Moderate Alcohol Consumption is Associated with Decreased Platelet Activity in Patients Presenting with Acute Myocardial Infarction

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Abstract. Moderate alcohol consumption (MAC) and platelet inhibition have been independently associated with a reduced risk for the development of acute myocardial infarction (AMI). The effects of MAC on the initial platelet status in patients presenting with AMI are not elucidated. Here we sought to define the effects of MAC on platelet characteristics in AMI patients before applying any reperfusion strategies. The study was designed as an analysis within the cohort study in 23 patients with AMI enrolled in the GUSTO-III. Platelets were investigated by different techniques, including aggregometry, flow cytometry, and ELISA. MAC patients exhibited mild, but consistent, inhibition of platelet aggregability, surface receptor expression, and released substances as compared to non-alcohol consuming patients. These differences were significant for 5 μM ADP (p = 0.04), 10 μM ADP-induced aggregation (p = 0.02); P-selectin (p = 0.01), and PECAM-1 (p = 0.02) platelet-bound expression. Our study confirms that moderate alcohol consumption is associated with diminished platelet activation in patients presenting with AMI. The ability of MAC to favorably modulate the pre-reperfusion platelet status in such patients is of clinical importance, and further investigation in large-scale clinical trials seem warranted.

Key Words. alcohol, platelets, acute myocardial infarction, human

Recent epidemiological studies have suggested that moderate alcohol consumption (MAC) is associated with a reduced risk for the development of coronary artery disease, angina pectoris, and acute myocardial infarction (AMI) [1,2]. The important role of platelets in ischemic cardiovascular disease has received increasing recognition. Inhibition of platelet aggregability remains a critical target for pharmacological interventions in patients with coronary artery disease, particularly in those with acute coronary syndromes including AMI [3,4]. There is substantial evidence from in vitro and animal studies that impaired platelet function occurs after thrombolytic therapy [5–7]. Intravascular platelet activation may limit reperfusion, or cause reocclusion of the recanalized infarct-related arteries, thus resulting in an overall decreased effectiveness of thrombolytic therapy [8,9].

It is also well established that alcohol, in physiologically relevant concentrations, has direct impact on platelets [10–13]. However, the effects of MAC on the baseline platelet status in patients presenting with AMI are not elucidated. The setting of a controlled clinical trial could be particularly useful to investigate this issue. The purpose of the present study was, therefore, to analyze the association of the alcohol drinking habits with the baseline platelet-related characteristics in patients presenting with AMI, who were later enrolled in the GUSTO-III trial. Our hypothesis was that social alcohol drinking can favorably influence platelet status in such patients.

Methods

Patients

Twenty three consecutive patients admitted to the emergency department between July and December of 1996 with a diagnosis of acute myocardial infarction (AMI) were included. All patients were enrolled in the randomized trial of reteplase (n = 13) versus accelerated alteplase (n = 10) for the treatment of acute myocardial infarction, (GUSTO-III trial). The inclusion criteria were previously reported [14]. In summary, patients of any age who presented within 6 hours of
symptom(s) onset, with more than 30 minutes of continuous symptoms of AMI, and who by 12-lead electrocardiogram had demonstrated at least 1 mm of ST segment elevation in 2 or more limb leads, or at least 2 mm ST segment elevation in 2 or more contiguous precordial leads, or bundle branch block were included in this trial. Patients were excluded if they had a history of bleeding diathesis, stroke, major surgery or significant trauma in the past six weeks, and hypertension above 200/110 mm Hg. Alteplase is a serine protease which has the property of fibrin-enhanced conversion of plasminogen to plasmin. It also produces limited conversion of plasminogen in absence of fibrin. Reteplase is a novel recombinant plasminogen activator which lacks carbohydrate side chains but contains the kringle 2 and protease domains of natural plasminogen activator.

Information on alcohol consumption was obtained before the beginning of the study protocol. MAC patients were defined as subjects reporting consumption of any alcoholic beverage regularly (at least once weekly) during the preceding year. Information about quantity and frequency of consumption was used to estimate the average amount of pure alcohol per day. Blood samples for platelet aggregation, for flow cytometric studies, and enzyme-linked immunosorbent assay, were taken in the emergency department immediately before administration of the thrombolytic therapy. To avoid possible observer bias, blood samples were coded and blinded. Sampling procedures and platelet studies were performed by individuals unaware of the protocol.

