Dose-escalation in the picture: pharmacological and imaging studies in depression
Ruhé, H.G.
Effect of the selective serotonin reuptake inhibitor paroxetine on platelet function is modified by a SLC6A4 serotonin transporter polymorphism

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Abstract

Background
Selective serotonin reuptake inhibitors (SSRIs) have been associated with an increased bleeding tendency.

Aim
To prospectively quantify the dose-response effects of paroxetine and the influence of the serotonin transporter gene (SLC6A4) promoter polymorphism (5-HTTLPR) on platelet function.

Methods
Nineteen drug-free psychiatric outpatients (44.5 ±10.8 years), were tested before and after 6 weeks of paroxetine treatment (20 mg/day). Based on clinical symptoms, paroxetine dosages were increased (40-50 mg/day) for 6 more weeks in 11 patients. Parameters related to platelet function were assessed by bleeding time, platelet function analyzer (PFA), platelet serotonin, platelet factor 4 (PF4), β-thromboglobulin (β-TG), and aggregation tests.

Results
Paroxetine 20 mg/day increased mean bleeding time by 1.2 minutes (95% confidence interval (95% CI) -0.2-2.7) and reduced median platelet serotonin level (463 ng/10^9 platelets; Inter Quartile Range (IQR) 361-666), and platelet β-TG concentration (3.1 IU/10^6 platelets; IQR 0.3-6.0). Other platelet parameters did not change significantly. Serial platelet aggregation tests did not become abnormal. Paroxetine dose-escalation did not further influence platelet function. However, 5-HTTLPR polymorphisms modified these effects: in L^-/- carriers, bleeding times did not change (-0.2 minutes; (95% CI -0.6 to 0.9)), while bleeding times significantly increased in <2L^- allele carriers (2.3 minutes (95% CI 0.5 to 4.07); p=0.032). Platelet serotonin decreases were larger in patients without L^- alleles (868 ng/10^9 platelets; (IQR 585 to 1213)) than in ≥1 L^- allele carriers (457 ng/10^9 platelets; (IQR 392 to 598); p=0.035). PFA closure time and PF4 increased significantly in patients without L^- alleles.

Conclusions
Paroxetine 20 mg/day does not increase overall bleeding time, but impairs platelet function by decreasing the levels of platelet serotonin and platelet β-TG. These paroxetine effects appear to be mediated by 5-HTTLPR, with most pronounced effects in patients without L^- alleles.
Introduction

Selective serotonin reuptake inhibitors (SSRIs) belong to the most widely prescribed classes of drugs and are often used for the treatment of depression and anxiety disorders. However, because of their extensive use, the impact of relatively infrequent side effects is of potential clinical importance. One of these harmful side effects is an increased bleeding tendency.

Over the years, numerous case reports and case control studies have suggested that SSRI use is associated with an increased risk of bleeding. The bleeding pattern varied from minor bleeding to spontaneous gastro-intestinal bleeding and excessive perioperative hemorrhage. One study showed that SSRI users who underwent orthopedic surgery had a nearly four-fold increased risk to require blood transfusion. In addition, large population based case-control studies have consistently shown an increased incidence of upper gastrointestinal bleeding associated with SSRI use, especially when co-medicating with nonsteroidal anti-inflammatory drugs, aspirin or coumarins.

The underlying pathophysiological mechanisms of the increased bleeding tendency of SSRIs were previously studied, and significant changes in platelet function related to SSRI use were consistently reported. These changes included a decreased aggregation with epinephrine and collagen, a prolongation of the PFA closure time, and a decrease in plasma beta-thromboglobulin (β-TG) and plasma platelet factor 4 (PF4).

Furthermore, SSRIs block serotonin transporters (SERTs), which increases serotonergic transmission between neurons and results in antidepressant effects. However, SSRIs also block the SERTs of blood platelets, causing decreased platelet uptake of serotonin. Serotonin mediates vasoconstriction, platelet aggregation, and platelet activation after vessel injury, and since platelets cannot synthesize serotonin, treatment with an SSRI could lead to depletion of platelet serotonin and impair hemostasis.

