

Platelet Reactivity in Depressed Patients Treated With Paroxetine

Preliminary Findings

Dominique L. Musselman, MD; Ulla M. Marzec; Amita Manatunga, PhD; Suzanne Penna, BA; Andrea Reemsnyder, BS; Bettina T. Knight, BSN; Aimee Baron, BA; Stephen R. Hanson, PhD; Charles B. Nemeroff, MD, PhD

Background: Alterations in platelet reactivity have been previously posited to underlie the increased vulnerability of patients with depression to ischemic heart disease (IHD). The present study sought to determine whether the increased platelet reactivity associated with major depression is reduced after antidepressant treatment.

Methods: Patients diagnosed as having DSM-IV major depression (n=15) (mean age, 37 ± 7 years; range, 23-48 years) and 12 normal comparison subjects (mean age, 36 ± 7; range, 23-48 years) were recruited. None of the controls or depressed group had evidence of IHD; 10 of 15 patients who were depressed had 1 or more traditional IHD risk factors. In vivo platelet activation, secretion, and dose-response aggregation of the controls and patients was measured after overnight bedrest under basal conditions, and after a mild exercise challenge. After 6 weeks of open-label treatment with the selective serotonin reuptake inhibitor paroxetine (20 mg/d), the patients with depression were readmitted and procedures of the first General Clinical Research Center admission repeated.

Results: In comparison with the control group, the depressed group exhibited greater procoagulant activity as detected by increased platelet binding of the monoclonal antibodies anti-ligand-induced binding site and GA6, and increased plasma concentrations of platelet factor 4 under basal conditions. After paroxetine treatment, the patients with depression exhibited significant reductions in all 3 parameters.

Conclusions: Normalization of platelet activation is associated with paroxetine treatment of patients with depression. Because this study design did not allow for the determination of whether this effect of paroxetine on platelet function is caused by a direct effect of the drug or placebo or, alternatively, because of recovery from depression, studies containing a placebo and/or psychotherapy treatment arm may resolve this issue.

Arch Gen Psychiatry. 2000;57:875-882

From the Departments of Psychiatry and Behavioral Sciences (Drs Musselman and Nemeroff and Mss Penna, Reemsnyder, Knight, and Baron) and Biomedical Engineering (Ms Marzec and Dr Hanson), School of Medicine, and the Department of Biostatistics, Rollins School of Public Health (Dr Manatunga), Emory University, Atlanta, Ga.

IN COMMUNITY studies, major depression is a major cause of emotional dysfunction, absenteeism, diminished productivity, and self-reported physical impairment.¹⁻⁶ Moreover, several studies have revealed that patients with depressive symptoms are at increased risk for developing ischemic heart disease (IHD)⁷⁻¹³ and cerebrovascular disease,¹⁴ although some recent reports differ.^{15,16} Indeed, of patients with IHD, 16% to 23% exhibit symptoms of major depression,¹⁷⁻²⁰ with the presence of depression predictive of future cardiac complications^{9,17,21} and diminishing survival time.^{19,22,23}

The increased risk for development of IHD of patients with depressive symptoms is postulated to be in part caused by increased platelet reactivity,²⁴⁻²⁶ because platelets play a central role in atherosclerosis, thrombosis, and acute coronary syndromes.^{27,28} Still unknown is whether the increased platelet activation observed in

patients with major depression is a state or trait variable, and whether this activation exists within patients with dysthymia or other mood disorders.

In this study, we hypothesized that patients with depression who had other commonly accepted risk factors for IHD would exhibit increased platelet activation when compared with normal comparison subjects. Indeed, the well-known predisposing factors for development of coronary heart disease, including essential hypertension, elevated plasma cholesterol, older age, and smoking, contribute to serotonin-mediated platelet activation.²⁹⁻³⁴ Because our previous study had used a mild physical stress (an orthostatic challenge) to increase platelet activation from basal levels, we wished to examine whether greater physical exertion would further enhance differences in platelet reactivity between the depressed and normal comparison groups.^{35,36} Another hypothesis to be tested in the present study was whether a

SUBJECTS AND METHODS

SUBJECTS

The research protocol was reviewed and approved by the Institutional Review Board of Emory University School of Medicine, Atlanta, Ga. Study subjects were recruited from the community by newspaper advertisements or word of mouth. Potential study participants, including the normal comparison individuals, were initially screened over the telephone for the presence (or absence) of current or past psychiatric disorders by a research interviewer. After informed consent was obtained, individuals meeting entry criteria (including the normal comparison individuals) were interviewed using the Structured Clinical Interview for DSM-IV³⁹ by a research nurse (B.T.K.) to ascertain the presence (or absence) of current, comorbid, and past psychiatric disorders. The severity of depressive and anxiety symptoms of study participants was assessed by the Hamilton Anxiety Rating Scale,^{40,41} Hamilton Depression Rating Scale (HDRS),^{42,43} Carroll Rating Scale for Depression,^{44,45} and the Clinical Global Impression Scale.

Fifteen patients (7 women and 8 men) with major depression (mean \pm SD age, 37 \pm 7 years; range, 23-48 years) were recruited, as well as 12 normal comparison subjects (NCS) (6 women and 6 men) (mean \pm SD age, 36 \pm 7 years; range, 23-48 years), who were similar in age and sex to the patients who were depressed. Those patients fulfilled DSM-IV⁴⁶ criteria for major depression, with a minimum score of 18 (mean \pm SD score, 25 \pm 3; range, 20-32) on the 21-item HDRS. The normal controls exhibited HDRS scores of less than 3 and were without any current or past personal history of psychiatric disorder. These patients were without other comorbid psychiatric disorders, with the exception of 2 patients who also fulfilled diagnostic criteria for generalized anxiety disorder. All subjects were in stable medical health with no evidence of endocrine, hematologic, hepatic, renal, or neurologic disease based on physical examination and routine laboratory screenings (complete blood cell count, chemistry panel,

thyroid-stimulating hormone, urinalysis, urine drug screen, chest roentgenogram and electrocardiogram). Study subjects were without any evidence of current or past IHD, diabetes, and alcohol or other drug abuse (**Table 2**).

