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Ultrastructural changes and immunolocalization of P-selectin in platelets from patients with major depression

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ABSTRACT

Depression is considered an important risk factor in patients with cardiovascular disease (CVD). Although the biological mechanism is unknown, it has been suggested that hyperactivity of platelets may have an important role in the onset and evolution of cardiovascular damage. The goals of this study were to evaluate by transmission electron microscopy and immunohistochemistry the presence of ultra-structural variations in platelets from individuals with recent diagnosis of major depression disease (MDD, patients without previous anti-depressant treatment and from healthy control subjects.). Platelets from depressed patients had a greater proportion of dendritic forms compared with those obtained from control subjects. Morphological changes, such as dilation of open canalicular and dense tubular systems, platelet vacuolization, electrodense pattern of membranes, and a different immunolocalization of P-selectin were observed in the platelets from depressed patients compared with those isolated from healthy subjects. Our results revealed ultra-structural changes in platelets isolated from patients with MDD suggestive of enhanced platelet activation.

1. Introduction

Depression is a major risk factor for cardiovascular morbidity and mortality (Musselman et al., 1998; Nair et al., 1999; Carney et al., 2002). It has been suggested that the increased mortality related to depressive states after a myocardial infarction may be induced by an increased activation of the hypothalamic–pituitary–adrenal axis (Stokes, 1995), a possible cardiotoxicity due to side effects of antidepressant drugs (Dziukas and Vohra, 1991; McKinney and Rasmussen, 2003; Von Kanel, 2004), or abnormalities in platelet function (Markovitz and Matthews, 1991; Carney et al., 2002).

It has been hypothesized that enhanced platelet activation could be the mechanism responsible for a high proportion of complications in patients suffering from ischemic diseases concurrently presenting a major depressive disorder (MDD) (Musselman et al., 1996, 1998; Nair et al., 1999; Nemeroff and Musselman, 2000; O'Connor et al., 2000; Ramasubbu, 2000). However, studies on functional platelet evaluation in depressed patients have produced contradictory results. Musselman et al. (1996) reported the presence of depression-related platelet activation measured by flow cytometry using antibodies directed against αIIbβ3 integrin and P-selectin. In addition, Lederbogen et al. (2001) and Shimbo et al. (2002) found that depressed patients presented enhanced platelet aggregation in response to ADP, thrombin, and serotonin stimulation assessed with platelet aggregometry. In contrast, Maes et al. (1996) found no differences in platelet aggregation, prothrombin time, or activated partial thromboplastin time between normal and depressed subjects, and Nugent et al. (1994, 1995) reported reduced platelet aggregation in depressed subjects, suggesting the presence of inhibitory plasma factors.

To our knowledge, only two studies have previously evaluated the ultrastructural characteristics of platelets from depressed subjects, with conflicting results (Kiktenko et al., 1992; Palmar et al., 1997). Since it is possible that major depression may affect platelet function, we conducted a study to evaluate depression-elicited changes in platelets using transmission electron microscopy. To analyze whether platelets from depressed subjects presented ultrastructural changes, their morphology was compared with that from healthy controls. In addition, the platelet functional state was indirectly evaluated

Abbreviations: MDD, Major depressive disorder; MINI, Mini International Neuropsychiatric Interview; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders; PRP, Platelet-rich plasma; TBS, Tris buffered saline; CAD, Coronary artery disease; CVD, Cardiovascular disease; ATP, Adenosine triphosphate.

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through the assessment of the immunocytochemical localization of P-selectin, a well-known marker of platelet activation (Walsh et al., 2002; Andre, 2004).

2. Methods

2.1. Study population

All participants live in Mexico City and have a Mexican-Mestizo ethnic background. We included patients with major depressive disorder (MDD) in accordance with the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, American Psychiatric Association, 1994), and based on evaluation with the clinician-rated 17-item Hamilton Depression Rating Scale Score (Hamilton scale) (Hamilton, 1967). Patients were seen at the outpatient clinic of the Instituto Nacional de Psiquiatría "Ramón de la Fuente" in Mexico City. Diagnosis of MDD was made by a psychiatrist using the validated Spanish version of the Mini-International Neuropsychiatric Interview (MINI) (Heinze, 2000), a standardized diagnostic interview based on DSM-IV criteria. They were further screened to rule out past and present neurological, psychiatric, or substance abuse problems. With the exception of anxiety, there were no other comorbidities in the depressed group, including those assessed by the MINI interview (i.e. dysthimic disorder, suicidal risk, manic and hypomanic episodes, panic disorder, agoraphobia, social phobia, obsessive-compulsive disorder, post-traumatic stress disorder, abuse and alcohol dependence, abuse and dependence on other substances, psychotic disorders, mood disorders with psychotic features, eating disorders, generalized anxiety disorders, and antisocial personality disorder). Patients were not included if they refused to participate, or if they had any of the following conditions: high suicidal risk, pregnancy, lactation, bipolar disorder, active smoking or alcohol consumption, hyperlipidemia, diabetes, use of non-steroidal anti-inflammatory or antidepressant drugs, and depression secondary to a medical condition. Non-depressed controls with similar characteristics to the patients were recruited (age, sex, social and demographic characteristics, etc.). The MINI interview confirmed that they did not suffer from any other mental disorder.

All controls and patients were free of medication for at least 1 year before to inclusion in the study. Additionally, all individuals underwent evaluation (complete blood count, full biochemical profile, urinalysis, thyroid function tests, and electroencephalogram) to rule out concurrent medical conditions. Other non-exclusion criteria included low coffee (two cups/day) and alcohol (three glasses/week) consumption. The protocol was conducted in accordance with the Helsinki declaration and with the approval of the institutional Ethics Review Board. All participants signed a written informed consent.