Polymorphisms of the SERT gene (SLC6A4) promoter region (5-HTTLPR) are associated with the transcriptional activity of the SERT gene and the rate of serotonin uptake. The 5-HTTLPR polymorphism is located approximately 1 kb upstream of the transcription initiation site and is composed of 16 repeat elements. Human lymphoblasts homozygous for the long (L) 5-HTTLPR allele produce higher concentrations of SERT mRNA than cells containing one or two copies of the short (S) allele. Furthermore, the rate of serotonin uptake by the transporter is more than twofold higher in cells homozygous for the L allele. In Caucasians the L allele is found more frequently (57%) than the S allele (43%), with a 5-HTTLPR genotype distribution of 32% LL, 49% LS and 19% SS. However, other populations show different prevalences, especially Asians, who have more than two-fold higher SS genotype frequencies. Nowadays, the 5-HTTLPR polymorphism is considered tri-allelic. The L allele can be subdivided in an L_C and an L_A variant by a common SNP (rs25531), which creates a functional transcription factor binding site (L_C), behaving like a short allele. This tri-allelic classification is probably more reliable to find associations between 5-HTTLPR polymorphisms and phenotypes (e.g. SSRI adverse effects). In Caucasians the S:L_A:L_C ratio is approximately 8:10:2, while in black patients this is 5:10:5.

Although an association between SSRI use and platelet dysfunction is undoubted, it remains uncertain whether a dose-response relationship exists between SSRI use and platelet function. In addition, since functional polymorphisms of the serotonin transporter influence the rate of serotonin uptake, they may also mediate the effects on hemostasis.

In this study, we evaluated the effect of standard and increasing dosages of the SSRI paroxetine on the platelet function of SSRI free patients. In addition, we assessed whether this effect is influenced by the 5-HTTLPR/rs25531 polymorphism.
Methods

Participants
After approval by the local ethics committee and written informed consent, we recruited 19 adult psychiatric outpatients (18-75 years) from February 2006 until February 2007. The inclusion criterion was indication of SSRI use (for mood or anxiety disorders) as determined by the treating physician. We excluded patients who used aspirin, NSAIDs (in the preceding 48 hours) or antidepressants (less than 4 weeks prior to inclusion), and patients who had a history of increased bleeding tendency.

Treatment and timing of measurements
After baseline assessment, patients were treated with paroxetine 20 mg/day for 6 weeks. If at 6 weeks the clinical symptoms of the patients had not improved by 50%, physicians could increase the dose (dose-escalation) to a maximum of 50 mg/day over the next 6 weeks, as recommended by current psychiatric treatment guidelines.1 As a measure to detect increased bleeding tendency, in all patients the bleeding severity score (Tosetto et al.)28 was assessed at baseline, after 6 weeks, and after 12 weeks of treatment (if a dose-escalation was prescribed). Platelet parameters were assessed at the same time points by technicians that were unaware of the treatment regimen.

Measurements of platelet parameters
Blood samples
Blood was collected in open 10 mL tubes containing 1 ml 3.2% sodium citrate by venapuncture from the antecubital vein using a 19-gauge needle. Within 60 minutes after collection, platelet rich plasma (PRP) was prepared by centrifugation at 190 g at room temperature for 10 minutes without braking. PRP was separated from the packed cells; after 30 minutes aggregation studies were started. PECT tubes for 4 ml blood containing 0.4 ml of a mixture of 94 nmol/L Prostaglandin E1, 90 mmol/L EDTA, 0.63 mmol/L sodium carbonate and 10 mmol/L theophylline were used for the determination of ß-TG and PF4 in plasma. Blood samples were collected in PECT tubes to prevent in vitro release of ß-TG and PF4 from platelets. K3EDTA 7.5% anti-coagulant tubes were used for the determination of platelet count.

