

## Sub-Optimal Response to ASA in Cardiovascular Ischemia Homocysteine-Related

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Received: August 30, 2015      Revised: November 19, 2015

Accepted: November 22, 2015

Published online: December 10, 2015

### ABSTRACT

Some epidemiological reports showed a significant association between the increased homocysteine serum levels (H-Hcy) and risk of ischemic cardiovascular disease (CVD). Acetyl-Salicylic Acid (ASA) is the drug commonly used to antagonize pro-thrombotic effect of Hcy. But, human and animal studies evidenced that ASA, given at habitual anti-ischemic dosage, may induce an incomplete inhibition of platelet (aspirin-resistance) in patients with CVD Hcy-related by several mechanisms. In these patients, doses higher than normal are requested to obtain an optimal ASA-response (aspirin-sensitivity). That happens because Hcy "per se" is a pro-aggregatory agent and acts as specific sensitizer of blood platelets with several mechanisms. On the contrary, its acetylated form (N-acetyl-homocysteine) obtained using high ASA doses, is able to reverse this effect restoring the platelets' sensitivity. That happens with a still unknown mechanism.

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**Key words:** Homocysteine; Ischemic vascular disease; Acetyl-salicylic acid; Aspirin-resistance; N-acetyl-homocysteine; Aspirin-sensitivity

Cacciapuoti F. Sub-Optimal Response to ASA in Cardiovascular

Ischemia Homocysteine-Related. *Journal of Cardiology and Therapy* 2015; 2(6): 456-458 Available from: URL: <http://www.ghrnet.org/index.php/jct/article/view/1515>

### INTRODUCTION

A growing body of evidence showed that, apart from traditional risk factors for cardiovascular disease, another powerful factor contributing to increased cardiovascular morbidity and mortality is the inherited or acquired hyperhomocysteinemia (H-Hcy)<sup>[1-3]</sup>. Several studies reported that this can be responsible for thrombotic disease, as myocardial infarction, stroke and peripheral vascular disease<sup>[4-6]</sup>. But, its pathological role isn't well-known yet. H-Hcy certainly promotes endothelial dysfunction and facilitates platelet activation<sup>[7,8]</sup>. A gene expression by DNA hypomethylation interacting endothelial and vascular smooth muscle cells must be also considered<sup>[9]</sup>. Increased lipid oxidation, enhanced inflammatory cytokine expression and reduction in serum antithrombin activity are other mechanisms<sup>[10]</sup>.

The drug employed for both primary and secondary prevention of these diseases is Acetyl-Salicylic-Acid (ASA), commonly known as aspirin. It is known that the leading antiplatelet effect of aspirin are related to irreversible inhibition of platelet Cyclooxygenase-1 (COX-1) by the acetylation of the potent vasoconstrictor and platelet activator thromboxane (TxA2)<sup>[11-13]</sup>. Aspirin exerts additional anti-thrombotic activity by reducing thrombin generation and acetylating lysine residues in fibrinogen. Referring to TxA2 production, an inhibition of more than 95% of thromboxane production is required to define the antiplatelet efficacy of ASA. Referring to its anti-thrombotic action, clinical practice demonstrated that daily aspirin-dosages of 75-160 mg is usually effective. In addition, a retrospective analysis from CURE indicated that doses  $\leq$ 100 mg/day were more effective than dosages  $\geq$ 200 mg/day<sup>[14]</sup>. On the other hand, prospective, placebo-controlled trials pointed out that an oral dose of 75 mg/daily obtains an excellent protection against vascular ischemia<sup>[15,16]</sup>. Nevertheless in ischemia induced by H-Hcy, an incomplete inhibition of platelet function can be obtained when

habitual ASA doses are supplied. But, an effective inhibition of platelet function is often obtained when higher ASA doses (about 150-160 mg) are used<sup>[17]</sup>.