Patients with depression were accepted into the study if they exhibited any of the following commonly accepted risk factors for IHD: hypertension⁴⁷; obesity (body mass index [BMI], >25) (BMI was calculated as weight in kilograms divided by the square of height in meters)^{48,49}; first-degree relatives with premature heart disease or stroke (first-degree male relative, <55 years of age, and/or first-degree female relative, <65 years of age); hypercholesterolemia⁴⁹; or daily nicotine use. The normal control subjects and 5 of the patients with depression (33%) did not have any of these risk factors for IHD, whereas most (10 of 15) of the patients with depression had 1 or more: 6 had only 1 IHD risk factor (2 patients with increased BMI, 2 with hypercholesterolemia, 1 with a family history of premature IHD, and 1 who used nicotine on a daily basis); 3 had 2 IHD risk factors (2 patients used nicotine and had a family history of premature IHD and 1 exhibited hypercholesterolemia and increased BMI); and 1 patient had 3 risk factors for IHD (hypertension, increased BMI, and family history of premature IHD). Four of the patients used nicotine on a daily basis. Both the normal comparison subjects and depressed individuals had first-degree family members with psychiatric disorders.⁵⁰

To minimize any potential confounding effect on platelet reactivity, all participants were instructed to avoid alcohol and medications (eg, aspirin, nonsteroidal anti-inflammatory agents), which might alter platelet activation. The patients were without psychotropic medications for 4 weeks (8 weeks for fluoxetine) before the study.

PROCEDURES

The study was conducted at the National Institutes of Health-funded General Clinical Research Center (GCRC), at Emory University Hospital, Atlanta. At 7 AM, after an overnight bedrest, subjects underwent venous blood sampling

commonly used antidepressant, the selective serotonin reuptake inhibitor (SSRI) paroxetine, would exert beneficial effects on platelet reactivity in patients with depression. We selected paroxetine because it is the most potent SSRI available in the United States, and, in contrast to nearly all of the other SSRIs, has no active metabolite.

Platelet activation can be scrutinized by flow cytometry, by the measurement of plasma concentrations of platelet-specific proteins β -thromboglobulin (β -TG) and platelet factor 4 (PF4), and by dose-response aggregometry. Fluorescence-activated flow cytometric analysis can provide in vivo measurement of platelet function in contrast to the latter 2 indirect methods,³⁷ and demonstrates superior sensitivity to classic aggregometry techniques in detecting early platelet activation events that occur before platelet-platelet aggregation. Activation of platelets, by thrombin or through adhesion to damaged subendothelium, generally results in 3 events (**Figure 1**): (1) Activation of the membrane integrin glycoprotein (GP) IIb/IIIa, which undergoes conformational changes to cre-

ate functional membrane receptors for fibrinogen. Calcium-dependent interplatelet linkage may then form between the activated receptors and bivalent fibrinogen to form platelet aggregates. The conformational change in GP IIb/IIIa is evidenced by the expression of new epitopes that can be detected by specific monoclonal antibodies (mAbs), such as PAC1 (which competes with fibrinogen), and anti-LIBS (which detects the ligand-induced binding site). (2) Secretion of proaggregatory mediators, including adenosine diphosphate (ADP), adenosine triphosphate (ATP), PF4, β -TG, thromboxane A₂, fibrinogen, and thrombospondin. Secretion of these mediators is followed by the translocation of α -granule membrane protein P-selectin (CD62) to the platelet surface (detected by mAb GE12 or GA6). (3) The exposure of the anionic phospholipid phosphatidylserine at the outer platelet surface membrane. Phosphatidylserine promotes assembly of 2 enzyme-substrate complexes of the coagulation cascade, the tenase complex (IXa, VIII, X, and calcium) and the prothrombinase complex (prothrombin, V_a, X_a, and calcium), which result in gen-

from an antecubital vein in 1 arm. Peripheral blood samples were drawn by clean venipuncture with a 21-gauge butterfly infusion set (Miniset; Baxter International Inc, Deerfield, Ill) with minimal tourniquet. After their first blood drawing, subjects underwent a mild exercise challenge (60 seconds of stepping on and off of a platform 22.9 inches off the ground).²⁴ After vital signs returned to resting values, venous blood samples were again obtained, this time from the antecubital vein of the other arm. Blood was collected using a 2-syringe sampling technique to avoid activation of platelets.

After their single overnight GCRC stay and morning blood sampling, the normal comparison patients were terminated from the study. The patients with depression received open-label treatment with paroxetine (20 mg/d) for 6 weeks. Patients were reevaluated on a weekly basis by a board-certified psychiatrist (D.L.M.) and instructed not to use adjunct psychotherapy or other psychoactive medications. After 6 weeks of paroxetine treatment, the patients with depression were readmitted for a second overnight GCRC stay. The next morning, the procedures and blood sampling of the first GCRC visit were repeated to determine the effect of treatment.