Platelet parameters
Bleeding time was measured with a standard incision using the Surgicutt device (Surgicutt Adult, ITC Edison, USA). Platelet function was measured with the PFA-100 (Siemens Healthcare Diagnostics, Marburg Germany) using epinephrine (EPI) and ADP containing cartridges. Platelet aggregation in platelet-rich-plasma with ADP, ristocetin, collagen and arachidonic acid was analyzed qualitatively with a standardized aggregometer (model 540-vs, Chrono-Log Corporation, Havertown, USA).

The amount of ß-TG and PF4 in platelets and plasma was measured using enzyme-linked immunosorbent assays (Asserachrom ß-TG and PF4, Diagnostica Stago, Roche). ß-TG and PF4 in plasma were determined in PECT samples. ß-TG and PF4 in platelets were determined after pelleting the platelets from PRP. The platelets were then destroyed by a combination of Triton (2% Triton X-100) and sonication for 15 seconds on ice (microtip, Branson, amplitude 50%). After 5 minutes of centrifugation at 13,000 rpm, the supernatant was used for analysis.

Serotonin
In the sonicated supernatant, the amount of serotonin was measured in an acidified (HClO4) environment by fluorimetry (Fluostar Galaxy, BMG Offenburg, Germany).
Paroxetine induced platelet function changes

Paroxetine serum concentrations
We collected blood samples for measurement of paroxetine serum concentrations (PSC) after 6 and 12 weeks of paroxetine treatment from 19 and 11 patients, respectively. PSC were measured using a validated HPLC-MS/MS method (details available on request). The lower limit of quantification was 5 µg/L, which was at the lower end of the therapeutic range of paroxetine in serum (5-75 µg/L). The lower limit of detection was 0.3 µg/L.

Genotyping procedures and analysis
Genotyping was performed as described earlier.23 In brief, genomic deoxyribonucleic acid (DNA) was isolated and the length of the 5-HTTLPR polymorphism was determined by gel electrophoresis. The region around the polymorphism was amplified by PCR. Genotyping of the rs25531 SNP was done by sequencing. The length of the 5-HTTLPR polymorphism was confirmed by looking at the length of the sequenced PCR product. Taking into account this SNP, we reclassified the tri-allelic genotypes into a modified bi-allelic classification: S'/S' (S/S, L_C/S, L_C/L_C = non L_A), S'/L_A (S/L_A, L_C/L_A = 1 L_A) and L_A/L_A (= 2 L_A). Because it is unknown which allele is dominant (heterozygosity), we either grouped S'/S' and S'/L_A as <2 L_A alleles versus ≥1 L_A or contrasted ≥1 L_A vs. non L_A genotypes in our analyses.

Statistical analyses
We first computed means for the platelet parameters at baseline, after 6 and 12 weeks of paroxetine administration. We compared patients who received a further dose-escalation from week 6 onwards with patients who remained at 20 mg/day with χ² tests for categorical data and independent T-tests for continuous data. We compared the changes in platelet parameters over time with paired T-tests (baseline vs. 6 weeks and 6 vs. 12 weeks). In case of a non-normal distribution (assessed by histograms and Shapiro Wilks’ test) we present medians with interquartile ranges (IQR) and used nonparametric Wilcoxon signed ranks tests for paired data. For the platelet parameters that appeared to change significantly after 6 weeks of treatment, we further explored the changes in linear mixed models;29 we examined the effects of paroxetine dosage by the significance of a dose*time interaction.

In addition, we investigated the relation of PSC with changes in relevant platelet parameters in linear regression models using all observations (after 6 and 12 weeks) at once. We introduced the 5-HTTLPR polymorphism to explore the genetic influence on these relations. Because of our sample size and non-normal distribution of bleeding times and platelet parameters, we tested differences between genotypes with nonparametric Mann-Whitney tests. All analyses were performed in SPSS v15.0.1.

