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# Effects of Quetiapine on Platelets in Major Depression

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## Abstract

**Objectives:** Test the prevailing hypothesis that depressive illness is associated with platelet hyperactivity and that treatment with an atypical antipsychotic with established antidepressant efficacy - quetiapine - will normalize platelet activity.

**Methods:** Forty-seven outpatients with major depressive disorder (MDD) and 27 healthy controls (HC) without evidence of cardiovascular disease were enrolled. Behavioral rating scales and medical tests preceded baseline assessments of (1) platelet-rich plasma (PRP) aggregometry and (2) whole blood flow cytometry (P-selectin surface labeling). The measures were repeated in those MDD subjects who completed 8 weeks (n=27) or 12 weeks (n=19) of treatment with quetiapine.

**Results:** Untreated MDD compared to HC subjects displayed more platelet aggregation when PRP was stirred agonist-free (p=0.021). Other platelet measurements at baseline such as *in vitro* agonist-stimulated PRP aggregometry or P-selectin expression by flow cytometry did not distinguish MDD from HC subjects. After 8 weeks on quetiapine, a reduced (now normal) agonist-free aggregatory response to stirring (p=0.035) was observed. By 12 weeks the aggregometry response to arachidonic acid (AA) was also lowered (p=0.016 vs. pretreatment; p=0.001 vs. HC). Other agonist additions (ADP, epinephrine, or collagen) failed to distinguish MDD from HC. There were no significant associations between mood rating scores and any form of platelet activity at any time point on quetiapine.

**Limitations:** High percentage of dropouts attributable to dose-related side effects limited the post-treatment assessments.

**Conclusions:** The hypothesis that untreated depression is associated with more active platelets, was confirmed, but this finding was confined to the "resting", or agonist-free state. Quetiapine treatment normalized this resting activity and led to a lower-than-normal response to AA-induced aggregation after 12 weeks of treatment. These findings confirm that 8 weeks treatment with quetiapine can normalize at least one form of platelet hyperactivity, but the lower-than-healthy response to AA after 12 weeks on quetiapine warrants further study.

**Keywords:** Major depression; Quetiapine; Platelets; Platelet aggregation; Flow cytometry; Pselectin

## Introduction

Numerous epidemiological studies have shown depressive illness to be an independent predictor of future cardiovascular disease (CVD) [1-5]. Platelets, being central to the atherosclerotic process, have been extensively investigated as a possible physiological link responsible for this association. Because platelets take up and secrete serotonin and norepinephrine, we hypothesized that platelet serotonergic and/or adrenergic receptors might be hypersensitive and provide not only a diagnostic biomarker but an explanation for the tendency of depressed patients to develop CVD. However, support for this hypothesis has been weak. At best there are slight elevations in 5-HT1A, 5-HT2A and α2-adrenergic receptors on platelets in depression [6], which is probably due to reduced peripheral serotonin [7,8]; however, none of these findings seem to be clinically robust enough to induce changes in platelet activation, adhesion and/or aggregation.

Contrary to those former platelet receptor studies, more consistent findings have emerged from studies of "resting" platelets, which were previously considered the unstimulated control samples. It is now realized that a small proportion of platelets even from healthy control subjects are circulating in an activated state, even without agonist additions, and the amount and intensity of their signal appears to be amplified in the unstimulated blood of depressed patients [9-13]. Evidence for heightened "resting" platelet activation in patients with untreated depression has been obtained in many studies and by various means: (1) Platelet-rich plasma (PRP) from depressed patients has been shown to be more aggregable than PRP from normal subjects, but only in the presence of sub-threshold agonist concentrations and provoked by stirring [14]. (2) Levels of platelet-released activation factors have been found to be elevated in the unstimulated blood plasma of depressed patients [11,15,16]. (3) Platelet surface markers of activation measured by flow cytometry have been found to be elevated in depressed patients, in the absence of exogenous agonists [12,13,17,18]. As far as we could determine, there are no studies of adult CVD-free MDD patients that have failed to reveal heightened platelet activation responses as compared to healthy controls (HC). We caution, however, that even in our own positive study of resting platelets [16], this conclusion was not revealed across all types of activation biomarkers measured. While platelet membranous P-selectin was not elevated in depressed patients

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as measured by whole blood flow cytometry, the plasma levels of the same protein were elevated. This was interpreted as being indicative of long-standing changes in platelet release rather than any acute platelet activation the subject may have experienced [16].

