Relationship between Inflammation and Aspirin and Clopidogrel Antiplatelet Responses in Acute Ischemic Stroke

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Objective: We measured serum levels of proinflammatory/prothrombotic markers P-selectin, CD40L, matrix metalloproteinase 9 (MMP-9), intracellular adhesion molecule 1 (ICAM-1), and interleukin (IL)-6 in ischemic stroke patients, correlating their levels with the results of aspirin (ASA) and clopidogrel antiplatelet responses, using 3 "point of care" platelet function instruments, thromboelastograph (TEG), Accumetrics (ACU), and impedance aggregometer (IMP). *Methods:* Patients on chronic ASA regimen at the time of stroke were switched to 300 mg clopidogrel loading dose and 75 mg clopidogrel maintenance dose. Serum levels of the aforementioned inflammatory mediators were measured in 51 patients at baseline (on ASA regimen), and at 26 ± 5 hours and 64 ± 18 hours postclopidogrel administration by enzyme-linked immunosorbent assay. *Results:* P-selectin, CD40L, and MMP-9 serum levels were reduced; ICAM-1 and IL-6 serum levels showed no difference postclopidogrel administration relative to baseline. Patients' stratification based on ASA dose showed more significant reductions in P-selectin, CD40L, and MMP-9 serum levels postclopidogrel administration in patients who were on baseline 81 mg ASA, as compared to patients on 325 mg ASA. Measurement with TEG was sensitive for correlating ASA antiplatelet responses to serum levels of inflammatory markers, whereas measurements with ACU and IMP were sensitive for correlating clopidogrel antiplatelet responses to serum levels of inflammatory markers. *Conclusion:* Clopidogrel exerts both platelet-dependent and platelet-independent anti-inflammatory effects. The association between platelet function and inflammation depends on the platelet function analyzer, the type of antiplatelet agent, the nature of the inflammatory marker, and the time of measurement relative to the time of drug administration. **Key Words:** Antiplatelet agents—inflammatory markers—platelet function analyzer—point of care.

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Introduction

Platelet aggregation plays a critical role in atherosclerosis and the formation of thrombus, leading to ischemic stroke. Antiplatelet agents such as salicylic acid (aspirin [ASA]) and clopidogrel are used for the prevention of stroke. However, up to 40% of patients experience recurrent ischemic stroke while receiving ASA.[1] These patients are often switched to the thienopyridine derivative, clopidogrel. Nevertheless, the antiplatelet response to clopidogrel is highly variable.[2]

Factors contributing to poor response to antiplatelet therapy include cellular and clinical factors,[3] high

baseline platelet reactivity,[4] and patients' compliance.[5] In addition, genetic polymorphism in platelet receptors such as the P2Y12, glycoprotein (GP) IIb/IIIa, GPIa/IIa, GPIb/IX/V, and the cytochrome P450 family of genes have been shown to be associated with poor response to antiplatelet therapy.[6] Furthermore, the type of agonist used to measure platelet function and different cutoff values[7] could determine the response to an antiplatelet therapy.

Inflammation is also known to contribute to stroke occurrence.[8] Platelet dysfunction in stroke patients has been shown to be more prevalent among those who present with high systemic inflammation.[9] The systemic inflammation, in turn, has the potential to influence the response to antiplatelet therapy.[10]

This study measured serum levels of platelet-dependent (P-selectin and CD40L) and platelet-independent (matrix metalloproteinase 9 [MMP-9], intracellular adhesion molecule 1 [ICAM-1], and interleukin [IL]-6) proinflammatory/prothrombotic mediators in acute ischemic stroke patients at baseline (on ASA regimen) and 24-96 hours postclopidogrel 300 mg loading dose and 75 mg maintenance dose. Furthermore, we analyzed the correlation between serum levels of these markers and ASA and clopidogrel antiplatelet responses, measured in the previous study, using 3 "point of care" platelet function analyzers, thromboelastograph (TEG) hemostasis, Accumetrics (ACU) VerifyNow, and impedance aggregometer (IMP) Chronolog 570VS.[7]

Subjects and Methods

Study Population

Patients who presented to the Emergency Stroke Center, Buffalo, New York, less than 24 hours after an acute ischemic event were recruited into the study. The clinical diagnosis of ischemic stroke was validated by either computed tomography or magnetic resonance imaging. The study duration was 19 months. Subjects were eligible if they were on a chronic dose of 81-325 mg ASA, but were switched to clopidogrel on the advice of the attending physician upon arrival to the hospital. While being treated with clopidogrel, the ASA regimen was discontinued. The data on serum levels of inflammatory markers were available for 51 patients (61% male), with a mean age of 72 ± 12 years (40-89). The patients' clinical demographics are described in Table S1.

Upon arrival to the emergency room, 31 patients (61%) were on a chronic 81 mg ASA regimen, while the remaining 20 (39%) were on a 325 mg ASA regimen. All patients received an oral maintenance dose of 75 mg clopidogrel 12-24 hours after a 300 mg clopidogrel loading dose. Exclusion criteria were as follows: patients treated with antiplatelet agents such as ticlopidine or Aggrenox within 1 month before the event; patients currently on or treated in the emergency room with GPIIb/IIIa inhibitors, nitrates or related drugs, warfarin, or heparin; and patients identified as suitable for thrombolytic drugs.

We could not measure ASA compliance; although we initially started to do so by using a specific urine test, we discontinued this due to ethical considerations related to patient discomfort in obtaining urine.

Blood Sampling

Blood sampling was obtained at baseline (on ASA regimen) and postclopidogrel. The postclopidogrel blood sampling was obtained at both 26 ± 5 hours after baseline sampling and 64 ± 18 hours after baseline sampling (before patient discharge). Peripheral blood samples were drawn from an indwelling catheter. Serum was stored for the analysis of the 5 inflammatory markers, whereas citrated blood and heparin tubes were used to measure platelet function by ACU, IMP, and TEG. The selection of serum for analysis, rather than plasma, was adopted to determine ASA and clopidogrel antiplatelet effects.

The study was approved by the Institutional Review Board at the State University of New York at Buffalo, and consent was obtained from each patient or family member before blood was drawn.

Platelet Function Analyzers

The detailed description of the 3 platelet function analyzers, the principle behind their operation, and the definition of poor response to antiplatelet therapy are discussed in Appendix S1.

Measurement of Inflammatory Markers

The measured markers included platelet activation markers, P-selectin and CD40L; the marker of blood–brain barrier disruption, MMP-9; the adhesion molecule, ICAM-1; and the proinflammatory cytokine, IL-6. The inflammatory markers were measured by enzyme-linked immunosorbent assay (R&D Systems), according to the manufacturer's instructions. The sensitivity ranges for P-selectin, CD40L, MMP-9, ICAM-1, and IL-6 were .82 ng/mL, 62.5 pg/mL, .31 ng/mL, 1.56 ng/mL, and 3.1 pg/mL, respectively.

Statistical Analysis

All statistical analyses were performed using SPSS 14.0 for Windows (SPSS Institute, Chicago, IL). The nonparametric Wilcoxon signed-rank test was used to measure the significant changes in the serum levels of inflammatory markers from baseline to postclopidogrel treatment. The results were adjusted for multiple comparison using the Benjamini and Hochberg method.[11]

Regression analysis determined the association between serum levels of inflammatory markers and platelet inhibition. Pearson correlation was used to examine the relationship between serum levels of inflammatory markers