PLATELET MEASURES

Venous blood samples were assayed for activated platelets as previously described.²⁴ Murine monoclonal antibodies directed to distinct activation-dependent epitopes on the platelet membrane were used: (1) anti-LIBS1 for LIBS on GP IIIa; (2) GA6 for P-selectin (CD62); and (3) recombinant placental protein annexin V detecting activation-dependent exposure of anionic phosphatidylserine determinants. The anti-LIBS mAb was donated by Edward F. Plow, PhD, of the Cleveland Clinic, Cleveland, Ohio. Because of excessive cost, the PAC1 mAb was not used as in our 1996 previous study. As supply of GE12 mAb had ceased, GA6 (donated by Biogen, Boston, Mass), an mAb with affinity identical to GE12 for P-selectin, was used. Additionally, we determine plasma concentrations of

platelet specific proteins secreted from platelet storage granules during platelet activation in vivo (ie, β -TG and PF4 and dose-response platelet aggregation induced by agonist ADP or thrombin receptor-agonist peptide (TRAP₁₋₆), expressed as the agonist concentration producing half-maximal aggregation (AC₅₀). Plasma concentrations of PF4 and β -TG were determined by enzyme immunoassay (Asserachrom^R PF4 and Asserachrom^R β -TG; American Bioproducts, Parsippany, NJ). Simulating circulating thrombin, TRAP₁₋₆ initiates the platelet thrombin-receptor activation and was used in this study rather than collagen (an agonist in our previous study). The laboratory personnel were blinded to the diagnostic identity of the samples.

STATISTICAL ANALYSES

Sample size for this study (patients vs normal controls) was calculated based on our previous study's results.²⁴ To determine whether there was a significant difference between the basal and postexercise challenge platelet measures, paired *t* tests were used. There were no significant differences between the basal and postchallenge scores in either the normal comparison or depressed patient group. Therefore, in subsequent analyses, we used the data obtained during the basal conditions. To determine whether the depressed group exhibited increased platelet activation compared with the normal comparison subjects, *t* tests were used. Nonparametric tests gave similar *P* values. For interpretation, as a percentage increase (or decrease), 95% confidence intervals (CIs) were calculated. We then divided the depressed group into those with 1 or more commonly accepted risk factors for IHD and those who did not have any risk factors for IHD. Because the number of subjects in the depressed group without risk factors for IHD was small, we do not report *P* values comparing the means of that group with the other groups. To determine whether platelet measures of the depressed group would decrease after 6 weeks of treatment with paroxetine, paired *t* tests were used. All of the statistical tests were 2-tailed, with a level of $\alpha = .05$.

eration of thrombin. This platelet procoagulant activity is detected by annexin V protein reacting with activation-dependent exposure of anionic phosphatidylserine determinants on the platelet surface (which serve as binding sites for the prothrombinase complex [prothrombin, factor V, and factor X]) and binding of V261 mAb against platelet-bound activated coagulation factor V fragment³⁸ (**Table 1**).

RESULTS

Compared with normal controls similar in age and gender, the depressed group exhibited the following: (1) increased binding of the mAb anti-LIBS, which attaches to the fibrinogen binding site of the activated GP receptor ($t_{25} = 3.86$, $P = .0007$) (**Figure 2**); (2) increased binding of the mAb GA6 to P-selectin ($t_{25} = 2.91$, $P = .01$); and increased plasma concentrations of the platelet-specific secretion protein PF4 ($t_{24} = 2.40$, $P = .02$) (**Table 3**). Thus, using these measures, the patients who were depressed exhibited nearly a 125% (95% CI, 63%-257%) increase in bind-

ing of the mAb anti-LIBS, a 50% (95% CI, 14%-96%) increase in binding of the mAb GA6, and a 150% (95% CI, 0.1%-284%) increase in plasma concentrations of PF4, relative to the baseline of the controls. These results of the total group of patients who were depressed ($n = 15$) are congruent with the results based on analyses of the patients with depression and risk factors for IHD ($n = 10$).

After 6 weeks of paroxetine treatment (20 mg/d), 12 of 15 patients were designated as "responders" (ie, they demonstrated a reduction of $\geq 50\%$ from their baseline HDRS score, and exhibited a positive response on the Clinical Global Impression score, defined as a score of ≤ 2 for those assessed with a score of ≥ 3 at baseline). After paroxetine treatment, the depressed group exhibited significant reduction of activation as demonstrated by diminished mAb anti-LIBS binding ($t_{14} = 4.32$, $P = .0007$), diminished mAb GA6 binding ($t_{14} = 2.75$, $P = .02$), and diminished plasma concentrations of PF4 ($t_{12} = 2.66$, $P = .02$) (**Table 4**). Therefore, those who were depressed exhibited a 48% decrease in anti-LIBS binding, a 17% decrease of GA6 binding, and a 54% decrease