A major prospective epidemiological study (SAD-HART) raised widespread attention to the potential role of antidepressants in the treatment of CVD [19]. This double-blind study enrolled patients who exhibited depressive symptoms post-myocardial infarction (MI) shortly after their admission to the intensive care unit. Patients received standard post-MI care and were randomized to receive either sertraline or placebo for treatment of their depression [20]. As early as 6 months into the study, the SSRI-treated group exhibited significantly lower numbers of adverse cardiovascular events compared to placebo, with no worrisome side effects. The major study outcome was prolongation of the lives of the patients [21]. Furthermore, blood tests during the course of treatment showed a reduction in platelet activation which was statistically significant after 16 weeks, suggesting that the benefit of sertraline was due directly to the action of the SSRI on platelet serotonin, leading to a normalization of platelet activation [22]. Unfortunately, this mechanism was difficult to prove because SSRIs are known to act rapidly on platelets, certainly not requiring 16 weeks of treatment. Other factors such as restored euthymic state, reduction in perceived stress and normalization of autonomic nervous system activity were likely at play. Nevertheless, there was an unquestionable therapeutic benefit of SSRI use as established in the SAD-HART trial [20].

Although classified as an atypical antipsychotic, there is strong evidence that quetiapine exerts antidepressant efficacy. In a large-scale, 6-week, placebo-controlled study by Bauer et al. [23], quetiapine-XR was effective at doses of 150 mg daily and 300 mg daily for augmentation therapy of Major Depressive Disorder (MDD). At least two other studies of quetiapine-XR monotherapy, prescribed also at 150 and 300 mg/day, have also shown statistically significant improvements in depressive symptoms versus either placebo or duloxetine, when studied in double-blind, randomized, parallel-group, placebo-controlled studies of MDD patients [24,25]. A primary reason for choosing quetiapine for this study was for comparison to our recently published study [26] in which we observed an elevation in resting platelet aggregation in depressed patients which normalized following 8 weeks of treatment with the highly selective SSRI, escitalopram. There was also a reduction in platelet response to ADP and AA by 12 weeks of treatment with escitalopram [26]. These positive findings, however, were clouded by a surprising negative correlation found with the depression symptom rating scores such that the more euthymic patients had the highest levels of platelet activation [26]. Quetiapine offered us an opportunity to reassess those findings using a compound with a completely different pharmacological mechanism of antidepressant drug action. Quetiapine is a dibenzothiazepine that was developed for the treatment of schizophrenia. Like other atypical antipsychotics, quetiapine shows good antagonistic affinities for the dopamine receptor type-2 sites (D2) and serotonergic (5-HT1A and 2A) receptors [27]. But, quetiapine has equal or higher affinities for muscarinic (M1), histaminergic (H1), and alpha-1 adrenergic (α1AR) receptors [27]. Quetiapine's activity and blockade of the H1 receptor has been linked to weight gain and strong sedative side effects.

Quetiapine appears to be devoid of hematological side effects but there are a few rare case reports of leucopenia and thrombocytopenia [28]. There is one report [29] that the *in vitro* incubation of gel-filtered platelets with quetiapine (>10 $\mu$M) leads to increased membrane phosphatidyinositol 4-phosphate (PIP), as well as the 4,5 *bis*phosphate metabolite ($PIP_2$) which results in higher platelet phosphatidic acid signaling in response to thrombin via a mechanism proposed to involve intercalation of quetiapine into the membrane phospholipid bilayer. There are no reports of any acute effects of quetiapine on platelet aggregation. Likewise, no prior study has examined the ability of quetiapine to normalize the platelet hyper-activation state observed in depression.

### Main hypothesis and ancillary hypotheses

We hypothesized that prior to antidepressant treatment heart-healthy MDD patients will exhibit higher levels of resting platelet activity relative to HC subjects. A secondary hypothesis was that quetiapine treatment would down regulate this platelet hyperactivity either by week-8 or week-12 of treatment. A tertiary hypothesis was that the degree of improvement in depressive symptoms, as reflected in rating scores, elicited by quetiapine would show positive correlation with the extent of platelet normalization, rather than the negative correlation we had reported earlier [26] between platelet activity and mood.