**Platelet aggregation**

Citrate and whole blood were immediately mixed 1:9 and then centrifuged at 1200 g for 2.5 minutes in order to obtain platelet-rich plasma (PRP) which was kept at room temperature for use within 1 hour. Platelet counts were determined for each PRP sample with Coulter Counter ZM (Coulter Co., Hialeah, FL). Platelet numbers were adjusted to 3.50 × 10^8/ml with homologous platelet-poor plasma. PA was induced by 5 μM ADP; 10 μM ADP; 1 μg/ml collagen; 1 mg/ml thrombin, and 1.25 mg/ml ristocetin. All agonists were obtained from Chronolog Corporation (Hawertown, PA, USA). PA studies were performed using a Chronolog Whole Blood Lumi-Aggregometer (model 560-Ca). PA was expressed as the maximum percentage of light transmittance change from the baseline using platelet-poor plasma as a reference at the end of the recording time. PA curves were recorded for 4 minutes and analyzed according to internationally established standards [15].

**Flow cytometry**

Procedures were described in detail previously [16,17]. Briefly, venous blood (8 ml) was collected in a plastic tube containing 2 ml of acid-citrate-dextrose (ACD) (7.3 g citric acid, 22.0 g sodium citrate × 2 H2O and 24.5 g glucose in 1000 ml distilled water) and mixed well. The blood-ACD mixture was centrifuged at 1000 r.p.m. for 10 minutes at room temperature. The upper 2/3 of the platelet-rich plasma (PRP) was then collected and adjusted to pH = 6.5 by adding ACD. The PRP was then centrifuged at 3000 r.p.m. for 10 minutes. The supernatant was removed and the platelet pellet was gently resuspended in 4 cc of the washing buffer (10 mM Tris/HCl, 0.15 M NaCl, 20 mM EDTA, pH = 7.4). Platelets were washed four times in the washing buffer, and an additional four times in TBS (10 mM Tris, 0.15 M NaCl, pH = 7.4). All cells were then divided into ten plastic capped tubes. Nine portions of washed platelets were incubated with 5 μl fluorescein isothiocyanate (FITC)-conjugated antibodies in the dark at +4°C for 30 minutes, and one part remained unstained and served as a negative control. Surface antigen expression was measured with monoclonal murine anti-human antibodies: CD9 (p24); CD41a (IIb/IIIa, α/β3); CD42b (Ib); CD61 (IIIa) (DAKO Corporation, Carpenteria California, USA); CD49b (VLA-2, or α/β3); CD62p (P selectin); CD31 (PECAM-1); CD 41b (Ib); and CD51/CD61 (vitronectin receptor, α/β3) (Pharmingen, San Diego California, USA). After incubation, the cells were washed three times with TBS and resuspended in 0.25 ml of 1% paraformaldehyde. Samples were stored in the refrigerator at +4°C, and analyzed on a Becton Dickinson FACScan flow cytometer with laser output of 15 mW, excitation at 488 nm, and emission detection at 530 ± 30 nm. The instrument was calibrated daily with fluorescence beads (CalIBRITE; Becton Dickenson) and measured FITC-conjugated fluorescence intensity. All parameters were obtained using four decade logarithmic amplification. The data was collected and stored in list mode, and then analyzed using CELLQuest™ (version 1.2.2) software.

**ELISA**

Platelet poor plasma was obtained by centrifugation at +4°C, at 3000 g for 10 minutes. Samples were stored at −80°C before final determination. Enzyme-linked immunosorbent assays (ELISA) for PECAM-1 (Bender MedSystems, Vienna, Austria), and P-selectin (Centocor, Inc., Malvern, Pennsylvania, USA) were used according standard techniques. Each sample was measured in duplicate.

**Statistical analysis**

A post hoc t-test comparison using the Bonferroni correction was performed to identify specific differences in aggregation, platelet-bound receptor expression, and plasma level of soluble substances between the AMI groups. A Mann-Whitney U test was used to analyze non-parametric data. Normally distributed data are expressed as mean ± SEM; and p < 0.05 was considered significant. Differences between individual flow cytometric histograms were assessed using the Smirnov-