Aspirin Resistance – Current Issues

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Abstract

In the last few years, the concept of aspirin resistance has been largely emphasized in the medical literature, although its definition, mechanism, and specific guidelines for its management remain unclear. Aspirin displays good antithrombotic activity. Various laboratory parameters assessing the efficacy of aspirin like bleeding time, platelet reactivity, thromboxane-A2 (TX-A2) production, and measurement of platelet aggregation, have confirmed the lack of its uniform effect on the platelets. Few studies have reported aspirin resistance to the tune of 5 - 45%. Various extrinsic and intrinsic factors influence the resistance. Numerous studies reveal that aspirin resistance can be overcome by combining it with another antithrombotic agent, i.e., clopidogrel. Further, clopidogrel resistance has also been reported. So, much is expected in the field of diagnostic tests in order to know the true picture of aspirin resistance.

Mechanisms of aspirin resistance

The exact mechanisms are not clear:

I. True aspirin resistance:

   The proposed factors for this type of resistance include:

   i. Decreased bioavailability of aspirin.
   ii. Accelerated platelet turnover introducing newly known antiinflammatory effects. Chronic administration of aspirin is associated with a modest increase in the incidence of gastrointestinal bleeding even while low-doses are used. It must be noted that despite the clear experimental evidence of its safety and efficacy, aspirin use continues to be less than optimal. There is no clear-cut definition of aspirin resistance. But it is widely defined as a mean aggregation of > 70% with 10 uM adenosine diphosphate (ADP) and a mean aggregation of ≥ 20% with 0.5 mg/ml arachidonic acid (AA). Aspirin semiresonders are defined as those meeting only one of the criteria.

Other definitions include failure of aspirin to prevent clinical aspirin resistance. But it should be termed treatment failure. These definitions are certainly unacceptable. The term “true aspirin resistance” is used for failure of aspirin to inhibit TXA2 production and the term “unproven aspirin resistance” is used for failure of aspirin to inhibit platelet function in vivo or in vitro (without demonstration of inadequate inhibition of TXA2 production).

Introduction

Aspirin exhibits good antithrombotic activity and is widely used in the management of coronary artery disease as well as in the prophylaxis of patients undergoing vascular grafting or percutaneous angioplasty and in the long-term prevention of cardiovascular and cerebrovascular events. The lowest cost and extreme safety of aspirin has the greatest impact on acute MI-associated events worldwide than any other – albeit very important – achievement in this field. Aspirin reduces the mortality from acute myocardial infarction (MI) to an extent which is similar to that of the thrombolytic agent streptokinase. The metanalysis of the Antiplatelet Trialists Collaboration involving 100,000 aspirin-treated patients, revealed a 25% reduction of vascular death, MI, or stroke, for antiplatelet therapy (mainly aspirin) versus placebo in patients with acute or previous cardiovascular or cerebrovascular events. Aspirin irreversibly inhibits Cox-1 by acetylating a serine residue at position 530, thus preventing the conversion of arachidonate to the unstable prostaglandin (PG) intermediate PGH2 which is converted to TXA2 – a potent vasoconstrictor and platelet agonist. A single dose of 160 mg aspirin completely abolishes the platelet TXA2 production and the same effect can be progressively achieved with low daily doses of 30 - 50 mg. High-doses of aspirin might have antithrombic effects which are independent of platelet Cox-1 inhibition including increased fibrinolytic activity, depression of prothrombin synthesis, improvement of endothelial function, and well known antiinflammatory effects. Chronic administration of aspirin is associated with a modest increase in the incidence of gastrointestinal bleeding even while low-doses are used. It must be noted that despite the clear experimental evidence of its safety and efficacy, aspirin use continues to be less than optimal. There is no clear-cut definition of aspirin resistance. But it is widely defined as a mean aggregation of > 70% with 10 uM adenosine diphosphate (ADP) and a mean aggregation of ≥ 20% with 0.5 mg/ml arachidonic acid (AA). Aspirin semiresponders are defined as those meeting only one of the criteria. Other definitions include failure of aspirin to prevent clinical aspirin resistance. But it should be termed treatment failure. These definitions are certainly unacceptable. The term “true aspirin resistance” is used for failure of aspirin to inhibit TXA2 production and the term “unproven aspirin resistance” is used for failure of aspirin to inhibit platelet function in vivo or in vitro (without demonstration of inadequate inhibition of TXA2 production).