Results

Patients
Nineteen subjects who were diagnosed with either a mood disorder (n= 13), an anxiety disorder (n= 2) or both (n= 4) participated in this study. The mean age was 44.5 years (SD 10.8) and 11 (58%) were female. Co-medication consisted of depakine (for epilepsy; n= 1), levothyroxine (n= 1) and omeprazol (n= 1).

All subjects received a standard dose of paroxetine 20 mg/day for 6 weeks (mean PSC 25.6 µg/L (95% CI 14.3 to 36.9)). In 11 subjects who failed to show sufficient clinical improvement after this period, paroxetine dosages were increased to 40 (n= 4) or 50 mg/day (n= 7) for another 6 weeks (mean PSC 107.2 µg/L (95% CI 42.2 to 172.2)). The group that only received standard dosage was comparable with the increased dosage group in type of psychiatric disorder, age and sex (Table 11.1).
Bleeding severity scores

At baseline, the bleeding severity score was low (<3) in all subjects. Six weeks at paroxetine 20 mg/day, or a subsequent dose-escalation for 6 weeks thereafter did not affect the bleeding score.

Platelet parameters

Six weeks of treatment with paroxetine non-significantly increased the mean bleeding time by 1.2 minutes (95% CI -0.2 to 2.7; Paired t-test; p = 0.083; Table 11.2). Furthermore, paroxetine resulted in a significant reduction of median platelet serotonin level of 463 ng/10⁹ platelets (IQR 361 to 666; Wilcoxon signed ranks test; p<0.001), and median platelet ß-TG level of 3.1 international units (IU)/10⁶ platelets (IQR 0.3 to 6.0; Wilcoxon signed ranks test; p= 0.016). Other changes in platelet parameters were not statistically significant, nor did the aggregation tests reveal qualitative abnormalities.

Dose-escalation of paroxetine to 40-50 mg/day for another 6 weeks did not lead to further reduction of platelet serotonin or platelet ß-TG levels or to a further increase of the bleeding time (Table 11.3). The absence of further changes in platelet ß-TG and serotonin levels were confirmed by non-significant dose*time interactions in mixed models.

There was no correlation between plasma paroxetine levels and changes in platelet function tests, including the change in platelet serotonin and platelet ß-TG levels. Platelet aggregation tests with ADP, ristocetin, collagen and arachidonic acid were not influenced by paroxetine administration (data not shown).

<table>
<thead>
<tr>
<th>Table 11.1. Baseline characteristics of patients starting treatment with paroxetine 20 mg/day.</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Age ±SD</td>
</tr>
<tr>
<td>M/F ratio</td>
</tr>
<tr>
<td>Psychiatric diagnosis</td>
</tr>
<tr>
<td>n (%)</td>
</tr>
<tr>
<td>Mood disorder*</td>
</tr>
<tr>
<td>Anxiety disorder†</td>
</tr>
<tr>
<td>Mood + anxiety disorder</td>
</tr>
<tr>
<td>Substance abuse‡</td>
</tr>
<tr>
<td>Eating disorder</td>
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</tbody>
</table>
* Unipolar depression (n= 16; 89.5%) and/or dysthymia (n= 3; 15.8%) † Panic disorder with or without agoraphobia (n= 4; 21.1%). ‡ Post traumatic stress disorder (n= 2; 10.5%)

