Aspirin Resistance in Stable Coronary Disease

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Abstract

Background: Coronary artery disease (CAD) has high morbidity and mortality rates. Coronary angioplasty and antiplatelet therapy with acetylsalicylic acid (ASA) are critical in treating CAD patients. Elective angioplasty lacks studies on the factors associated with resistance to ASA.

Objective: To evaluate the prevalence and ASA resistance related factors in patients undergoing elective angioplasty.

Methods: Retrospective cohort study of 198 patients undergoing elective angioplasty, evaluated for resistance to ASA by optical aggregometry with arachidonic acid as agonist.

Results: Resistance to ASA in 6.56% of the cohort (13/198). C-reactive protein (CRP) levels indicated association with resistance to ASA (p=0.02).

Conclusions: Low prevalence of ASA resistance in patients undergoing elective angioplasty; C-reactive protein high levels are related to greater frequency of this resistance.

Keywords: Aspirin; Platelet aggregation; Coronary balloon angioplasty

Introduction

Coronary artery disease (CAD) is the leading cause of death in the world¹; in Brazil, it accounts for 28.9% of all deaths². In stable CAD, the use of acetylsalicylic acid (ASA) in patients undergoing elective percutaneous coronary intervention (PCI) is a grade of recommendation I, and level of evidence A³. For these patients, resistance to ASA is related to the worst outcome possible⁴. Therefore, a better understanding of the factors associated with ASA resistance is required.

ASA resistance or a high platelet reactivity to ASA or a non-response to ASA are common names for the same laboratory phenomenon, which can set a higher incidence of treatment failure related to this antiplatelet⁵. Treatment failure is a broader phenomenon, which refers to cases in which, even with proper use of the antiplatelet, the patient suffers a cardiovascular event potentially preventable by the use of antiplatelet⁶.

The concept of non-response to ASA was introduced in early 90’s, based on studies with patients with prior...
encephalic vascular accident. These studies evidenced the frequent occurrence of cardiovascular outcomes, such as nonfatal myocardial infarction, in non-responsive patients. Recent studies confirmed such findings, proving that ASA resistance determines worse prognosis even in patients with stable CAD.

The effects of this antiplatelet can be evaluated by quantification methods of ASA resistance; dosage of thromboxane A2 (TXA2) metabolism byproducts, or by quantification of the cyclooxygenase-1 (COX1) blocking degree, either by optical aggregometry, impedance or flow cytometry.

Correlation between the different methods is low, with prevalence of resistance to ASA ranging from 60% to less than 4% in different methods. Among them, optical aggregometry, which uses arachidonic acid as agonist at a concentration of 0.5 mg/mL, is the test less influenced by laboratory and genetic variability. Thus, today it is the gold standard for the study of ASA resistance. Nevertheless, the intratest variability is great in most tests, occurring to a lesser extent with blood dosage of TXA2.

In the study with stable CAD patients, resistance to the use of ASA defined by VerifyNow test, in the mean follow-up of 200 days, determined twice the chance of occurrence of the following outcomes requiring hospitalization: cardiovascular death, acute myocardial infarction (AMI), stroke, transient ischemic attack or unstable angina. In CHARISMA clinical trial, the baseline urinary concentrations of 11-dehydrothromboxane B2 (11-DH-TXB2), a derivative of TXA2 production in the highest quartile, has been related to the worst clinical outcome compared to patients with 11-DH-TXB2 dosage in the lowest quartile.

Despite the importance of ASA resistance in patients with stable CAD undergoing elective PCI, there is still no full understanding of the factors related to the occurrence of a high platelet reactivity to ASA in this population.

Therefore, the purpose hereof is to evaluate the prevalence and ASA resistance related factors in patients with stable coronary artery disease undergoing elective PCI.

**Methods**

Cohort study with 198 patients using ASA and clopidogrel, undergoing elective PCI between January 2007 and January 2010, at the Hemodynamics Service of quaternary hospital in southern Rio de Janeiro.

This study was approved by the Committee for Ethics in Research of Casa de Saúde São José, under no. 4351/20131113404 in Plataforma Brasil (Brazil Platform). Since this is a retrospective and observational study, no informed consent form was signed.

Inclusion criteria: 1) prolonged use (>5 days) of ASA (100 mg/day), clopidogrel (75 mg/day); 2) angioplasty with stent placement of at least one vessel, and angiographic success in covered injuries; 3) platelet aggregation, one hour after the procedure.

Exclusion criteria: 1) use of anticoagulant or other antiplatelet; 2) hematocrit <30%, and platelet count <100,000 cells/mm³.

Patients (coming from their homes) were admitted by the coronary unit staff, which took notes on their relevant clinical data and performed the preparation for angioplasty (venoclysis and shaving). Angioplasties were performed in the catheterization unit Siemens Artis Zee (Siemens ArtisZee, Siemens, Wittelsbacherplatz, Munich, Germany) always in the presence of the main hemodynamicist, an auxiliary hemodynamicist, an anesthetist, a nurse and a hemodynamic technician. The responsible hemodynamicist and the assistant physician.
of the patient chose the catheter introduction pathway and the caliber of the sheath used; they defined what injury would be addressed, the technique used in angioplasty, the stent type and number. Following the institution’s protocol, 10,000 units of unfractionated heparin were administered during the procedure. After the angioplasty, the patient returned to the coronary unit to undergo ECG and blood testing within one hour after the procedure (including a sample for the aggregation study).

