Aspirin Resistance: Fact or Fiction?

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Abstract

A meta-analysis of clinical studies of patients with cardiovascular disease demonstrated that the use of aspirin was associated with a 22% decrease in death rates and relevant ischemic vascular events. However, clinical studies demonstrated that patients that regularly took aspirin presented recurrence of cardiovascular events. Such observation led to the question whether, in some patients, the aspirin was not effective in blocking platelet aggregation and these patients were called unresponsive to aspirin or aspirin-resistant.

The clinical aspirin resistance is characterized as the occurrence of cardiovascular events in patients during treatment with aspirin, whereas the laboratory resistance is defined as the persistence of platelet aggregation, documented by laboratory test, in patients regularly taking aspirin. Patients that are aspirin-resistant presented, according to laboratory tests, on average 3.8 times more cardiovascular events when compared to non-resistant ones.

Platelet function

In 1963, in the Journal of Physiology, Born and Cross[1] published a pioneer study on platelet aggregation. That was the consistent start of the study of platelet function in coagulation. Currently, this function is of great importance in the physiopathology of coronary syndromes[2]. Platelets adhere, activate and aggregate on a ruptured atherosclerotic plaque or on the damaged endothelium, significantly contributing to the formation of thrombi within the coronary arteries, which can be totally or partially obstructed[3,4].

Initially, the platelets adhere to the extracellular matrix and this adherence is mediated by glycoproteins Ib, V, IX, with the Von Willebrand factor as the major ligand. It is noteworthy the fact that glycoproteins VI and Ia, important collagen receptors, also have a relevant role in platelet adherence[5].

After the adherence, the ADP, thrombin, epinephrine and thromboxane A2 (TXA2) amplify the mechanism and recruit more circulating platelets, which contribute to the formation of the thrombus[5,6].

At this moment, the platelets activate and secret several substances such as ADP, serotonin, fibrinogen, von Willebrand factor, fibronectin, growth factors (platelet-derived growth factors, alpha-growth factor, beta-growth factor), pro-coagulants (platelet factor 4 and factor V) and TXA2[6].

Another relevant event for the formation of the thrombus is platelet aggregation, that is, the binding of platelets. The final pathway for platelet aggregation is the glycoprotein IIb/IIIa receptor, which is also the main adherence receptor. The fibrinogen also has an important role in this process, considering that it stabilizes the platelet thrombus and functions as bridge between platelets[5,6].

The vascular endothelium controls platelet reactivity through three main mechanisms: the arachidonic acid-prostacyclin pathway, the L-arginine nitric oxide and the endothelial ectoadenosine diphosphate (Ecto-ADPase)[5,6].

Endothelial cells convert the arachidonic acid into prostacyclins through the action of cyclooxygenase 1 (COX1), cyclooxygenase 2 (COX 2) and prostacyclin synthetase. The prostacyclins inhibit platelet function, increasing the levels of intracellular cyclic adenosine monophosphate (cAMP)[5,6].

The nitric oxide diffuses into the platelets and stimulates the production of guanosine monophosphate, causing a decreased intracellular calcium flow. Such calcium reduction modifies the conformation of the glycoprotein IIb/IIa, allowing the formation of fibrinogen bridges and platelet aggregation[5,6].

The Ecto-ADPase limits the plasma level of nucleotides. The activity of this enzyme annuls the critical phase of recruitment of platelet reactivity, as it removes the nucleotides from the fluid environment[5].

Platelet antiaggregation

The blocking of platelet aggregation is considered an effective strategy for the prevention and treatment of cardiovascular events caused by atherosclerotic disease[7].

The search for therapeutic strategies capable of blocking platelet aggregation in patients with atherosclerotic disease, with the objective of decreasing the occurrence of clinical events, has been the objective of several studies[8-10].

Due to the fact that aspirin inhibits cyclooxygenase 1, which prevents the formation of thromboxane A2, is considered an excellent platelet antiaggregant drug[11].

The effectiveness of aspirin in preventing cardiovascular events has been demonstrated by a meta-analysis that

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evaluated 287 studies with more than 200,000 patients and revealed a 22% decrease in deaths and vascular ischemic events in the group of patients taking aspirin, when compared to those who did not take the drug[12].

American, European and Brazilian guidelines recommend the use of acetylsalicylic acid (ASA) in the treatment of patients with atherosclerotic disease and as a measure of prevention of future events[3,4,13,14].