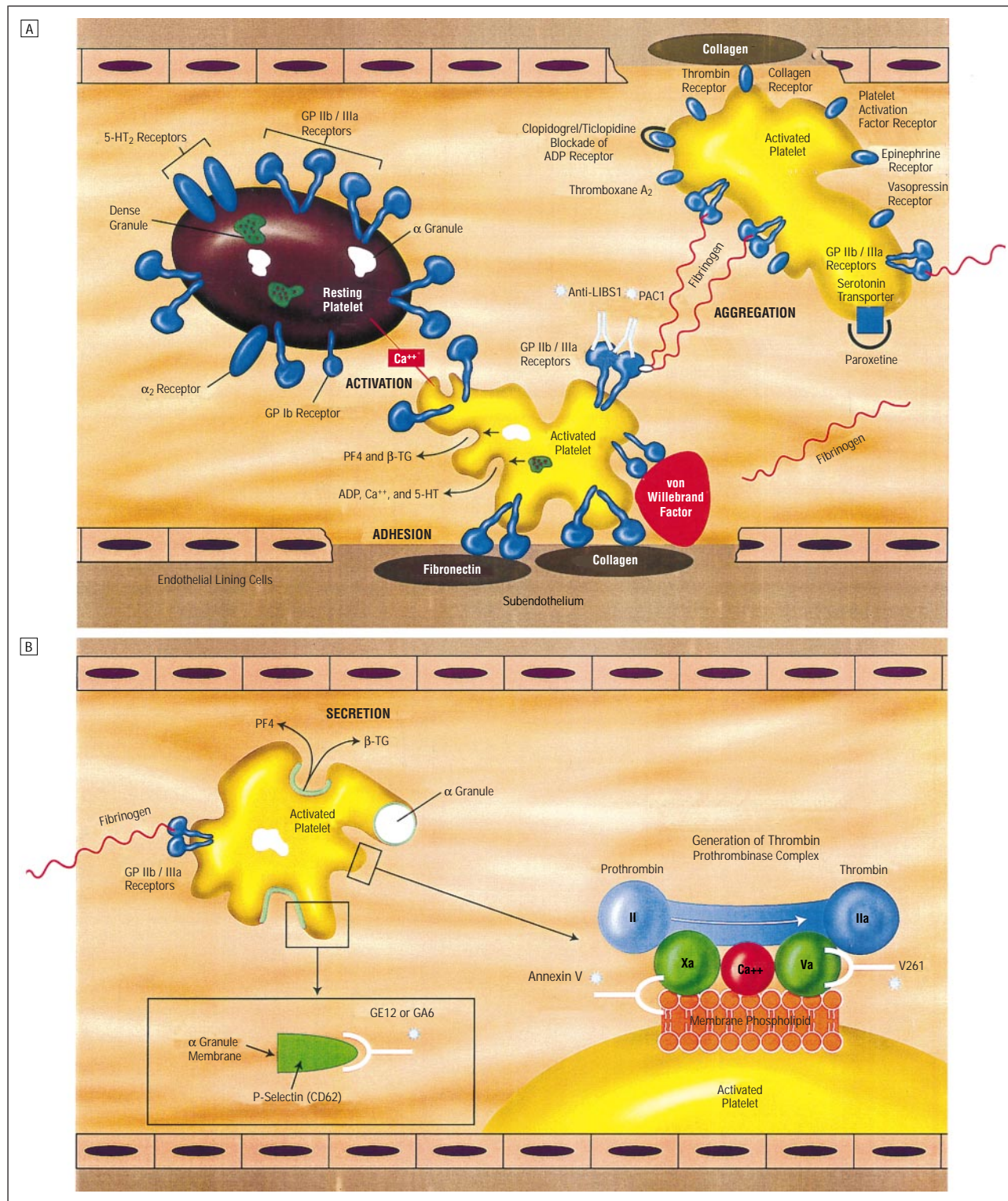


Figure 1. Platelets may be activated through several receptor-mediator pathways. Collagen exposed within the denuded area of vascular endothelium has stimulated platelet activation and adhesion to vessel wall. During activation platelet storage granule contents are extruded and induce irreversible platelet-platelet aggregation and thrombus formation. Activated circulating platelets can be identified by fluorescent-labeled monoclonal antibodies, including PAC1, anti-ligand-induced binding site (LIBS1), GE12, or GA6, V261, and the protein annexin V. Ca^{++} indicates calcium; PF4, platelet factor 4; β -TG, β -thromboglobulin; ADP, adenosine diphosphate; and 5-HT, serotonin.

of PF4 plasma concentrations, relative to their baseline pretreatment values. These posttreatment platelet measures were not significantly different from the baseline values of the normal controls.

The patients who were depressed did not differ significantly from the normal comparison subjects before or after paroxetine treatment in their platelet binding of annexin V, plasma concentrations of β -TG, or AC_{50} of

Table 1. Flow Cytometric Detection of Activated Platelets*

Antibodies and Activation-Dependent Epitopes	Clinical Significance	Relation to Cardiovascular Risk
PAC1 for the activated GP IIb/IIIa complex†	Platelet-platelet accumulation	Thrombus formation
<i>Anti-LIBS1</i> for fibrinogen binding sites on GP IIIa†‡	Platelet adhesion to thrombogenic surfaces	Thrombus formation
GE12 or GA6 for P-selectin†	Secretion of intraplatelet contents including thromboxane A ₂ , histamine, 5-HT, and PDGF	Coronary vasoconstriction (thromboxane A ₂ , histamine, and 5-HT), smooth muscle migration, proliferation in the intima, thereby narrowing vessel lumen and enhancing growth of atheromatous plaque (PDGF)
<i>Annexin V</i> reacts with activation-exposed platelet phosphatidylserine residues†	Platelet attachment to prothrombinase complex	Generation of thrombin
V261 reacts with platelet-bound activated coagulation factor V fragment (Va)†	10 ⁵ increased generation of thrombin	Increased endothelial cell proliferation and scarring of vessel wall

*LIBS1 indicates ligand-induced binding site; GP, glycoprotein; 5-HT, serotonin; and PDGF, platelet-derived growth factor.

†Indicates the monoclonal antibodies used in the flow cytometry analysis of platelet activation in our 1996 study. The monoclonal antibodies (in italics) were repeated in the present study.

‡Increased binding of the anti-LIBS1 antibody in patients with depression compared with controls was observed in both the 1996 study and this study.

Table 2. Demographic, Psychiatric, and Cardiovascular Characteristics of Study Patients*

	Normal Controls (n = 12)	Patients With Depression	
		No Risk Factors (n = 5)	Risk Factors (n = 10)
Age, y, mean (SD)	36 (7)	32 (7)	42 (6)
White/African American, No.	12/0	5/0	9/1
Sex (male/female), No.	6/6	2/3	6/4
Recurrent MDD, No.	...	5	8
Family history, No.			
Psychiatric disorder†	3	1	5
Premature atherosclerotic heart disease or stroke‡	0	0	4
IHD risk factors, No.			
Obesity§	0	0	3
Hypercholesterolemia	0	0	3
Nicotine use¶	0	0	4
Hypertension#	0	0	1
Diabetes**	0	0	0

*MDD indicates major depressive disorder; IHD, ischemic heart disease; and ellipses, that the value was not applicable.

†First-degree family member with psychiatric disorder.

‡First-degree male relative younger than 55 years and/or first-degree female relative younger than 65 years.