Platelet aggregation was evaluated by optical aggregometry using the four channel aggregometer Chronolog 470VS® (Chronolog, Havertown, PA, USA). Resistance to acetylsalicylic acid was evaluated using the arachidonic acid (AA) as agonist, at a concentration of 0.5 mg/mL (Helena Platelet Aggregation System, Helena Laboratories Corp, Beaumont, TX, USA). ASA resistance was defined by a percentage of basal aggregation >20%. C-reactive protein dosage (Siemens Laboratory Diagnostics, Newark, Delaware, USA) was evaluated in a sample collected one hour after angioplasty, at a concentration measured in mg/L.

Clinical and laboratory data were collected through the review of physical and electronic records of patients, by means of a standardized collection form. Angiographic data were also collected in a standardized form, through the review of angioplasty films. This review was blindly and independently made by two experienced hemodynamicists. Any disagreement found was compared to the exam’s official report. Objective technical data of the procedure (e.g. stent length and type, procedure duration and inflation pressure) were collected directly from the official reports of angioplasties performed by the main investigator.

Continuous variables were expressed as mean ± standard deviation when normally distributed, and as mean and interquartile range when not. Kolmogorov test was applied to check the presence of normal distribution of these variables. The Student t test was applied to compare the parametric variables, and the Mann-Whitney test was applied to compare nonparametric variables. Categorical variables were expressed in percentage, and compared by either the chi-square test or Fisher’s exact test. The SPSS (Statistical Package for the Social Sciences) software, version 17.0, performed the statistical analysis.

Results

Thirteen patients were resistant to ASA, representing 6.56% of the cohort (13/198). The most found aggregation value was 4, averaging 7.7±11.4, and median of 5. First and third quartile were 3 and 8, respectively.

Mean age of resistant patients: 64.4±12.0 years. Of the 13 resistant patients, 69.23% had hypertension, 23.0% had diabetes, and 76.9% had dyslipidemia. The occurrence of all clinical characteristics analyzed was similar between resistant and non-resistant patients, with no statistically significant difference (Table 1).

The use of statins, calcium channel blockers and proton pump inhibitor have not been decisive for greater or lesser chance of ASA resistance diagnosis.

Among ASA resistant patients, none had hemoglobin drop >2 mg/dL; smaller drops occurred in 84.62% of patients. More complex injuries (C-type) were observed in 84.62% of the resistant patients. One, double and triple-vessel coronary artery disease occurred in 15.38%, 30.77% and 53.85% of patients, respectively. Resistance to ASA was not a predictor of any of these characteristics.

As for laboratory data, blood glucose and platelet levels were not associated with the occurrence of ASA resistance. It was observed, however, a statistically significant association between ASA resistance and greater CRP values (p=0.02).
Discussion

Hamberg et al.\textsuperscript{17} and Smith et al.\textsuperscript{18}, in 1974, discovered the role of prostaglandins, particularly of one of their derivatives: thromboxane A2 in thrombus formation. Weiss et al.\textsuperscript{19}, O’Brien\textsuperscript{20}, Zucker and Peterson\textsuperscript{21} have defined the antiplatelet action of ASA, a non-steroidal anti-inflammatory, inhibitor of TXA2 formation, and initially used only as analgesic. Studies by Fitzgerald et al.\textsuperscript{22} and Patrignani et al.\textsuperscript{23} defined the ASA mechanism in blocking the synthesis of TXA2. They have also determined that using 70-100 mg/day would be enough to inhibit the most selective platelet COX1\textsuperscript{22,23}.

Over time, it was found that the use of ASA was critical in the treatment of several medical conditions related to atherosclerosis. In a meta-analysis from 2002\textsuperscript{24}, the authors reviewed 195 clinical trials testing the use of AAS vs. placebo in more than 135,000 patients with several clinical conditions (acute coronary syndrome, encephalic vascular accident, transient ischemic attack, atrial fibrillation); most patients had known CAD, showing a 22.0% relative risk reduction in the composite outcome consisting of nonfatal AMI, nonfatal EVA, and cardiovascular death. Given the great importance of ASA, studies have emerged to