## ASPIRIN-RESISTANCE

Really, a sub-optimal response to ASA is observed in up to 40% of all aspirin users. The unsuccessful response was defined as aspirin-resistance<sup>[18]</sup>. This condition consists in the inability of aspirin to reduce the platelet production of thromboxane-2 and thereby platelet activation and aggregation<sup>[19]</sup>. Aspirin-resistance may be defined as both laboratory and clinical resistance. The laboratory aspirin-resistance is the failure of aspirin to inhibit platelet thromboxane A2 production or inhibit tests of platelet function (eg. platelet aggregation) that are dependent on thromboxane production. Instead, the clinical aspirin resistance is defined as the failure of drug to prevent clinical thromboembolic events<sup>[20]</sup>. Potential causes of aspirin resistance include inadequate dose, drug interactions, genetic polymorphisms of COX-1 and other causes involved in thromboxane biosynthesis and in platelet turnover<sup>[21]</sup>.

## INADEQUACY OF NORMAL DOSES IN ISCHEMIA BY H-HCY

The unlike anti-platelet response to ASA in normal and in hyper-homocysteinemic patients was respectively evidenced in Antithrombotic Trialists' Collaboration study<sup>[22]</sup> and in a multicenter randomized trial of Karolczak *et al*<sup>[23]</sup>. More recently, the influence of Hcy levels on platelet reactivity in CAD patients treated with ASA was confirmed by Verdoia *et al*<sup>[24]</sup>. It must be added that sub-optimal response to aspirin was frequently found also in diabetic and/or elderly patients with elevated Hcy levels<sup>[25,26]</sup>. Although the exact mechanism of aspirin resistance in patients with H-Hcy remains to be established, likely the high Hcy serum concentration will induce an exaggeration of platelets' adhesion and aggregation. The sub-optimal response in these was also reported to the action of glutamate Hcy-stimulated. This is an excitatory neurotransmitter that bind the methyl-aspartate (NMDA) receptor and 3-hydroxy-5-methyl-4-propionic acid (AMPA) receptor. In turn, NMDA and AMPA stimulate and strengthen platelet aggregation<sup>[27,28]</sup>. Another hypothesis reports the prevalence of aspirin resistance in H-Hcy patients to higher levels of P-selectin, also named CD62P in these<sup>[24]</sup>. CD62P is a cell adhesion molecule (CAM) normally present on the surface of activated endothelial cells and activated platelets, and it is useful to promote platelet aggregation through platelet-fibrin and platelet-platelet binding. In confirmation of that, another study previously found that the levels of CD62P were significantly higher in the aspirin-resistant group in comparison to aspirin-sensitive group<sup>[29]</sup>. In this connection, Silverman *et al.* have hypothesized that Hcy enhances monocyte/human aortic endothelial cell interactions, by upregulating endothelial adhesion molecules<sup>[30]</sup>. Other causes of increased platelet aggregation could be the concentration-dependent inhibition of l-arginine transport<sup>[31]</sup>, Hcy-mediated platelet fibrinogen receptor<sup>[32]</sup>, calcium mobilization from intracellular stores<sup>[33]</sup> and endoplasmic reticulum stress<sup>[34]</sup>.

Contrarily to the sub-optimal response obtained with lower doses in ischemia Hcy-related, when higher doses of anti-ischemic ASA are administered, the platelets' response is re-established. In fact, the study of Karolczak *et al.* showed that ASA given at 160 mg/daily, contrarily to lower doses (75 mg/daily), effectively inhibits blood platelet reactivity in ischemia H-Hcy-related. These authors

hypothesized that in hyperhomocysteinemic patients, ASA reacts with an amino group of Hcy inducing the formation of N-acetyl-homocysteine. This new compound in turn is able to induce the reduction of platelet aggregation with unknown mechanism<sup>[23]</sup>. In conclusion, in hyper-homocysteinemic patients ASA seems to exert a twofold behaviour: at habitual doses, ASA can have sub-optimal antiplatelet response, and may carry out a pro-aggregatory effect too. On the contrary, the excessive acetylsalicylic acid obtained at higher doses seems to induce the Hcy acetylation generating N-acetyl-homocysteine, a compound carrying out a prevalent antiplatelet effect. Nevertheless, how the Hcy-acetylation restores aspirin-sensitivity remains unknown and other studies performed in this topic are requested.

## CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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