§Obesity as indicated by a body mass index higher than 25 (calculated as weight in kilograms divided by the square of height in meters).

||Hypercholesterolemia was determined by a nonfasting total cholesterol level higher than 200 mg/dL (>5.17 mmol/L).

¶Nicotine use was defined as daily cigarette or pipe use.

#Hypertension was defined as a persistent diastolic blood pressure remaining persistently at or higher than 90 mm Hg, or systolic blood pressure at 140 to 180 mm Hg.

**Diabetes was defined as 2 venous plasma glucose concentrations higher than 200 mg/dL (>11.1 mmol/L) during an oral glucose tolerance test.

ADP, or TRAP₁₋₆. A linear regression analysis revealed no significant relationship between the change in HDRS scores and change in platelet measures.

COMMENT

The results of this study indicate that those with depression but not IHD exhibit increased platelet activation under basal conditions, as evidenced by increased binding of

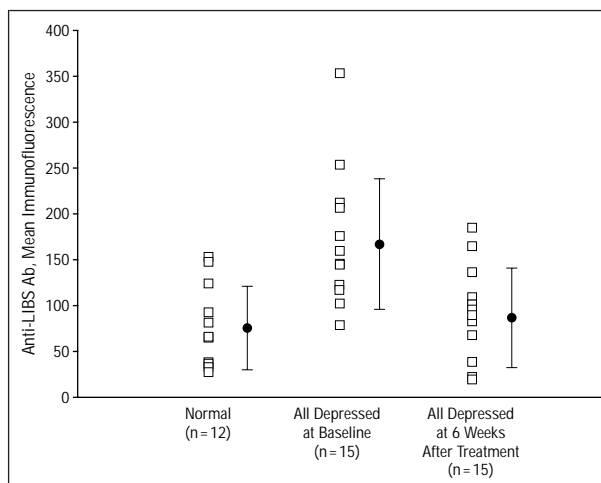


Figure 2. Before paroxetine treatment, platelet anti-ligand-induced binding site (LIBS1) of patients with major depression is significantly increased ($t_{25} = 3.86$, $P = .0007$) compared with normal controls. After 6 weeks of open-label paroxetine treatment (20 mg/d), platelet anti-LIBS1 binding was not significantly greater than that of the normal controls ($t_{25} = 0.59$, $P = .56$). The vertical lines are SE bars, with a mean of 1 SE.

mAb anti-LIBS to GP IIIa, GA6 to P-selectin, and increased plasma concentrations of PF4 compared with normal controls. Evidence of increased GP IIb/IIIa activation of platelets in patients with major depression as demonstrated by increased platelet binding of the mAb anti-LIBS was originally observed in our preliminary²⁴ and current study, and confirmed by Markovitz and colleagues.³⁶ The increased anti-LIBS binding of the platelets of patients who were depressed was observed not only in patients with depression and other risk factors for IHD, but in those without those factors, as in our 1996 study, suggesting that the increased anti-LIBS binding is not a surrogate marker for other commonly accepted risk factors for IHD.

The lack of significant differences in platelet activation and aggregation after the exercise challenge (even in the normal comparison subjects) may be caused by compensatory inhibitory processes associated with exercise. The increased platelet binding of annexin V exhibited by the patients who were depressed in our previous study was not replicated, perhaps in part because of the marked variability we have observed in our labo-

Table 3. In Vivo Platelet Activation, Secretion, and Dose-Response Aggregation in Patients With Depression and Normal Comparison Subjects at Baseline and After Exercise Challenge*

Variable	Normal Comparison Subjects (n = 12), Mean (SD)		Patients With Depression, Mean (SD)						t Test	
	Basal	Post	Total (N = 15)		No Risk Factors (n = 5)		Risk Factors (n = 10)		t (df)	P Value†
			Basal	Post	Basal	Post	Basal	Post		
Annexin‡	28.23 (21.39)	26.58 (25.77)	26.90 (17.17)	34.22 (31.15)	23.16 (8.99)	14.93 (8.47)	28.77 (20.27)	43.87 (34.17)	0.18 (25)	.86
Anti-LIBS‡	75.31 (44.89)	83.46 (46.51)	166.96 (71.48)	177.27 (90.87)	217.34 (87.35)	178.27 (119.14)	141.76 (49.41)	176.77 (80.84)	3.86 (25)	.0007§
GA6‡	4.15 (1.22)	3.80 (1.20)	6.39 (2.42)	6.06 (2.50)	5.11 (1.17)	4.26 (1.63)	7.03 (2.67)	6.96 (2.41)	2.91 (25)	.0075§
β-Thromboglobulin, IU/mL	12.29 (6.18)	12.55 (5.98)	24.73 (26.25)	13.61 (14.67)	20.78 (34.42)	9.24 (13.16)	26.70 (23.07)	15.37 (15.95)	1.60 (25)	.12
Platelet factor 4, IU/mL	6.05 (3.26)	6.80 (4.06)	15.05 (12.05)	10.05 (8.35)	12.76 (17.03)	6.42 (9.00)	16.19 (9.62)	11.86 (7.85)	2.40 (24)	.0244§
ADP, half-maximal aggregation	2.80 (1.61)	4.19 (4.28)	3.26 (4.48)	3.78 (3.02)	1.55 (0.84)	2.45 (1.64)	4.11 (5.33)	4.45 (3.40)	0.33 (25)	.74
TRAP, half-maximal aggregation	1.77 (1.04)	1.51 (0.56)	1.77 (1.84)	2.01 (1.89)	0.98 (0.30)	2.90 (2.75)	2.16 (2.18)	1.57 (1.25)	0.01 (25)	>.99
Platelet count (in thousands)	235.17 (49.50)	250.0 (50.13)	230.07 (77.30)	232.73 (82.47)	220.0 (75.15)	248.2 (69.97)	235.10 (81.86)	225.0 (90.58)	0.20 (25)	.84

*LIBS indicates ligand-induced binding sites; ADP, adenosine diphosphate; and TRAP, thrombin receptor-agonist peptide.