## Material and Methods

### Standard operating procedures

The Institutional Review Board of Loyola University Medical Center approved and monitored this study conducted according to the principles of the Declaration of Helsinki. Our standard operating procedures (SOPs) imposed certain restrictions on all subjects prior to arrival for drawing of experimental blood samples to protect against ways in which platelets might become activated by factors other than the disorder of depression, itself. These restrictions stipulated that subjects not take aspirin (previous 240 hours), antihistamines (previous 72 hours), acetaminophen (previous 72 hours), vitamins C or E (previous 72 hours), sleeping pills (previous 72 hours), caffeinated beverages (previous 8 hours), tobacco products (previous 8 hours), or engage in excessive physical activity (previous 8 hours) before the experimental blood collection. We also considered psychological stressors that are known to activate platelets [30,31] and implemented a multiday screening and work-up process to allow subjects to become habituated to the clinic setting and blood drawing procedures and personnel. Specifically, there were two extensive work-up visits before the first experimental blood drawing: (1) Work-up visit-1 was for blood and urine collection to determine a complete blood count with differential, metabolic panel with electrolytes, thyroid function, lipid profile, hCG pregnancy test and a toxicology screen. (2) Work-up visit-2 occurred 1-2 weeks later and involved a physical examination followed by a structured diagnostic interview and a battery of mood rating instruments that included the Mini International Neuropsychiatric Interview (MINI), Family History Questionnaire, Gynecologic History Questionnaire, HAM-D, HAM-A (for anxiety scores), BDI, Beck Scale for Suicide (BSS) and a Clinical Global Impression (CGI). During these two work-ups, depressed subjects were allowed to continue taking anti-anxiety and/or hypnotic medication (if applicable), because these agents are not known to affect platelet function and in order to keep the patient calm to avoid psychological factors inducing platelet activation. The first experimental platelet blood draw was scheduled 2-3 days after the second work-up visit unless a mandatory four-week antidepressant washout period was required.

### Patient population

Prospective study subjects were recruited from the outpatient clinics of the Department of Psychiatry at Loyola University Medical

Center. Study subjects were between 20 and 65 years of age and met DSM-IV criteria for primary MDD, first episode or recurrent type, were physically healthy and capable of giving informed consent. Their depressive index episode had to be ≥1 month in duration. If they had been using psychopharmacological agent(s) for depression, a four-week washout was instituted while they remained under close oversight by the study team to assess suicidality. A minimum score of 18 on the 17-item Hamilton Depression Scale (HAM-D17) was required for admission to the study. Inclusion criteria also stipulated normal range laboratory values for complete blood count, complete metabolic panel, lipid profile, thyroid function and urinalysis (including a pregnancy test for females). Exclusion criteria stipulated that all subjects be free of the following potential confounding conditions: any inflammatory condition (including gum disease), hypertension, dyslipidemia, diabetes mellitus, history of smoking or substance abuse in the preceding 6 months, and a history of heart disease. A BMI score greater than 35 was an exclusion criterion. Female subjects could not be lactating or taking oral contraceptives. Sexually active females were also not enrolled unless they agreed to practice reliable contraception for the duration of the study. The presence of active suicidality, or for that matter any other Axis I diagnosis, were additional exclusion criteria. During the informed consent process, the subjects were given up to date information about quetiapine; namely, its potential side effects and lack of FDA approval as monotherapy for MDD. Forty-seven MDD patients were enrolled in the study and demographic information is presented in Table 1 and juxtaposed to that of HC subjects.

| Variable | Major Depression (N=47) | Healthy Controls (N=27) | T score or $X^2$ value (depending*) | P Values |
|---|---|---|---|---|
| **Age (years)** | 43.3 (12.0 SD) | 38.4 (12.2 SD) | 0.17 | 0.08 |
| **Sex** | | | | |
| # Females | 26 (=55.3%) | 20 (=74.1%) | 2.56 | 0.10 |
| # Males | 21 (=44.7%) | 7 (=25.9%) | | |
| **Ethnic group** | | | | |
| Caucasian | 22 (=46.8%) | 16 (=59.3%) | 1.06 | 0.30 |
| Non-Caucasian | 25 (=53.2%) | 11 (=40.7%) | | |
| **Tobacco Use** | | | | |
| Non smoker | 22 (=100%) | 24 (= 88.9%) | NA | 0.24 |
| Smoker | 0 (=0%) | 0 (= 0%) | | |
| **Height (m)** | 1.6 (0.1 SD) | 1.6 (0.0 SD) | -0.39 | 0.69 |
| **Weight (kg)** | 88.4 (73.7 SD) | 73.7 (17.1 SD) | 2.78 | 0.001 |
| **BMI** | 31.8 (6.6 SD) | 26.5 (6.1 SD) | 3.50 | 0.001 |
| **Female status** | | | | |
| # Premenopausal | 20 (=83.3%) | 15(=78.9%) | NA | 1.00 |
| # Postmenopausal | 4 (=16.7%) | 4(=21.1%) | | |