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formed, non-aspirinated platelets into the blood stream\textsuperscript{13}.

iii. Competition of aspirin with other NSAIDs (like ibuprofen) preventing aspirin access at Serine 530 of Cox-I\textsuperscript{14}.

iv. Transcellular formation of TxA\textsubscript{2} by aspirinated platelets from PGH\textsubscript{2} released by other blood cells or vascular cells\textsuperscript{15}.

v. TxA\textsubscript{2} production by aspirin insensitive Cox-2 in newly formed platelets or other cells\textsuperscript{16}.

vi. (Theoretical) presence of variant Cox-I which is less sensitive to aspirin inhibition\textsuperscript{17}.

vii. Poor compliance by the patient\textsuperscript{18}.

II. Unproven aspirin resistance:

These factors include:

i. Increased sensitivity to ADP-induced GP IIb/IIIa activation.

ii. Increased resistance to collagen\textsuperscript{20}.

iii. High plasma levels of von Willebrand factor\textsuperscript{21}.

iv. GPIIb/IIIa polymorphisms\textsuperscript{22}.

v. Potent proaggregatory activity exhibited by non-enzymatic and oxidation-dependent pathway for the synthesis of arachidonic acid derivatives isoprostanes\textsuperscript{23}.

vi. Hyperlipidaemia\textsuperscript{24}.

vii. Cigarette smoking\textsuperscript{25}.

viii. Physical and mental stress\textsuperscript{25}.

It has been shown that the extent of inhibition of platelet aggregation by aspirin progressively decreased over time in some patients, thus suggesting that some kind of aspirin tolerance might develop during chronic aspirin therapy\textsuperscript{26}. Contrary to this study, another study reported that 100 patients on chronic aspirin treatment had consistently reduced platelet aggregation over time\textsuperscript{27}.

Thus, the proposed mechanisms of aspirin resistance can be summarised as:-

\begin{itemize}
  \item \textbf{I. Extrinsic mechanisms:}
    \begin{itemize}
      \item A. Accentuation of platelet thrombi by exogenous substances (e.g., smoking).
      \item B. Drugs, e.g., NSAIDs may interact with aspirin’s acetylation of Cox-I.
      \item C. Increased platelet turnover
      \item D. Inadequate aspirin dosing
    \end{itemize}
  \item \textbf{II. Intrinsic mechanisms:}
    \begin{itemize}
      \item A. Inducible Cox-2, not adequately inhibited by low-dose aspirin, thus allowing for platelet thromboxane A2 production despite inhibition of Cox-1.
      \item B. Polymorphisms in the Cox-1 gene.
      \item C. Uninhibited Cox-1 in nucleated cells, e.g., macrophage and vascular endothelium producing PGH\textsubscript{2} is shunted into platelet and bypasses platelet Cox-1.
      \item D. Polymorphisms in the GPIIb/IIIa receptor complex.
    \end{itemize}
\end{itemize}

\section*{Measurement of aspirin resistance}

At present there is no clinically validated, uniformly accepted method to assess the effect of aspirin on platelet aggregation. Various methods include measurement of platelet aggregation with optical aggregometer, estimation of urinary II-dehydro-TxB\textsubscript{2} levels, platelet function analyser 100 and Verify Now Aspirin Assay.