†P value corresponds to the t test comparing baseline values of normal subjects (n = 12) and those with depression (n = 15).

‡Indicates the mean immunofluorescence.

§Values indicate statistical significance.

||One observation was missing.

Table 4. Baseline and Postparoxetine Treatment Values of In Vivo Platelet Activation, Secretion, and Dose-Response Aggregation in Patients Who Were Depressed, With and Without Risk Factors for Ischemic Heart Disease*

Variable	Patients With Depression, Mean (SD)					Comparison of Baseline Normal vs Depressed After 6 Weeks Treatment, P‡ (t, df)
	After 6 Weeks of Treatment (N = 15)		No Risk Factors After 6 Weeks of Treatment (n = 5), Basal	Risk Factors After 6 Weeks of Treatment (n = 10), Basal		
	Basal	P† (t, df)				
Annexin V§	25.83 (15.49)	.83 (0.22, 14)	20.37 (8.54)	28.56 (17.78)	.74 (0.34, 25)	
Anti-LIBS§	86.85 (53.95)	.0007 (4.32, 14)	91.10 (71.54)	84.71 (47.31)	.56 (0.59, 25)	
GA6§	5.29 (2.06)	.016 (2.75, 14)	4.40 (1.34)	5.73 (2.27)	.10 (1.69, 25)	
β-Thromboglobulin, IU/mL	15.56 (10.70)	.27 (1.16, 13)	11.70 (10.32)	16.16 (11.5)	.36 (0.93, 24)	
Platelet factor 4, IU/mL	6.98¶ (5.05)	.021 (2.66, 12)	7.49 (7.33)	6.75 (4.24)	.61 (0.52, 22)	
ADP, half-maximal aggregation	2.26 (1.28)	.38 (0.90, 14)	1.44 (0.55)	2.67 (1.37)	.34 (0.98, 25)	
TRAP, half-maximal aggregation	1.22 (0.64)	.17 (1.44, 14)	1.08 (0.34)	1.29 (0.76)	.10 (1.69, 25)	
Platelet count (in thousands)	214.67 (52.21)	.25 (1.20, 14)	210.4 (68.84)	216.80 (46.04)	.31 (1.04, 25)	

*LIBS indicates ligand-induced binding sites; ADP, adenosine diphosphate; and TRAP, thrombin receptor-agonist peptide.

†P value corresponds to the t test comparison of values at baseline and after 6 weeks of treatment for patients with depression.

‡P value corresponds to the t test comparison of the population means of patients who were depressed after treatment and the normal controls.

§Indicates the mean immunofluorescence.

||One observation was missing.

¶Two observations were missing.

ratory with this particular assay. Moreover, the means and SDs of the flow cytometry and platelet secretion variables are greater than in our prior study, because of the sample analyses having been performed with a different flow cytometry apparatus.

The increased platelet binding of mAb GA6 in the patients who were depressed under basal conditions reflects the increased exposure of the α-granule membrane glycoprotein P-selectin during secretion of α-granule contents, including PF4 and β-TG. Increased plasma

concentrations of the platelet-specific proteins, β-TG and PF4, have been reported in patients with IHD and comorbid depression compared with patients with IHD alone and control subjects.²⁵ Indeed, such perturbations may be observed in certain groups of patients with depression (ie, those with clinical evidence of IHD, those with risk factors for IHD [as reflected by increased plasma concentrations of PF4], but not in relatively young patients with depression but no risk factors for IHD [as observed in our original 1996 study]).

There were no significant differences in platelet aggregation (as detected by the AC₅₀ of the platelet agonists ADP and TRAP₁₋₆) between patients who were depressed and normal comparison subjects at baseline, or in the patients with depression after paroxetine treatment compared with pretreatment values. Our previous study reported that the patients who were depressed exhibited an initially decreased AC₅₀ of the platelet agonist collagen compared with controls, and a resulting increase (AC₅₀) after orthostatic challenge (because previously activated platelets in vivo required higher concentrations of agonist in vitro to induce aggregation). As in our previous study, less potent agonists, such as ADP (and TRAP₁₋₆), do not detect alterations in platelet aggregation of those with depression compared with normal comparison subjects. Nevertheless, our results support an increase in platelet activation of patients who were depressed under basal conditions, because platelet reactivity (or responsivity) after the stimulus of the mild exercise challenge was not significantly increased in those patients (or normal comparison subjects).

Platelet activation in the depressed group was significantly reduced after 6 weeks of paroxetine treatment (20 mg/d), as demonstrated by diminished platelet binding of the mAb anti-LIBS to GP IIIa, GA6 to P-selectin, and plasma concentrations of PF4. Our data do not inform as to whether the reduction in platelet activation after paroxetine administration is caused by remission of depression, a direct pharmacological action of the drug on platelets, or some other pharmacological effect of this SSRI. Indeed, the effects of paroxetine on platelet activation could be entirely independent of its reduction of depressive symptoms in the patients. The effects of paroxetine treatment on platelet function of nondepressed subjects might reveal such important information.

The limits of this study include a relatively small sample size (and the associated possibility of a type II error), lack of psychiatric control subjects (eg, patients with anxiety disorders), and patients with more mild forms of depression. Moreover, normal comparison subjects with other risk factors for IHD were not recruited in this study, as we sought to determine the differences between the groups of patients with depression (those with and without risk factors for IHD). Platelet activation of a group of patients without depression with other risk factors for IHD will be scrutinized in future investigations. Subsequent studies will also seek to determine whether patients who are depressed treated with non-pharmacological modalities (eg, psychotherapy, electroconvulsive therapy) exhibit similar normalization of platelet function.

Accepted for publication May 23, 2000.