<table>
<thead>
<tr>
<th>Table 11.2. Changes in platelet function after 6 weeks of treatment with paroxetine 20 mg/day (total n= 19).</th>
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</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Platelets (x10⁹/l)</td>
</tr>
<tr>
<td>Bleeding time (min)</td>
</tr>
<tr>
<td>PFA-ADP (sec)</td>
</tr>
<tr>
<td>PFA-epinephrine (sec)</td>
</tr>
<tr>
<td>PF4 platelets‡ (IU/10⁶ platelets)</td>
</tr>
<tr>
<td>PF4 plasma‡ (IU/mL)</td>
</tr>
<tr>
<td>ß-TG platelets‡ (IU/10⁶ platelets)</td>
</tr>
<tr>
<td>ß-TG plasma‡ (IU/mL)</td>
</tr>
<tr>
<td>Platelet serotonin‡ (ng/10⁹ platelets)</td>
</tr>
</tbody>
</table>
* n for pairs without missing values † p-values for paired t-tests for normally distributed data and paired Wilcoxon signed ranks test for non-normally distributed data ‡ due to non-normal distribution medians and interquartile ranges (IQR) are shown.
Paroxetine induced platelet function changes

Effects of the 5-HTTLPR polymorphism

Genotype was analyzed in eighteen patients, of whom 3 had the S'/S', 9 the S'/LA, and 6 the LA/ LA genotype, respectively. For these 18 patients, 26 observations after 6 and 12 weeks were available when combining data from these time points. In patients with the 2LA genotype, paroxetine treatment did not increase bleeding time (-0.2 minutes; 95% CI -0.6 to 0.9), while in patients carrying the <2LA genotype, bleeding time increased by 2.3 minutes (95%CI 0.5 to 4.1, Mann-Whitney test; p= 0.032). In a linear regression model the <2LA genotype significantly predicted the change in bleeding time (p<0.001). This difference in bleeding time between the two genotype groups was not explained by a decrease in platelet serotonin, dosage or PSC (linear regression; pchange> 0.37).

The median decrease in platelet serotonin levels of patients with paroxetine treatment was not different between the <2LA and 2LA genotypes (<2LA; 587 ng/10⁹ platelets; IQR 393 to 841; 2LA: 457 ng/10⁹ platelets; IQR 373 to 582; Mann-Whitney test; p= 0.244). However, in patients without LA alleles, we found significant decreases in serotonin, and increases in PFA-ADP, PFA-EPI and platelet PF4 after 6 and 12 weeks of paroxetine treatment (Mann-Whitney test; Table 11.4; Figure 11.1).

Table 11.3. Changes in platelet parameters in patients who received a secondary dose-escalation (40-50 mg) paroxetine after 6 weeks (n= 11).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Difference baseline vs. 6 weeks (mean ±SD)</th>
<th>6 weeks (mean ±SD)</th>
<th>12 weeks (mean ±SD)</th>
<th>n</th>
<th>Difference 6 vs.12 weeks (mean 95% CI)</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets (x10⁹/l)</td>
<td>-9.9 (-23.5 - 2.7)</td>
<td>242 ±61</td>
<td>249 ±53</td>
<td>11</td>
<td>7.0 (-13.8 - 27.8)</td>
<td>0.470</td>
</tr>
<tr>
<td>Bleeding time (min)</td>
<td>1.3 (-0.1 - 3.1)</td>
<td>5.6 ±2.3</td>
<td>5.8 ±2.3</td>
<td>9</td>
<td>0.4 (-1.1 - 2.0)</td>
<td>0.523</td>
</tr>
<tr>
<td>PFA-ADP (sec)</td>
<td>-1.4 (15.8 - 13.1)</td>
<td>88.0 ±16.4</td>
<td>91.7 ±15.3</td>
<td>11</td>
<td>3.7 (8.2 - 15.6)</td>
<td>0.502</td>
</tr>
<tr>
<td>PFA-epinephrine (sec)</td>
<td>9.5 (14.7 - 33.6)</td>
<td>122.6 ±39.7</td>
<td>124.5 ±30.0</td>
<td>10</td>
<td>0.9 (19.5 - 21.3)</td>
<td>0.923</td>
</tr>
<tr>
<td>(median IQR)</td>
<td>(median IQR)</td>
<td>(median IQR)</td>
<td>(median IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF4 platelets (IU/10⁶ platelets)</td>
<td>-0.2 (-2.3 - 2.7)</td>
<td>16.0 (12.5 - 18.4)</td>
<td>15.0 (14.3 - 17.0)</td>
<td>10</td>
<td>-0.8 (-2.2 - 2.2)</td>
<td>0.799</td>
</tr>
<tr>
<td>PF4 plasma (IU/mL)</td>
<td>0.1 (-6.8 - 8.9)</td>
<td>6.4 (4.3 - 18.2)</td>
<td>5.2 (3.7 - 12.2)</td>
<td>10</td>
<td>-2.6 (-13.4 - 9.9)</td>
<td>0.575</td>
</tr>
<tr>
<td>β-TG platelets (IU/10⁶ platelets)</td>
<td>-3.7 (-9.4 - -0.3)</td>
<td>31.2 (27.4 - 35.3)</td>
<td>32.8 (28.6 - 35.4)</td>
<td>10</td>
<td>1.0 (-2.2 - 2.5)</td>
<td>0.575</td>
</tr>
<tr>
<td>β-TG plasma (IU/mL)</td>
<td>0.9 (-15.5 - 22.0)</td>
<td>28.0 (20.9 - 65.2)</td>
<td>22.7 (19.3 - 38.9)</td>
<td>10</td>
<td>5.6 (-39.6 - -0.1)</td>
<td>0.074</td>
</tr>
<tr>
<td>Platelet serotonin (ng/10⁹ platelets)</td>
<td>-499 (-780 - -360)</td>
<td>118 (74 - 143)</td>
<td>100 (86 - 145)</td>
<td>10</td>
<td>1.50 (23.50 - 14.00)</td>
<td>0.799</td>
</tr>
</tbody>
</table>