**Note:** * Depending: For continuous variables, the student's t test was applied, mean (SD) given, and a t score obtained. For categorical variables, the chi square test was applied, N (=percent,%) given, and $X^2$ score obtained. P values are from the relevant statistical test.

**Table 1:** Demographics of the study groups.

## Healthy control subjects

HC subjects were recruited by word of mouth and with flyers posted on the campus of the Medical Center. The flyer had been reviewed and approved by the Institutional Review Board (IRB). They were screened in like manner; hence, they also underwent a multi-day habituation work-up and were enrolled only if their screening physical exams and blood tests were in normal range. The exclusion criteria for HC subjects included presence or history of any serious medical condition (including gum disease) or any diagnosed mental illness (including substance use, mental illness or substance use amongst first degree relatives), or if they were pregnant or lactating females. Scores for HCs on the HAM-D17 and the Beck Depression Inventory (BDI) had to be less than 5. HC blood drawings were made during the same time period and according to identical procedures as the MDD group. A total of 27 HC subjects were enrolled (Table 1).

## Depression and anxiety ratings

Two rating scales were used to quantify depression: the 17-item HAM-D and the 21-item Beck Depression Inventory (BDI) [32]. Because anxiety often accompanies depression, and anxiety is known to promote platelet activation, we also quantified anxiety using the HAM-A and the Beck Anxiety Inventory.

## Treatment

Immediately after their baseline blood drawings, the patients received the first dose of oral quetiapine-XR (Seroquel$^{R}$). It was administered in open-label fashion. Besides quetitapine, no other type of therapeutic intervention (e.g., psychotherapy) was offered during the study. Patients returned for follow-up visits at weeks 1, 2, 4, 8 and 12 at which times the following rating scales were repeated: HAM-D, HAM-A, BDI, BSS, CGI and Adverse Events (AE) inventory. No serious AE's occurred. The most common side effects, which also accounted for most of the dropouts, were hypersomnia, fatigue, and drowsiness. An end-of-study (week-12) HAM-D17 score ≤ 7 constituted "remission". Patients whose baseline HAM-D17 score decreased by at least 50% at week-12, but remained above 7, were classified as "partial responders." Patients who failed to meet either of these criteria were deemed to be "non-responders." In the final analysis of treatment completers, there were at total of 22 treatment responders (78.6% of total) of which 18 patients were remitters, and 6 patients (21.4%) were non-responders.

## Sampling conditions

Two types of platelet assays were performed: (a) platelet aggregometry using stirred plasma-rich plasma (PRP), and (b) P-selectin detection using whole blood flow cytometry. Prior to the blood draw for these assays, the subjects reclined in a quiet air-conditioned room for 20 minutes. A 21 gauge butterfly needle was inserted into the antecubital vein and the first 3 ml of blood was discarded to avoid tissue factors. Additional blood was collected from the same venipuncture into four 12 cc plastic syringes. From the 4 syringes the blood was distributed into seven evacuated tubes: 6 citrate "blue-tops" at room temperature (total = 27 ml) and 1 heparin "green-top" on ice (4.5 ml). After mixing by inversion, 1 ml of the citrated blood was aliquoted for flow cytometry. The remainder was centrifuged (180 *g* for 10 min at RT) to yield PRP, and subsequently the loose pellet was centrifuged (2,000 *g* for 10 min at RT) to yield platelet poor plasma (PPP). Platelet aggregometry was performed within 4 h after venipuncture. The heparinized tube on ice (4.5 ml blood) was centrifuged (2,000 g for 10 min at 3˚C) and the plasma was frozen at -80˚C for future analyses.

