. Thromb Haemost 2007; 98: 201-209.