\subsection*{I. Platelet aggregation with optical aggregometer:}

It measures the increase in light transmission through a platelet suspension that occurs when platelets are aggregated by an agonist at standard concentrations. Various agonists used include arachidonic acid (AA), collagen epinephrine and thrombin. Aspirin almost completely inhibits platelet aggregation induced by AA and collagen, but partially inhibits aggregation induced by epinephrine and ADP\textsuperscript{28}. The studies are done with platelet-rich plasma prepared from a citrated whole blood sample\textsuperscript{29}. The amount of platelet aggregation is directly related to the amount of light which is allowed to be transmitted through the solution. There are many pre-analytical and analytical variables which affect the results of platelet aggregation. The results within one laboratory can
hardly be compared with those obtained in a different laboratory because of lack of standardisation. For examination, the source of platelet agonists, the scales of the recorder, and the geometry of the optical system, all influence the results of platelet aggregometry. Platelet aggregation is sensitive to changes in temperature and hydrogen-ion concentration and must be conducted within hours of sample collection. Although aggregation-dependent TxA2 production can potentiate ADP-induced platelet aggregation in citrated PRP, ADP at 10 μmol/l induces full platelet aggregation that is largely independent of TxA2 production. Arachidonic acid (AA) being the precursor of TxA2, is a more suitable platelet agonist than ADP for studying the effects of aspirin. However, the final platelet aggregation induced by AA is the sum total of the effects of synthesised TxA2 and other agonists secreted by platelet granules.

II. Urinary II-dehydro-TxB2 levels:

TxA2 is rapidly hydrated to form the more stable TxB2, which is subsequently converted by the liver into two major metabolites, i.e., 2, 3-dinar TxB2 and II-dehydro TxB2. Both metabolites, along with TxB2 are excreted unchanged in the urine. TxB2 is a stable metabolite which can be measured in the urine and can serve as an indirect measure of TxA2 activity in vivo. The advantage of this method is that it is non-invasive and is normalised with standard controls. However, it is based on a retrospective case control study in which the frequencies of significant risk factors for cardiovascular disease were higher in the case group than the controls.

III. Platelet function analyzer 100 system:

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 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 the injured part of a vessel wall).

A platelet plug is formed which gradually occludes the aperture and ultimately the blood flow through the aperture gradually decreases and eventually stops. The time needed for blood flow interruption (closure time) is recorded. PFA-100 system is more reproducible and more sensitive to type I von Willebrand disease as compared to bleeding time. However, like the bleeding time, it is sensitive to many variables including platelet function, platelet count, red blood cells, and plasma VWF. It has been shown that high plasma VWF levels are the main determinant of short PBA-100 closure time in patients with cardiovascular disease on aspirin treatment. Anderson et al. in their study reported that aspirin treatment abolished TxB2 production to the same extent in patients with short closure time ("aspirin resistance") and patients with long closure time ("aspirin sensitive"). In a study by Grundman et al., aspirin resistance measured by the PFA-100 has been only weakly correlated with an increased risk of clinical events.

IV. Verify Now Aspirin Assay also known as Ultegra Rapid Platelet Function Assay-ASA:

This assay is a whole blood, point of care device which measures platelet aggregation using different cartridges for different applications. It can detect only platelet dysfunction on account of exposure to antiplatelet agents including aspirin, clopidogrel, and GPIIb/IIIa inhibitors. Platelet aggregation detection is based on the agglutination of platelets on fibrinogen-coated beads stimulated by an agonist in citrated whole blood. Although the specificity of the test for aspirin inhibition is reported to be 85% by the manufacturer, yet it is also sensitive to GP IIb/IIIa inhibitors like clopidogrel and dipyridamole as well as streptokinase, suggesting that it is an ideal test for measuring the effect of aspirin on platelets.

Management of aspirin resistance

Currently there are no specific guidelines for the management of aspirin resistance. The first step is to enquire about the patient’s compliance. Regarding
optimal aspirin dosing, it is controversial. No convincing data are available showing that the antithrombotic effect of aspirin is dose related. The meta-analysis by Anti-Thrombotic Trialist’s Collaboration refuted the claim that high doses of aspirin (500 - 1,500 mg/day) were effective than low doses (75 - 150 mg/day). Other method to manage aspirin resistance is by addition of another antiplatelet agent – clopidogrel, because CAPRIE trial has shown greater benefit of combination of aspirin and clopidogrel compared with aspirin alone. The combination of aspirin with clopidogrel is an ideal one since clopidogrel inhibits another pathway of platelet activation. However, till date, it is not clear whether the superiority of a combination of clopidogrel and aspirin over aspirin is due to clopidogrel compensation for aspirin non-responders. Resistance to even clopidogrel has been reported, which is associated with an increased risk of recurrent thrombotic events in patients with acute MI.