* n for pairs week 6 – week 12 without missing values
† p-values for paired t-tests for normally distributed data and paired Wilcoxon signed ranks test for non-normally distributed data
‡ due to non-normal distribution medians and interquartile ranges (IQR) are shown.
§ Wilcoxon signed ranks test p= 0.051
¶ Wilcoxon signed ranks test p= 0.008

Table 11.4. Changes in platelet parameters after 6 and 12 weeks of paroxetine treatment, relative to baseline, stratified for genotype.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Platelet serotonin (ng/10⁹ platelets)</th>
<th>PFA-ADP (sec)</th>
<th>PFA-epinephrine (sec)</th>
<th>PF4 platelets (IU/10⁶ platelets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No LA/LA</td>
<td>-868 (-1213 - -585)</td>
<td>23.0 (17.0 - 48.0)</td>
<td>47.0 (26.5 - 63.5)</td>
<td>3.2 (0.7 - 4.7)</td>
</tr>
<tr>
<td>≥1 LA p*</td>
<td>-457 (-598 - -392)</td>
<td>-0.5 (-15.5 - 5.0)</td>
<td>4 (-16.0 - 19.0)</td>
<td>-0.4 (-1.5 - 1.7)</td>
</tr>
</tbody>
</table>

Values represent changes of platelet parameters of combined observations after 6 and 12 weeks of paroxetine treatment (relative to baseline; 29 observations for 18 patients). Negative numbers indicate a decrease of the parameter.

* Mann-Whitney non-parametric test
Figure 11.1. Reduction of platelet serotonin levels after paroxetine exposure stratified by SERT genotype (n=18).

Boxplots show mean (+), median (-), interquartile range, and minimum-maximum reductions in serotonin levels after 6 and 12 weeks of paroxetine treatment (compared with baseline). For 18 patients observations after 6 weeks were available, for 8 patients (secondary receiving higher doses) observations after 12 weeks were available. Data are stratified for genotype: S’/S’ (S/S, S/L_A, L_A/L_A; n=3; 3 observations after 6, and 2 after 12 weeks), S’/L_A (S/L_A, L_A/L_A; n=9; 9 observations after 6, and 1 after 12 weeks) and L_A/L_A (n=6; 6 observations after 6, and 5 after 12 weeks). There was a significant difference between the genotype group S’/S’ vs. S’/L_A + L_A/L_A (Mann-Whitney; p=0.035).