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|}
\hline
 & ASA resistant & ASA non-resistant & p-value \\
\hline
Study population n (%) & 13 (6.6) & 185 (93.4) & \\
\hline
Male n (%) & 10 (77) & 128 (69.2) & 0.758 \\
\hline
Age in years (mean±SD) & 64.4±12.9 & 66.8±10.9 & 0.533 \\
\hline
Weight in kg (mean±SD) & 82.0±12.6 & 77.2±15.1 & 0.144 \\
\hline
AMI n (%) & 2 (23.1) & 33 (17.8) & 0.709 \\
\hline
CABG n (%) & 1 (7.7) & 27 (14.6) & 0.698 \\
\hline
PCI n (%) & 5 (38.5) & 57 (30.8) & 0.55 \\
\hline
Stroke n (%) & 0 (0.0) & 7 (3.8) & 1 \\
\hline
SAH n (%) & 9 (69.2) & 135 (73.0) & 0.753 \\
\hline
DM n (%) & 3 (23.1) & 33 (17.8) & 0.709 \\
\hline
Dyslipidemia n (%) & 10 (76.9) & 131 (70.8) & 0.761 \\
\hline
COPD n (%) & 1 (7.7) & 4 (2.2) & 0.219 \\
\hline
Smoking n (%) & 4 (30.8) & 78 (42.2) & 0.42 \\
\hline
Statin use n (%) & 9 (69.2) & 123 (66.5) & 1 \\
\hline
PPI use n (%) & 0 (0.0) & 23 (12.4) & 0.176 \\
\hline
Ca++ blocker use n (%) & 2 (15.4) & 17 (9.19) & 0.361 \\
\hline
Blood glucose (mg/dL) (mean±SD) & 112.0±14.3 & 105.0±8.8 & 0.12 \\
\hline
CRP (mg/L) (mean±SD) & 3.2±2.2 & 0.8±0.6 & 0.02 \\
\hline
Platelets (cells/mm³) (mean±SD) & 205,0±66.7 & 213.3±61.2 & 0.639 \\
\hline
Hemoglobin (mg/dL) (mean±SD) & 13.3±1.7 & 12.8±2.3 & 0.093 \\
\hline
\end{tabular}
\caption{Univariate analysis: resistance to AAS vs. clinical data}
\end{table}

ASA – acetylsalicylic acid; AMI – acute myocardial infarction; CABG – coronary artery bypass graft surgery; PCI – percutaneous coronary intervention; SAH – systemic arterial hypertension; DM – Diabetes mellitus; COPD – chronic obstructive pulmonary disease; PPI – proton pump inhibitor; CRP – C-reactive protein; SD – standard deviation
improve antiplatelet therapy with this drug. Among the several points raised, understanding the importance of resistance to ASA still remains a major topic for many studies.

In a study on the resistance to ASA, 326 patients of a stable CAD cohort, taking 325 mg/day of aspirin for at least seven days, were tested through optical aggregometry using adenosine and arachidonic acid as agonists. In that study, the resistance was detected in 5.2% of patients, who showed higher rates of composite outcome consisting of death, AMI and EVA in 1.9 years (OR: 4.14). In this study, the prevalence of resistance to ASA was 6.56%, a value close to others found in medical literature; however, the degree of adherence was not evaluated. But a study that evaluated ASA resistance before and after a period of monitoring the adherence to treatment did not detect differences in resistance rates in both periods evaluated.

Such non-response can be explained by several possible reasons. Although they do not yet have their role on ASA resistance well established, some genetic characteristics seem to explain some cases of non-response. In an in vitro resistance analysis through optical aggregometry with arachidonic acid as agonist, patients undergoing elective PCI showed 3.4% of resistance to the use of ASA (7/203). However, of resistant patients, 6 admitted not using ASA as recommended, i.e., only 1 (0.4%) proved to be truly resistant. The use of enteric-coated aspirin increases the chance of a patient being identified as resistant after a first dose, but this chance almost becomes void if the patient does prolonged use of such aspirin formulation.

In a study evaluating the resistance to ASA in 900 patients with stable CAD, through VerifyNow® test and Multiplate Analyzer®, type 2 diabetes, previous myocardial infarction, smoking, and previous coronary bypass surgery were independent determinants for the occurrence of resistance to ASA. These relations were not observed in this study, which used the optical aggregometry, still the gold standard for the study of ASA resistance.

The relationship of inflammation with resistance to ASA is still an emerging field of study. Larsen et al. revealed a relationship between higher levels of C-reactive protein and the highest tertile resistance evaluated by VerifyNow® and Multiplate Analyzer® tests. This cohort also observed that higher levels of CRP are related with greater chance of ASA resistance. High levels of CRP determine the worst prognosis in patients suffering from cardiovascular diseases with atherothrombotic pathophysiology, but the exact explanation of this is still controversial. The association of high levels of CRP with resistance to ASA, a drug of widely recognized benefit in preventing outcomes among that profile of patients, can raise a hypothesis for understanding the role of inflammation as a determinant of poor prognosis of atherothrombotic cardiovascular diseases.

This study has some limitations: its retrospective character reduces the statistical power of associations found; it was developed based on data from a single institution, limiting the ability to generalize any conclusion here found. In addition, there has been no evaluation of inter and intraobserver variability in the collection of angiographic data, although this collection has been made by experienced hemodynamicists. Nevertheless, the study provides information that add up to the knowledge about the factors associated with variability in response to ASA in patients with stable CAD.

Conclusions
The prevalence of ASA resistance in patients undergoing elective percutaneous coronary intervention is low; high levels of CRP relate to greater frequency of such resistance. Further studies are needed to better understand the associations found in this study.

Potential Conflicts of Interest
No relevant potential conflicts of interest.

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References