2.2. Preparation of samples for electron microscopy

Blood samples (10 ml) were collected between 8:00 and 9:00 a.m. after overnight fasting in Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) containing 1.5 ml of ACD solution (22 g/l trisodic citrate, 8 g/l of citric acid, and 24.5 g/l dextrose) as anticoagulant. The blood samples were centrifuged at 180*g* for 15 min. The platelet-rich plasma thus obtained was re-centrifuged at 2500×*g* for 10 min to obtain a platelet pellet that was fixed in Karnovsky solution (4% paraformaldehyde, 2.5% glutaraldehyde), subsequently placed in 2% osmium tetraoxide solution, and gradually dehydrated with alcohol and propylene oxide. The platelets were embedded in EPON-ARALDITE resin, and included in BEEN capsules. Ultrathin sections (90 nm) were obtained using an MT-7000 RMC Ultramicrotome (RMC Products, Boeckeler Instruments Inc., Tucson, AZ), and contrasted with uranyl acetate and lead citrate.

2.3. P-selectin immunocytochemistry

P-selectin immunocytochemistry was performed, with slight modifications, as previously described (Bendayan et al., 1987; Merighi, 1992). Briefly, 90-nm sections were pre-incubated for 30 min in 1% bovine serum albumin, 0.1% Triton X-100, and 1:10 human serum in TBS buffer (0.05 M, pH 7.6). Then, they were incubated with 1:50 rabbit anti-human P-selectin antibody (R&D Systems Inc., Minneapolis, MN). After washing, sections were incubated with 1:25 colloidal gold-conjugated polyclonal anti-rabbit antibody (Amersham Biosciences Ltd., Buckinghamshire, UK). Finally, the samples were washed again, dried, and contrasted with uranyl-lead.

2.4. Platelet sample evaluation

Platelet sections were evaluated using a transmission electron microscope EM900 (Carl Zeiss AG, Oberkochen, Germany). Morphology was assessed using microphotographs (7000×) of equivalent areas. Quantification and localization of P-selectin positive binding spots were determined by direct observation of 100 platelets in each studied sample at 20000×. All evaluations were conducted by two independent observers without knowledge of group assignment, and major disagreements were resolved by consensus.

2.5. Statistical analysis

Data were analyzed using Microsoft Excel XP (Microsoft Corp. Redmond, WA) with an add-in statistical software package (Analyse-it, version 1.67. Analyse-it software LTD., Leeds, UK). Data are presented as median and interquartile range (IQR). Mann-Whitney *U*-tests were used for group comparison; a 2-sided *P*-value$<$0.05 was considered as statistically significant.

3. Results

3.1. Patients

We included 10 patients (1 man, 9 women, median age 28 years; range, 18–51) and 10 normal controls (1 man, 9 women, median age 24 years; range, 18–48). Among depressed patients, the minimum total score on the Hamilton Depression Rating Scale was 22 (mean score 24.2 ± 1.98) implying the presence of major depression with moderate to severe intensity. The laboratory results of complete blood counts, biochemical profile, urinalyses, and thyroid function tests did not show significant differences between groups.

3.2. Platelet morphology

Platelets from depressed subjects showed different densities in their somas (Fig. 1), and a low percentage of discoid shapes (2.8%; IQR 2.1–4.1) (Table 1), whereas platelets from control subjects showed a similar density among them, and a high percentage of discoid shapes (14.3%; 14.0–15.4) (Table 1). The proportion of discoid platelets was significantly lower among depressed subjects ($U=2.0$; $P<0.001$) (Table 1). In addition platelet vacuolization and a severe dilation of the open canalicular and dense tubular systems were frequently found in platelets from depressed patients as compared with those from controls (Fig. 1A, B, C). Platelets from depressed patients showed an electrodense pattern of the mitochondrial and cell membranes suggestive of an activation process. Such a pattern was not observed in platelets from control subjects (Fig. 1D). No differences were observed between patients and controls in the number of alpha granules ($U=16.0$; $P=0.093$), dense bodies ($U=22.5$; $P=0.368$), or mitochondria ($U=32.5$; $P=0.713$) (Table 1).

3.3. Immunocytochemical analysis

Platelets from the 10 depressed patients ($n=1000$) and the 10 controls ($n=1000$) were analyzed. The total number of P-selectin binding sites did not show a significant difference between the two groups (Table 1). However, in platelets from depressed patients, P-selectin was more frequently localized within the open canalicular system (Fig. 1F) ($U=1.4$; $P<0.001$), whereas in platelets from control subjects P-selectin was mainly located in non-canalicular areas (Fig. 1E) (Table 1).

4. Discussion

Previous studies assessing ultrastructural changes in platelets from patients with depression are scarce. Kiktenko et al. (1992) reported the presence of pseudopodia 2 to 3 times larger than the cell body and a tendency to form aggregates in platelets from depressed subjects before receiving treatment with antidepressants; interestingly, these changes disappeared after starting such treatment. In contrast, Palmar et al. (1997) reported that platelets isolated from patients with depressive disorders showed a greater number of platelets with discoid form, as well as platelets with dilated canalicular and dense tubular systems. In addition, they found that the mitochondria in these platelets showed severe swelling. However, these authors interpreted their results as indicative of a 'quiescent state', and hypothesized that there is no secretion or transport of 5-HT in these platelets.