## Flow cytometry analysis

The flow cytometry protocol was identical to that previously described [26]. Blood was incubated with either saline (for the resting platelet assay), 5 μM ADP (BioData, Horsham, PA), 500 μg/ml arachidonic acid (BioData), 10 μg/ml collagen (ChronoLog, Havertown, PA) or 6.25 μM TRAP (Thrombin Receptor Activating Peptide; Sigma, St. Louis, MO) for 3 minutes at 37ºC and then fixed by addition of 1% paraformaldehyde. All agonist concentrations are final

concentrations. Immunolabeling occurred with CD61-FITC antibody-conjugate (exclusively tagging platelets) and with antibody targeting P-selectin (a human platelet CD62P-PE antibody-conjugate that tags just activated platelets), followed by analysis with an EPICS XL flow cytometer (Beckman-Coulter, Miami, FL). The coefficients of variation for this assay using repeated blood drawings were less than 15% [26].

### Platelet aggregometry

The platelet aggregometry protocol has been described previously [26], Platelet aggregation was measured using a four-channel optical aggregometer (BioData PAP-4, BioData Corp., Horsham, PA). PRP (450 µl) was added to cuvettes containing stir bars and incubated at 37°C for 3 minutes. 50 µl of the following agonists were added immediately prior to initiation of the measurement of aggregation: saline (for the resting platelet assay), ADP (2.5 x $10^{-5}$M), arachidonic acid (AA: 5.0 mg/ml), collagen (1.9 mg/ml), or epinephrine (EPI: 100 µg/ml). The aggregation of platelets was determined from the chart recordings and expressed as a percentage of aggregation, representing the percentage of light transmission standardized to untreated PRP and PPP samples yielding 0% and 100%, respectively.

### Statistical analyses

Demographic differences were noted between the groups of MDD patients and the HC in terms of body weight and BMI (Table 1). For this reason we used statistically adjusted marginal means in most of the final analyses. Group means and any differences (i.e. pre- versus post-treatment) were regressed on sex, age, and BMI to yield marginal means. Analysis by repeated measures ANOVA was done to test changes in biomarkers over time (followed by post-hoc Student's t-tests of the predicted pre- versus post-treatment comparisons). Pearson correlation coefficients were determined to correlate absolute values obtained from the platelet assays with HAM-D and HAM-A scores. The changes in depression rating scores (i.e. HAM-D from pre- to post-treatment) were examined for correlations with changes in each platelet biomarker. Then, after examining the data thoroughly, we decided for the sake of brevity to omit the values derived from epinephrine, collagen, and TRAP stimulations in the Results section because there were no statistical group differences or treatment findings with these agonists.

## Results

### Quetiapine dosing regimen

Based on the available literature at the start of the study, we used the following dosing schedule [23-25]. Days 1-3 50 mg/day; days 4-7 100 mg/day; days 8-21 150 mg/day; thereafter the patient would be maintained at 150 mg/day. If the response was not satisfactory, at the discretion of the physician, the dose could be increased to 200-300 mg/day from day 22 until end of treatment with the possibility of rolling back the dose to 150 mg/day, if not well tolerated. After this dosing schedule was implemented with the first 10 patients, it became apparent that many patients could not tolerate the morning drowsiness which frequently extended into the afternoon hours even if quetiapine was taken as a single dose at bedtime. Because of this side effect, we had 5 dropouts from amongst the first 10 enrolled patients. These drop-outs tended to be higher functioning individuals who had jobs or families to attend. Consequently, after the first 10 enrollees we revised the dosing schedule as follows:

Days 1-3: 25 mg 1-2 hours before bedtime.

Days 4-7: 50 mg 1-2 hours before bedtime. Patients were instructed to carefully consider whether they felt ready to move the dose up to 50 mg. They were encouraged to call the study physician, if they felt unsure how they should proceed. Many patients availed themselves of this possibility and were guided appropriately. There were also instances where the dosing increases were delayed until the drowsiness subsided.

Days 8-14: At the end of week-1 there was a face-to-face assessment by the physician to make a determination whether to increase the dose to either 75 mg or 100 mg for the following week. This determination was highly individualized.

Week-2 to week-12: Again the dosing was highly individualized depending on response and tolerability.