**Aspirin dosage**

According to the Antithrombotic Trialists’ Collaboration, daily doses of aspirin (75 - 150 mg) are as effective as higher doses for prevention of thrombotic events and are associated with low risk of bleeding. Bornstein et al in their study have shown that even 100 mg of aspirin completely inhibits Cox-1 enzyme, thus further substantiating the fact that patients with resistance established during low dose aspirin therapy may respond to higher doses. The results of this study showed that aspirin in doses of 500 mg/day significantly prolonged the time between first and second stroke (p = 0.002) compared with lower doses. Helgason et al revealed that an increase in the dose of aspirin to 625 mg/day in five patients who were aspirin resistant with 325 mg/day showed aspirin sensitivity. Another study has revealed that these patients remained resistant with aspirin 1,300 mg. This shows that inadequate dose cannot explain aspirin resistance in all subjects.

**Current evidence and its clinical implications**

Till today there is scarce data on aspirin resistance which ranges from 5 - 50% depending upon the type of test used. Gurn et al in their study reported that aspirin resistance was found in 5.5%, and 23.3% were semi-responders, thus giving an inadequate response of 28.8%. The patients were taking 325 mg of aspirin and patients who showed inadequate response were more likely to be females (34.4% V/s 17.3%, p = 0.001) and less likely to be smokers (0% V/s 8.3%, p = 0.004). There was a trend towards increased age of patients showing inadequate response (65.7 V/s 61.3 years, p = 0.06). Gun et al also showed that among stable patients with CAD over a mean follow-up period of 679 ± 185 days, aspirin resistance was associated with an increased risk of composite end-points of death, MI, or cerebrovascular accident (p = 0.03). Coma-Canella et al have reported higher aspirin resistance among men (p = 0.02) and especially those using tobacco (p = 0.03). Few more studies have reported aspirin non-responder status, i.e., 30% and 34% respectively. In a study Sadiq et al involving 50 patients taking 150 mg of aspirin at least for the past seven days, 2.08% were found to be aspirin resistant while 39.58% were aspirin semi-responders. Thus, 41.66% patients were found to show inadequate response to aspirin, i.e., aspirin resistant plus semi-responders. There was no difference related to age (p = 0.2) but there was a trend for females to be semi-responders (31.67% V/s 10.7%, p = 0.08). No statistically significant difference related to smoking could be found among the groups (p = 0.2). Eikeboom et al showed that suboptimal reduction of urinary 11-dehydro TxB2 level during aspirin treatment is associated with increased risk for future MI and cardiovascular death, thereby suggesting that “true aspirin resistance” may be a clinically relevant phenomenon. Inadequate inhibition of TxA2 biosynthesis by aspirin can be seen in patients on ibuprofen therapy, because of competition of these 2 drugs at Cox-1 level. An association between suboptimal platelet function inhibition during aspirin treatment and increased incidence of cardiovascular or cerebrovascular events has also been reported. To confirm these findings, larger studies are required because monitoring platelet function during antiplatelet therapy can be of greater use to predict the risk of treatment failures. However, the phenomenon that they describe should not be termed “aspirin resistance” because it is determined to a large extent by variables that cannot be inhibited by aspirin.
Conclusion

Inadequate response to aspirin is prevalent in Indian subjects and there are no predictors for this condition. The diagnosis is mainly based on laboratory investigations. Various intrinsic and extrinsic factors influence aspirin resistance. According to a statement by Marco Cattaneo, there are no predictors for this condition. Derry et al. state that risk of gastrointestinal haemorrhage varies in intrinsic and extrinsic factors. According to a statement by Marco Cattaneo, "true" or "unproven" aspirin resistance is insufficient to recommend laboratory monitoring of patients on aspirin treatment in the clinical setting. In such situations, a clear-cut policy on aspirin is required to ascertain whether all patients on aspirin be investigated, whether all patients with so called aspirin resistance be put on clopidogrel, and whether these patients can also face the danger of clopidogrel resistance. So, much is expected in the field of diagnostic tests in order to know the true picture of aspirin resistance.

References

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