However, clinical studies have shown that patients who regularly took aspirin still presented cardiovascular events[15,16]. Such observation led to the question whether, in some patients, the aspirin was not effective in blocking platelet aggregation and these patients were called unresponsive to aspirin or aspirin-resistant.

Aspirin resistance

The observation of cardiovascular events in patients receiving aspirin represented the basis for the establishment of the concept of aspirin resistance, which can be clinical or laboratory resistance[17,18].

Although there is no consensus definition, the concept of clinical aspirin resistance refers to the occurrence of cardiovascular events in patients during treatment with aspirin, with a prevalence of 5% to 45%[17], whereas laboratory resistance is defined as the persistence of platelet aggregation documented by laboratory assessment (in patients receiving aspirin regularly), with a prevalence of 5% to 60%[18].

There is no standard laboratory test that can be used as the ideal test to evaluate platelet antiaggregation determined by aspirin. The laboratory tests used to assess the aspirin action on platelets evaluate the function (*in vivo*) and platelet aggregation (*ex vivo*)[19].

The main tests used to evaluate aspirin action on platelet function are: urinary levels of TXB2, quantification of the expression of P-selectin and soluble P-selectin. The main tests that evaluate aspirin action on platelet aggregation are: optical platelet aggregation (OPA) test, platelet function analyzer (PFA), rapid verification of aspirin action (RVAA) and thromboelastogram[18].

The tests that assess aspirin action can be or not specific for COX1. The specific ones use arachidonic acid as a platelet aggregation stimulant or measure the metabolites of TBX2 (P-selectin) in urine or blood. The non-specific ones use collagen, ADP or epinephrine as platelet aggregation inducers[19]. The OPA test uses a blood sample in citrate, which is centrifuged and two types of plasma are obtained: one is platelet-rich and the other is platelet-poor. Subsequently, arachidonic acid (AA), ADP and collagen, which are platelet aggregation stimulants, are added. The aggregation is assessed through the comparative analysis of the change in color of the platelet-rich plasma.

Aspirin resistance is considered when there is a change in color > 20% with AA or > 70% with ADP or collagen[20].

At the platelet function analyzer, whole blood goes through an orifice that contains a fine membrane coated by collagen and epinephrine. The time of the orifice occlusion, which occurs due to platelet aggregation, is used to evaluate platelet aggregation[21]. The rapid verification of aspirin action test consists in placing whole blood in a tube of which bottom contains a membrane coated by fibrinogen and AA. Platelet function is assessed through the binding of the platelets to fibrinogen and aspirin resistance is considered when there are more than 550 reaction units[22].

At the thromboelastogram, reptilase, factor VII, heparin and AA are added to whole blood. This test initially assesses the thromboelastogram of the intrinsic and extrinsic pathways and when there is any alteration (altered thrombus formation time), the fibrin thromboelastogram must be assessed. The abnormality of this thromboelastogram indicates a quantitative or qualitative platelet alteration[23].

The comparative analysis of the prevalence of aspirin resistance, according to these laboratory tests, showed conflicting results. In the ASPECT study[24], there was no concordance regarding the prevalence of laboratory resistance to ASA when comparing the OPA test, PFA, RVAA, TXB2 and thromboelastogram. However, the study carried out by Karon et al[25] demonstrated good sensitivity and concordance between the OPA test, PFA, RVAA and TXB2 for the diagnosis of resistance.

Inadequate platelet antiaggregation mechanisms in patients receiving aspirin

There are clinical, pharmacodynamic, biological and genetic causes for inadequate platelet antiaggregation in patients receiving aspirin[19,26].

It is known that, in spite of the prescription of ASA and the recommendations that the physicians give to their patients, a considerable number of these patients does not take the drug adequately, for either they take it irregularly or the patient is not adherent after some time of treatment[19,26].

Smoking, according to some authors, would increase platelet activation, thus accentuating the formation of platelet thrombi. However, the scientific information does not consistently support such hypothesis[19,26].

Regarding the pharmacodynamics of ASA, it is believed that in some patients, the antiaggregatory effect is dose-dependent; that the longer therapy duration decreases the antiaggregatory potency; and that non-steroidal anti-inflammatory agents attenuate the effect of aspirin on platelets in the long term[19,26].