Discussion

This study confirms that treatment with the SSRI paroxetine at 20 mg/day decreases platelet serotonin and platelet β-TG levels, and increases bleeding time. Paroxetine treatment did not affect other platelet parameters or bleeding tendency. Dose-escalation of paroxetine to 40-50 mg/day did not further change platelet parameters. Additionally, the 5-HTTLPR polymorphism of the patients strongly affected the effect of paroxetine on bleeding time, platelet serotonin and PF4, PFA-ADP and PFA-EPI. In patients with the L_A/L_A genotype, paroxetine did not influence bleeding time, whereas patients with non-L_A/L_A genotypes had an increased bleeding time. Especially patients with the S’/S’ genotype had a greater reduction in platelet serotonin level, and increased PFA-ADP, PFA-EPI and platelet PF4.

Previous studies did not investigate dose-escalation. Because of the large inter-individual difference of plasma SSRI levels at stable doses, proper investigation of a dose-response relation require quantification of within-subject changes, instead of comparisons of (small) groups. By doing so, paroxetine at 20 mg/day already maximally prolonged bleeding time and decreased platelet serotonin, which was not altered by consecutive dose-escalation (to 40-50 mg/day). This is in accordance with the recent finding that SERT occupancy in the brain is not increased by dose-escalation.

Of interest are two in vitro studies that reported dose-response relations for sertraline and venlafaxine. Serebruany et al. reported a significantly prolonged in vitro PFA-ADP and PFA-epinephrine with sertraline concentrations that were claimed to mimic the plasma levels observed in patients using sertraline 50, 100 or 200 mg/day. Increased platelet aggregation with ADP, arachidonic acid, epinephrine and collagen was found in vitro, but with venlafaxine
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concentrations 1000-fold higher than usual plasma concentrations of treated patients.\footnote{31} However, these in vitro studies incubated samples with antidepressants immediately before assessing platelet function. Normally platelet serotonin levels decrease \textit{in vivo} only after >7 days, since the circulating platelet pool must be renewed before effects of SSRIs on platelet function are measurable.\footnote{31} This raises concern about the validity of \textit{in vitro} experiments.

The effect of the SERT polymorphism on platelet function

The effect of paroxetine on platelet function highly depended on the SERT polymorphism of the patients. In mice the influence of the serotonin transporter can be studied in strains without (knockout Slc6a4\textsuperscript{-/-}), with 1 (Slc6a4\textsuperscript{+/-}) or with 2 alleles (Slc6a4\textsuperscript{++}) of the SERT gene. In knockout mice the serotonin level in peripheral tissues (e.g. platelets) is <10\% compared with non-knockouts. In Slc6a4\textsuperscript{-/-}-mice (best resembling humans with S/S genotypes), peripheral serotonin levels were unchanged compared with Slc6a4\textsuperscript{+/-} mice. Thus, in Slc6a4\textsuperscript{+/-}-mice overall serotonin homeostasis can be retained, despite a 4 to 5 fold decrease in serotonin reuptake.\footnote{32} Furthermore, a recent platelet aggregation study in Slc6a4\textsuperscript{-/-}-mice showed 80\% reduction in ADP-induced aggregation, not observed in Slc6a4\textsuperscript{+/-}-mice, while results of this study also indicated a more prominent role of the requirement of ongoing SERT transport of serotonin in maintaining platelet aggregation.\footnote{33} In other words, apart from the direct effect of SSRIs reducing serotonin granules in platelets, blockade of the SERT will also impair the functioning of the SERT at the platelet surface, which is required for the aggregation process itself. This might explain our findings: despite compromised serotonin reuptake in S'/S' subjects, platelets manage to achieve adequate, normal serotonin levels under normal circumstances. However, blocking the SERT by paroxetine might affect this compromised transporter system more than in patients with the most effective L\textsubscript{A}L\textsubscript{A} genotype, resulting in larger decreases of serotonin, reduced aggregation capacity (increased PFA-ADP and PFA-EPI closure times), and longer bleeding times.