To ensure compliance, a precise number of tablets was distributed each visit and the subjects were required to return the vial with any unused medication at the following visit. Based on this, plus some spot-checks of quetiapine blood levels (using the heparinized blood plasma), we were able to ensure compliance and calculate the following range of doses overall:

- Quetiapine doses used from week-0 to week-12 were 25-300 mg daily.
- Mean dose between baseline and week-1: 30.85 mg (SD=15.82)
- Mean dose at week-12: 169.23 mg (SD=94.15)

### Course of treatment

Of the 47 patients who signed the informed consent and gave baseline platelet samples, 4 dropped out before even receiving a single dose of quetiapine for personal reasons unrelated to the study. The remaining 43 patients received at least 1 dose of quetiapine. Unfortunately not all subjects were able to give a sufficient volume of blood for both categories of platelet measures (due to occasional phlebotomy problems) and consequently the post-treatment number of aggregometry samples (especially at week-12) was n=19.

### Pre-quetiapine platelet comparisons

Concerning flow cytometry (Table 2), the citrated blood from untreated MDD patients showed 6.7 ± 1.3% platelets labeled with P-selectin compared to 4.7 ± 1.9% of platelets from the HC subjects. That is, MDD patients showed a baseline surface P-selectin level in unstimulated blood which was higher than in the healthy controls but this difference did not reach statistical significance. The *in vitro* addition of agonists led to higher absolute values of surface P-selectin in each group. The induced levels due to +ADP or +AA were lower

| Groups | Week-0 | Quetiapine Week-8 | Quetiapine Week-12 |
|---|---|---|---|
| **MDD subjects** | | | |
| **Saline** | 6.7 ± 1.3 (n=47) | 7.0 ± 1.6 (n=25) | 3.9 ± 0.5 (n=23)* |
| **+ADP** | 20.0 ± 1.5 (n=47) | 18.0 ± 1.7 (n=25) | 20.2 ± 1.5 (n=23) |
| **+AA** | 22.1 ± 1.5 (n=47) | 18.3 ± 1.9 (n=25) | 19.6 ± 1.7 (n=23) |
| **Healthy controls** | | | |
| **Saline** | 4.7 ± 1.9 (n=26) | NA | NA |
| **+ADP** | 25.0 ± 2.1 (n=24)$^{NS}$ | NA | NA |
| **+AA** | 25.5 ± 2.2 (n=24) | NA | NA |

Note: * P= 0.035 compared to week-8.
NS not significantly different from healthy controls (P=0.067).
No significant differences were noted comparing baseline to week-8 or week-12 values.
All values represent mean ± SEM. "NA" not applicable.

**Table 2:** Whole Blood Flow Cytometry: Percent Platelets Labeled with P-Selectin.

in patients compared to healthy controls but the difference was not statistically significant.

Table 3 shows the PRP aggregation data. The baseline aggregatory response to + saline (with stirring) showed significantly higher resting platelet activity in untreated MDD patients. The mean value was 3 times higher than in HC subjects (p=0.021). The *in vitro* addition of agonists at baseline yielded lower levels of aggregation in untreated MDD patients compared to HC subjects but the difference was not statistically significant (+ADP and +AA shown in Table 3). Finally, platelet endpoints were evaluated in relation to dose. No significant correlations were determined.

## Post-quetiapine group comparisons

Changes from baseline were observed after quetiapine treatment as evaluated by flow cytometry of surface P-selectin in the saline-control blood. Control (+saline) values in MDD subjects slightly but non-significantly increased at week-8, but then decreased significantly at week 12. The difference between week-8 and week-12 was statistically significant (P=0.035) (Table 2). However, +ADP-induced platelet surface P-selectin expression in patients on quetiapine showed no significant change throughout the 12 weeks of treatment (Table 2). Similar insignificant changes were noted with +AA throughout the 12 weeks of treatment (Table 2).

Results obtained from PRP aggregometry were quite different from the above (Table 3). In MDD subjects +saline aggregation response of PRP was 7.8% and 6.5% after 8 and 12 weeks of quetiapine treatment, respectively. Both time points differed significantly from baseline (week-0) (p=0.035). They did not differ from each other and they did not differ from +saline in HC subjects (p=0.359 paired t test). Results with +ADP showed no significant change at any time point. Percent maximal response with +AA was not changed at week-8 of treatment. However, at week-12 response was significantly lower when compared to week-0 (P=0.016) and Week-8 9P=0.044) and also significantly lower than HC subjects (P=0.001).