Some patients have TXA2 production induced by COX2, or from a new formation of COX1 from macrophages or endothelial cells. It is also possible that there is platelet activation through other pathways, such as: increased sensitivity to collagen, which also increases platelet adherence and failure in the inhibition of platelet activation mediated by catecholamines, or through mechanisms that are not mediated by TXA2[19,26].

Some isoprostanes (similar to prostaglandins) are produced from the arachidonic acid or peroxidation of lipids and possibly have similar properties to TXA2, thus being capable of stimulating platelet aggregation[19,26].

The increased expression in the CD40 ligand of the platelet membrane possibly represents a new platelet aggregation

pathway and can be associated with the triggering of platelet aggregation in situations of vascular inflammation[19,26].

Genetic mutations or polymorphisms of the COX1 genes and polymorphisms of the glycoproteins IIb/IIa codifying genes represent genetic mechanisms of ASA resistance[19,26].

Clinical impact of laboratory resistance to aspirin

As mentioned before, although there is no ideal, standard and exclusive laboratory test to assess the ASA action on platelet aggregation, several studies have assesses the clinical implications of laboratory resistance to ASA, according to the available laboratory tests[27-29].

Snoep et al[30] carried out a meta-analysis that included 16 evaluation studies of the ASA action on platelet aggregation, according to laboratory tests and their clinical implications.

The population of the studies, a total of 1,813 patients, was submitted to myocardial revascularization surgery (MRS) and percutaneous coronary intervention (PCI). This population had coronary artery disease or had suffered a cerebrovascular accident (CVA). The ASA dose varied from 80 to 1,500 mg and the clinical follow-up ranged from one day to 4 years.

The prevalence of laboratory aspirin resistance was 27.4%. Patients who were ASA-resistant, when compared to non-resistant ones, presented 4.37 times more ischemic clinical events (CI = 2.19 - 8.73); 2.43 times (CI = 0.4 - 14.2) more reocclusion of the treated artery; 3.11 times (CI = 1.6 - 5.4) more myonecrosis; and 3.78 times (CI = 2.34 - 6.11) more clinical events.

Krasopoulos et al[31] recently published a meta-analysis including 20 studies that evaluated ASA action on platelet aggregation, according to laboratory tests.

A total of 2,930 patients were studied, who had been submitted to PCI and MRS or that had presented acute coronary syndrome, CVA or cardiovascular disease. ASA dose varied from 75 to 1,500 mg. The prevalence of laboratory aspirin resistance was 27.6%. Patients resistant to ASA, when compared to the non-resistant ones, presented 3.85 times (CI = 3.08 - 4.8) more cerebrovascular events ; 5.99 times (CI = 2.28 - 15.72) more deaths; and 4.06 times (CI = 2.96 - 5.56) more acute coronary syndrome. The sub-analysis of patients receiving ASA and clopidogrel showed that the ones resistant to ASA presented 4.12 times (CI = 2.87 - 5.92) more cardiovascular events than the non-resistant ones, that is, clopidogrel use did not attenuate the risks of clinical events in ASA-resistant patients. The prevalence of aspirin resistance was lower among men when compared to women and higher in patients with chronic kidney disease.

Table 1 - Aspirin resistance and occurrence of cardiovascular events

Events	N. events Pts resistant to ASA	N. events Pts sensitive to ASA	OR (95%CI)
Cardiovascular	151/497	167/1,316	3.78 (2.34 - 6.1) *
Cardiovascular	316/810	357/2,812	3,85 (3,08 4,8)**

*pts - patients; ASA - acetyl salicylic acid; *reference 30; **reference 31.*

These studies support the concept that, although there is no standard and exclusive method to diagnose laboratory aspirin resistance, patients who present this resistance, according to the laboratory test, present a higher risk of cardiovascular ischemic events (Table 1), including death.

Conclusions

It is an indisputable fact that some patients that take aspirin present the recurrence of cardiovascular events. These patients have been called aspirin-resistant or unresponsive and the mechanisms responsible for this resistance are multifactorial.

There is no laboratory test considered to be ideal one for the assessment of the action of the acetylsalicylic acid on platelet aggregation. Additionally, there is no homogeneity regarding the tests used for the diagnosis of ASA resistance in studies with clinical outcomes.

However, ASA-resistant patients, according to the currently available laboratory tests, present a higher occurrence of cardiovascular events, when compared to non-resistant ones.

Further studies, with standardization of the laboratory tests used to test ASA resistance and the clinical outcomes and larger sample sizes will contribute to a better understanding of the aspirin-resistance phenomenon.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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