A recent study failed to find an effect of 5-HTTLPR polymorphisms on PFA-closure time.\footnote{27} Consistent with our findings, this study reported no significant differences in bleeding tendency, but unfortunately platelet serotonin levels were not measured. Furthermore, in this study the bi-allelic variant of 5-HTTLPR was genotyped, while the reclassification of the ‘tri-allelic’ A to G SNP in the long allele as an S'-variant is probably more precise to detect differences.

Limitations

Due to the small number of patients, the results of dose-escalation and genotype must be interpreted with some restraint. However, despite the modest sample size of our study, the effects of the genetic variants on platelet serotonin and bleeding time were demonstrated in conservative nonparametric tests. We furthermore consider our findings as valid, because our bleeding time abnormalities and platelet serotonin findings for subgroups of SERT genotypes clearly coincide. We did not test changes in platelet parameters over time in control patients without SSRI, nor did we investigate dose-escalation in a randomized design. Additionally, our sample size did not allow further exploration of large versus small changes in platelet parameters within genotype groups.\footnote{34}

Clinical relevance

Two potential clinical implications emerge. First, dose-escalation of paroxetine above 20 mg/day does not further impair platelet function, as it is already maximally impaired at a standard dose of 20 mg/day. In other words, increased bleeding tendency associated with SSRI use will occur irrespective of the administered dose. Second, and most important, the impairment of platelet function appears to depend on the common functional SERT promoter polymorphism. Although we only investigated paroxetine, we think this finding, if replicated, can be extrapolated to other SSRIs.\footnote{35}

The absence of an increased bleeding tendency or abnormal bleeding severity score may represent low sensitivity of the bleeding severity score to detect changes. However, this may also suggest that the observed decrease in platelet function is not clinically relevant in patients.
without trauma or surgery, unless a preexisting platelet abnormality is present. However, because of their enhanced antiplatelet response to SSRIs, patients with a 2L genotype may be at increased risk of bleeding complications during surgery or while using concomitant anticoagulants.

With a low prevalence of clinically important bleeding complications associated with SSRI use, determination of the SERT polymorphism in all patients who start with an SSRI will not be cost-effective. However, for patients with a previous severe bleeding episode (either with or without SSRI use) genotyping may be considered. When an S/S genotype is found, switching to another, non-serotonergic antidepressant might be advisable. Generally, non-serotonergic antidepressants (i.e. mirtazapine, maprotiline, doxepin or bupropion) are associated with fewer hospitalizations due to bleeding complications than intermediate serotonin reuptake inhibitors (e.g. venlafaxine and amitriptyline; Odds ratio 1.9, 95% CI 1.1 to 3.5), and high degree serotonin reuptake inhibitors (fluoxetine, sertraline, clomipramine and paroxetine; Odds ratio 2.6, 95% CI 1.4-4.8), although calculated odds ratios differed for individual antidepressants. The decision to switch must then depend on the risks (e.g. oncoming surgery) and benefits (clinical response) for individual patients.

**Conclusion**

This study shows that the SSRI paroxetine already maximally impairs platelet function at 20 mg/day, with no overall effect on bleeding time, but significant decreases in platelet serotonin and β-TG levels. Dose-escalation does not further influence hemostasis. In addition, the effect of paroxetine on platelet function appears to be largely mediated by the common functional 5-HTTLPR SERT polymorphism, with patients with the S/S polymorphism having the largest hemostatic impairment with larger decreases in serotonin, and increases in PFA closure time and platelet PF4.

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**Conflicts of interests**

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References