Supported by grants MH-01399, MH-42088, MH-49523, and RR-00039 from the National Institutes of Health, Bethesda, Md, and an Established Investigator Award from the National Alliance for Research on Schizophrenia and Depression, Chicago, Ill (Dr Nemeroff).

Paroxetine was provided by SmithKline Beecham, Philadelphia, Pa.

We thank George Cotsonis, PhD, the staff of the General Clinical Research Center at Emory University, and the

staff of the Investigational Drug Service of the Pharmacy Department at Emory University Hospital, Atlanta, Ga, for all of their assistance.

Corresponding author: Charles B. Nemeroff, MD, PhD, Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, 1639 Pierce Dr, Suite 4000, Atlanta, GA 30322.

REFERENCES

- Greenberg PE, Kessler RC, Nells TL, Finkelstein SN, Berndt ER. Depression in the workplace: an economic perspective. In: Feighner JP, Bayer WF, eds. *Selective Serotonin Re-Uptake Inhibitors*. New York, NY: John Wiley & Sons Inc; 1996.
- Murray CJL, Lopez AD, eds, for the World Health Organization. *The Global Burden of Disease*. Cambridge, Mass: Harvard University Press; 1996.
- Berndt ER, Koran LM, Finkelstein SN, Gelenberg AJ, Kornstein SG, Miller IM, Thase ME, Trapp G, Keller MB. Lost human capital from early-onset chronic depression. Presented at: the Annual Meeting of the American Psychiatric Association; June 2, 1998; Toronto, Ontario.
- Finkelstein SN, Berndt ER, Gelenberg A, Keller MB, Koran LM, Miceli R, Miller IV, Russell JM, Kocsis J, Verner J. Patterns of functional and symptomatic response over two years of sertraline therapy for chronic depression. Presented at: the 37th Annual Meeting of the American College of Neuropsychopharmacology; December 16, 1998; Las Croabas, Puerto Rico.
- Kessler RC, Mickelson RC, Barber C, Wang P. The effects of chronic medical conditions in work impairment. In: Rossi AS, ed. *Caring and Doing for Others: Social Responsibility in the Domains of Family, Work, and Community*. Chicago, Ill: University of Chicago Press; 2000.
- Kessler RC, Barber C, Birnbaum HG, Frank RG, Greenberg PE, Rose RM, Simon GE, Wang P. Depression in the workplace: effects on short-term work disability. *Health Aff (Millwood)*. September-October 1999;18:163-171.
- Murphy JM, Monson RR, Olivier DC, Sobol AM, Leighton AH. Affective disorders and mortality: a general population study. *Arch Gen Psychiatry*. 1987;44:473-480.
- Anda R, Williamson D, Jones D, Marcera C, Eaker E, Glassman A, Marks J. Depressed affect, hopelessness, and the risk of ischemic heart disease in a cohort of US adults. *Epidemiology*. 1993;4:285-294.
- Aromaa A, Raitasalo R, Reunanen A, Impivaara O, Heliovaara M, Knecht P, Lehtinen V, Joukamaa M, Maatela J. Depression and cardiovascular diseases. *Acta Psychiatr Scand Suppl*. 1994;377:77-82.
- Everson SA, Goldberg DE, Kaplan GA, Cohen RD, Pukkala E, Tuomilehto J, Salonen JT. Hopelessness and risk of mortality and incidence of myocardial infarction and cancer. *Psychosom Med*. 1996;58:113-121.
- Barefoot JC, Schroll M. Symptoms of depression, acute myocardial infarction, and total mortality in a community sample. *Circulation*. 1996;93:1976-1980.
- Pratt LA, Ford DE, Crum RM, Armenian HK, Gallo JJ, Eaton WW. Depression, psychotropic medication, and risk of myocardial infarction: prospective data from the Baltimore ECA follow-up. *Circulation*. 1996;94:3123-3129.
- Ford DE, Mead LA, Chang PP, Cooper-Patrick L, Wang NY, Klag MJ. Depression is a risk factor for coronary artery disease in men: the Precursors Study. *Arch Intern Med*. 1998;158:1422-1426.
- Simonsick EM, Wallace RB, Blazer DG, Berkman LF. Depressive symptomatology and hypertension-associated morbidity and mortality in older adults. *Psychosom Med*. 1995;57:427-435.
- Callahan CM, Wolinsky FD, Stump TE, Nienaber NA, Hui SL, Tierney WM. Mortality, symptoms, and functional impairment in late-life depression. *J Gen Intern Med*. 1998;13:746-752.
- Mendes de Leon CF, Krumholz HM, Seeman TS, Vaccarino V, Williams CS, Kasl SV, Berkman LF, for the Established Populations for the Epidemiologic Studies of the Elderly. Depression and risk of coronary heart disease in elderly men and women: New Haven EPESE, 1982-1991. *Arch Intern Med*. 1998;158:2341-2348.
- Carney RM, Rich MW, Freedland KE, Saini J. Major depressive disorder predicts cardiac events in patients with coronary artery disease. *Psychosom Med*. 1988;50:627-633.
- Schleifer SJ, Macarini-Hinson MM, Coyle DA, Slater WR, Kahn M, Gorlin R, Zucker HD. The nature and course of depression following myocardial infarction. *Arch Intern Med*. 1989;149:1785-1789.
- Frasure-Smith N, Lesperance F, Talajic M. Depression following myocardial infarction: impact on 6-month survival. *JAMA*. 1993;270:1819-1825.
- Gonzalez MB, Snyderman TB, Colket JT, Arias RM, Jiang JW, O'Connor CM, Krishnan KR. Depression in patients with coronary artery disease. *Depression*. 1996;4:57-62.