Platelet endpoints were not associated with response status of MDD subjects.

| Groups | Week-0 | Quetiapine Week-8 | Quetiapine Week-12 |
|---|---|---|---|
| **Major Depressives** | | | |
| **Saline** | 31.8 ± 4.4 (n=47) * | 7.8 ± 1.3 (n=27)** | 6.5 ± 1.0 (n=19) **£ |
| **+ADP** | 58.5 ± 3.4 (n=47) | 60.4 ± 4.5 (n=27) | 61.5 ± 5.9 (n=21) |
| **+AA** | 63.7 ± 4.1 (n=47) | 56.6 ± 6.7 (n=27) | 37.1 ± 8.2 (n=21)*** |
| **Healthy Controls** | | | |
| **Saline** | 10.6 ± 7.5 (n=17) | NA | NA |
| **+ADP** | 61.8 ± 4.8 (n=25) | NA | NA |
| **+AA** | 72.3 ± 5.6 (n=26) | NA | NA |

**Note:** *Significantly higher than healthy controls (P=0.021).
** Not significantly different than healthy controls (P=0.352), but significantly lower (P= 0.035) compared to themselves at week-0 (paired t-test, n=27).
*** Significantly lower than healthy controls +AA (P=0.001), significantly lower (P=0.044) than themselves at week-8, and significantly lower (P=0.016) compared to themselves at week-0 (paired t-test, n=21).
£ Not significantly different than healthy controls (p=0.438) or Week-8 (p=0.359: paired t-test).
No other significant differences were noted between week-8 and week-12 values.
All values represent estimated marginal means ± SEM. "NA" means not applicable.

**Table 3:** Platelet-Rich Plasma Aggregometry: Percent Maximal Response.

## HAM-D correlations

The changes in depression rating scores (i.e., HAM-D from pre- to post-treatment) were examined for correlations with changes in each platelet biomarker. Then, after examining the data thoroughly, we decided for the sake of brevity to omit the values derived from epinephrine, collagen, and TRAP stimulations in the Results section because there were no statistical group differences or treatment findings with these agonists. In our previous study with escitalopram [26], a number of *strong negative* correlations had emerged when PRP aggregation values were plotted in association with subjects' mood symptoms, at pre- or post-treatment and with multiple agonists; however, no significant correlations were found amongst the flow cytometry responses in that study [26]. Therefore, we sought to determine if the same finding would be obtained in the study of quetiapine. It was not. The closest to a negative correlation (or any significant correlation with any of the study parameters) was regarding the HAM-D scores and baseline ADP-induced aggregation across subjects; correlation r = -0.216, p =0.144 (not significant). To conclude the analysis, we also explored whether the flow cytometry or PRP aggregation responses to the *in vitro* additions of collagen, epinephrine, or TRAP that had shown no hint of group effects, might nonetheless be correlated with HAM-D scores. No significant correlations emerged. These findings are congruent with our inability to detect any significant correlations between treatment response and any of the flow cytometry or aggregometry parameters.

## Discussion

The main hypothesis underlying this study was that platelets from untreated MDD patients would show higher than normal platelet activation as assessed by PRP aggregation and P-selectin flow cytometry. More specifically, this increased platelet activation would be detectable primarily in the platelet resting state (no agonists added), but possibly also in *in vitro* agonist stimulations. We formulated this hypothesis despite the fact that our most recent study [26] had in some regards failed to statistically confirm the presumptive pre-treatment elevation of platelet activation, yet knowing that the prevailing literature still supported this view, it was reasonable to reexamine this issue. With respect to post-treatment effects on platelet activation, our three previous studies using different types of antidepressants [9,16,26] have led us to the tentative conclusion that there is a class-dependent treatment effect. A review of the literature also reveals that SSRIs and non-SSRIs alike (notably norepinehrine reuptake inhibitors-NRI's), and even psychotherapy, may be able to down regulate platelet hyperactivity in heart-healthy depressed patients [8,33,34]. One of the studies showed an effect on platelet aggregation following 12 weeks of either escitalopram (SSRI) or nortriptyline (NRI), but the finding was confined to mood responders [33].

Another important factor to consider in interpreting the reported findings is that acute life stressors can physiologically elicit activation of circulating platelets [30,31]. Some studies have shown that platelet responses to acute life stressors are more exaggerated in depressed patients relative to healthy subjects [31,35]. Aschbacher and colleagues first reported [35] that platelet activation responses to psychological stress are *positively* correlated with rating scores of depression and in a subsequent study they showed that a robust platelet activation to psychological stress is a stable trait exhibited over several years [36].