21. Carney RM, Rich MW, Tevelde A, Saini J, Clark K, Jaffe AS. Major depressive disorder in coronary artery disease. *Am J Cardiol.* 1987;60:1273-1275.
22. Frasure-Smith N, Lesperance F, Talajic M. Depression and 18-month prognosis after myocardial infarction. *Circulation.* 1995;91:999-1005.
23. Ahern DK, Gorkin L, Anderson JL, Tierney C, Hallstrom A, Ewart C, Capone RJ, Schron E, Kornfeld D, Herd JA, Richardson DW, Follick MJ, for the CAPS Investigators. Biobehavioral variables and mortality or cardiac arrest in the Cardiac Arrhythmia Pilot Study (CAPS). *Am J Cardiol.* 1990;66:59-62.
24. Musselman DL, Tomer A, Manatunga AK, Knight BT, Porter MR, Kasey S, Marzec U, Harker LA, Nemeroff CB. Exaggerated platelet reactivity in major depression. *Am J Psychiatry.* 1996;153:1313-1317.
25. Laghrissi-Thode F, Wagner WR, Pollock BG, Johnson PC, Finkel MS. Elevated platelet factor 4 and β -thromboglobulin plasma levels in depressed patients with ischemic heart disease. *Biol Psychiatry.* 1997;42:290-295.
26. Markovitz JH, Shuster J, Tolbert L. Effects of sertraline treatment of depression on platelet activation in an open-label study: implications for coronary disease [abstract]. *Psychosom Med.* 1999;61:93.
27. Vorchheimer DA, Badimon JJ, Fuster V. Platelet glycoprotein IIb/IIIa receptor antagonists in cardiovascular disease. *JAMA.* 1999;281:1407-1414.
28. Alexander JH, Harrington RA. Antiplatelet and antithrombin therapies in the acute coronary syndromes. *Curr Opin Cardiol.* 1997;12:427-437.
29. Kamal LA, Le Quan-Bui KH, Meyer P. Decreased uptake of 3H-serotonin and endogenous content of serotonin in blood platelets in hypertensive patients. *Hypertension.* 1984;6:568-573.
30. Fetkovska N, Amstein R, Ferracin F, Regenass M, Buhler FR, Pletscher A. 5HT-kinetics and sensitivity of human blood platelets: variations with age, gender and platelet number. *Thromb Haemost.* 1988;60:486-490.
31. Fetkovska N, Fedelesova V, Dzurik R. Low-density lipoprotein amplifies the platelet response to serotonin in human plasma. *J Hypertens Suppl.* 1989;7:S164-S165.
32. Fetkovska N, Amstein R, Ferracin F, Regenass M, Buhler FR, Pletscher A. 5-hydroxytryptamine kinetics and activation of blood platelets in patients with essential hypertension. *Hypertension.* 1990;15:267-273.
33. Amstein R, Fetkovska N, Buhler FR. Platelet serotonin-LDL interaction in essential hypertension. *J Cardiovasc Pharmacol.* 1991;17(suppl 5):S35-S40.
34. Lassila R, Laustiola KE. Cigarette smoking and platelet-vessel wall interactions. *Prostaglandins Leukot Essent Fatty Acids.* 1992;46:81-86.
35. Andreasen NC, Endicott J, Spitzer RL, Winokur G. The family history method using diagnostic criteria: reliability and validity. *Am J Psychiatry.* 1977;34:1229-1235.
36. Markovitz JH, Matthews KA, Kiss J, Smitherman TC. Effects of hostility on platelet reactivity to psychological stress in coronary heart disease patients and healthy controls. *Psychosom Med.* 1996;58:143-149.
37. Matzdorff AC, Kemkes-Matthes B, Voss R, Pralle H. Comparison of β -thromboglobulin, flow cytometry, and platelet aggregometry to study platelet activation. *Haemostasis.* 1996;26:98-106.
38. Musselman DL, Evans DL, Nemeroff CB. The relationship of depression to cardiovascular disease: epidemiology, biology, and treatment. *Arch Gen Psychiatry.* 1998;55:580-592.
39. First MB, Spitzer RL, Gibbon M, Williams JBW. *Structured Clinical Interview for DSM-IV Axis I Disorders, Version 2.0, 1998 Revision.* New York: Biometrics Research Dept, New York State Psychiatric Institute; 1998.
40. Hamilton M. The assessment of anxiety states by rating. *Br J Med Psychol.* 1959;32:59-65.
41. Bruss GS, Gruenberg AM, Goldstein RD, Barber JP. Hamilton Anxiety Rating Scale Interview Guide: joint interview and test-retest methods for interrater reliability. *Psychiatry Res.* 1994;53:191-202.
42. Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry.* 1960;23:56-62.
43. Potts MK, Daniels M, Burnam MA, Wells KB. A structured interview version of the Hamilton Depression Rating Scale: evidence of reliability and versatility of administration. *J Psychiatr Res.* 1990;24:335-350.
44. Smouse PE, Geinberg M, Carroll BJ, Park MH, Rawson SG. The Carroll Rating Scale for Depression, II: factor analyses of the feature profiles. *Br J Psychiatry.* 1981;138:201-204.
45. Carroll BJ, Feinberg M, Smouse PE, Rawson SG, Greden JF. The Carroll Rating Scale for Depression, I: development, reliability and validation. *Br J Psychiatry.* 1981;138:194-200.
46. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.* Washington, DC: American Psychiatric Association; 1994.
47. Guidelines Subcommittee of the WHO/ISH Mild Hypertension Liaison Committee. 1993 guidelines for the management of mild hypertension: memorandum from a World Health Organization/International Society of Hypertension meeting. *Hypertension.* 1993;22:392-403.
48. Willett WC, Dietz WH, Colditz GA. Guidelines for healthy weight. *N Engl J Med.* 1999;341:427-434.
49. Department of Agriculture, Department of Health and Human Services. *Nutrition and Your Health: Dietary Guidelines for Americans.* Vol 232. 4th ed. Washington, DC: Government Printing Office; 1995.
50. National Cholesterol Education Program Expert Panel. Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. *Arch Intern Med.* 1988;148:36-69.