In Tables 2 and 3 it can be seen that only the aggregometry method distinguished MDD from HC. What might explain this disconnect between Table 2 (showing a mere small flow cytometry change in depression) and Table 3 (showing higher aggregation at baseline as

well as quetiapine's effects on AA-induced aggregation by week-12)? Several possibilities may be considered, but certainly flow cytometry is a gentler technique because it uses undisturbed whole blood. Aggregometry invokes centrifugal force (during PRP preparation) as well as mechanical stirring in the aggregometer. With stirring there is probably some release of 5-HT, to which the platelets from depressed subjects are more sensitive. This could explain the extent of aggregation seen with saline at baseline in depressed subjects. The normalization of this endpoint could be due to serotonergic antagonism by quetiapine.

Flow cytometry can also be viewed as a "snap-shot" of P-selectin, whereas the aggregometer follows platelet changes over 5 min. There is the primary wave which is known to be the immediate agonist-mediated clumping response, and then there is the secondary wave which is believed to be linked to subsequent dense granule secretion. It is during the second wave that serotonin is released. Most likely, there is no serotonin release during flow cytometry, and, on this basis, the aggregometry assay may be particularly better suited to studying antidepressant effects. However, this may not be such a critical difference in this regard. With flow cytometry we report P-selectin expression after 3 minutes of incubation; with aggregation, the results presented are a final level of aggregation after 5 minutes.

In the whole blood flow cytometry studies, while the agonist induced aggregation responses were not significantly different in the MDD subjects at 8 and 12 weeks, at 12 weeks the P-selectin expression on platelets was significantly lower at week 12. This is probably due to the indirect effect of quetiapine on the platelet activation process. While the exact mechanism of this down regulation is not known, this finding is consistent to the notion that the treated patients exhibit lower platelet activation. Additionally in the platelet aggregation studies the auto aggregation responses in the MDD group were markedly higher in comparison to the healthy controls. This may be due to the activation of platelets in the MDD group which is consistent with the previously reported data on endogenous auto activation of platelets in depressed patients [37].

In our previous study with escitalopram [26] we found a negative correlation with HAM-D scores which was difficult to explain. We speculated it aligned with an elevation of plasma epinephrine and other stress hormones in depression. We proposed that the platelets of depressed patients may have undergone pre-blood drawing compensatory mechanisms to these stress hormones which had desensitized their platelet responses and given the negative correlation observed [38]. By contrast, our present study which allowed our patients to better habituate before the blood drawing, did not detect any such evidence. Nor, however, did we observe the positive association with mood change scores that we had hypothesized. There was also no evidence in this study for any dose relationship between the degree of platelet normalization following quetiapine and the extent of mood improvement.

To summarize, we have found that untreated MDD patients display higher platelet aggregation responses in the absence of added agonists, and that following quetiapine treatment there was a normalization of this "resting" auto aggregation. Furthermore, the finding did not extend to measures obtained by flow cytometry. We suggest that this pattern of results may be explained by the serotonin (and other mediators) release stage of platelet aggregation which is likely to be over-shadowed in the presence of added agonists, but which becomes prominent during the secondary wave of platelet aggregometry when no agonists are added. A surprising finding was the AA-induced platelet aggregation pattern which was normal in untreated MDD patients and progressively decreased after 8 and 12 weeks on quetiapine to abnormally low values at 12 weeks. The platelet aggregometry changes may not be due to quetiapine's activity at platelet receptors or uptake sites in view of the negative findings with flow cytometry and given that the concentrations required would exceed free levels of the drug reported to act on the known platelet receptors. Nevertheless, quetiapine's reported [29] ability to intercalate into platelet membranes may be relevant. Based on the 8 week time course of the effects, it appears more likely that the mechanism of quetiapine on platelet aggregation may be secondary to CNS mediated mood improvement.

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### Author contributions

JP and AH designed the study and wrote the protocol, which was approved by AstraZeneca and the Loyola IRB. AH and EM were responsible for all clinical aspects of the study and clinical data acquisition. JP, DH, WJ and JF were responsible for the assays and the interpretation of the findings. JS was responsible for statistical analyses. JP wrote the first drafts of the manuscript. A complete description of the findings was submitted to AstraZeneca in 2011. All authors have contributed and have approved the final version of the manuscript.

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