Increased Serotonin Receptor Density and Platelet GPIIb/IIIa Activation Among Smokers

Jerome H. Markovitz, Lelland Tolbert, Suzan E. Winders

Abstract—This study sought to determine whether depressive symptoms and/or platelet serotonin receptor (5HT2A) density are associated with increased platelet activation (PA) found among smokers. Flow cytometric detection of PA was used to study 36 smokers and 16 nonsmokers, aged 18 to 48 years. Subjects were tested at baseline and after either smoking 2 cigarettes (smokers) or a similar resting interval (nonsmokers). Assessment of PA included both platelet secretion and fibrinogen receptor (GPIIb/IIIa) binding. Platelet 5HT2A receptor binding and saturation were tested using [3H]LSD, and depressive symptoms were measured using the Beck Depression Inventory. Platelet 5HT2A receptor density was increased among smokers versus nonsmokers (82.7 ± 67.7 versus 40.0 ± 20.2 fmol/mg protein; P < 0.005), and there was a dose-dependent relationship between receptor density and packs/d among smokers. Baseline wound-induced GPIIb/IIIa binding at 1 minute and GPIIb/IIIa binding in response to collagen stimulation in vitro was increased among smokers (P < 0.05); there were no changes in PA among smokers after smoking, and platelet secretion was not elevated among smokers. Depressive symptoms were associated with 5HT2A receptor density among nonsmokers (P < 0.005), but no such relationship was evident among smokers; PA was unrelated to 5HT2A receptor density in either group. The findings indicate that smoking is associated with increased platelet serotonin receptor density and with increased GPIIb/IIIa receptor binding, although these 2 factors are not related to each other or to depressive symptoms among smokers. Serotonergic dysfunction may be an important factor in the development of cardiovascular disease among smokers. (Arterioscler Thromb Vasc Biol. 1999;19:762-766.)

Key Words: smoking ▪ platelet activation ▪ thrombosis ▪ serotonin receptors ▪ depression

Cigarette smoking is a well-documented risk factor for cardiovascular disease.1–3 The specific mechanisms involved in this relationship, however, are not entirely clear. One proposed mechanism is an increased propensity toward thrombosis among smokers,4–6 including increased platelet activation (PA). The connection between smoking and platelet activation has been frequently studied. Although animal studies have shown increased PA in response to cigarette smoke exposure,7–9 the connection between PA and smoking has not been found in all studies in humans.10–16 Perhaps in part because of the variety of methods used to assess platelet function among smokers. Thus, despite compelling evidence from animal studies, the relationship between smoking and PA has not been consistently demonstrated in humans. The mechanisms by which smoking increases PA are also not clearly established. Increases in plasma catecholamines are known to occur with smoking,17–18 and this has been the primary mechanism suggested.7–8 However, serotonergic dysfunction also plays a role in PA,19–20 and serotonergic dysfunction has been found in some studies of smokers.21–24 Interestingly, both adrenergic and serotonergic dysfunction have been associated with clinical depression,25–29 and depression is far more prevalent among smokers,30–31 suggesting a possible link between PA and depression among smokers. The increase in platelet serotonin receptor (5HT2A) density consistently found in clinically depressed patients26–28 may have a direct impact on PA.

The present investigation sought to determine the relationship between smoking and PA, using wound-induced flow cytometric detection of PA as previously measured in our laboratory.32–34 Platelet 5HT2A receptor binding and saturation, and depressive symptoms were also assessed as factors that might mediate between PA and smoking. The study examined PA both at baseline and after smoking (among smokers only). The major hypotheses for the study were (1) smokers have greater PA, higher platelet 5HT2A receptor density, and more depressive symptoms than nonsmokers; and (2) the relationships of depressive symptoms and/or serotonergic dysfunction with PA account in part for the increased PA seen among the smokers.

Methods

Subjects

A total of 52 subjects (36 smokers and 16 nonsmokers), aged 18 to 48 years participated. Subjects were recruited through local advertisements and leaflets circulated in the university community. Sub-
Platelet serotonin receptor studies

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Platelet activation measures

In addition to venous blood for resting and in vitro activation studies, a modified bleeding time procedure was performed as described previously, with samples taken at 1 and 2 minutes after incision for activation levels. After the samples were taken, the wound was bandaged. The bleeding time procedure was repeated after smoking or, for nonsmokers, an additional 10-minute rest period as described above. This method has been shown previously to be highly reproducible in our laboratory.

Platelet function testing was performed as described previously, with 3 smokers reporting smoking 1.5 packs/d, 29 smokers reporting smoking 1 pack/d, and the remaining 7 smokers reporting smoking more than 1 pack/d.

Results

Characteristics of Smokers and Nonsmokers

Table 1 indicates that there were no substantial differences between groups in age, sex, or racial composition. As predicted, smokers had more depressive symptoms than the nonsmokers (P < 0.01). Of the smokers, 7 had Beck Depression Inventory scores ≥10 (indicating possible clinical depression), whereas all nonsmokers had scores <10. Most of the smokers (29) reported smoking 1 pack/d, and the remaining 7 reported smoking 1.5 packs/d.
Platelet Serotonin Receptor Measures, Platelet Activation, and Smoking

Smokers had significantly higher B_max than nonsmokers, but there were no differences in K_d (Table 2). Furthermore, smokers who reported smoking 1.5 packs/d had higher B_max and K_d than smokers reporting 1 pack/d (130.6 ± 75.3 versus 71.6 ± 61.9 fmol/mg protein, P < 0.05; 0.96 ± 0.22 versus 0.69 ± 0.31 nmol/L, P < 0.05), suggesting that the relationship was dose-dependent.

There were no differences in platelet secretion between smokers and nonsmokers before or after smoking. However, as shown in Table 3 and the Figure, GPIIb/IIIa receptor binding was significantly higher among smokers relative to nonsmokers for collagen activation in vitro (Z score = 2.4; P < 0.05) and for wound-induced activation at 1 minute (Z score = 2.0; P < 0.05). There were no changes in PA after smoking or resting in either group (P > 0.1; data not shown). As in previous studies, no racial or sex differences were seen in PA.

Smoking and Relationships Among Serotonin Receptor Density, Depressive Symptoms, and Platelet Activation

Among the nonsmokers, depressive symptoms were significantly correlated with B_max (r = 0.73, P < 0.005). However, among smokers, there were no significant relationships between depressive symptoms and either B_max or PA (data not shown). There were also no significant relationships between depressive symptoms and PA among the nonsmokers (data not shown).

Discussion

The present results indicate that platelet serotonin receptor density and platelet GPIIb/IIIa receptor binding are elevated among smokers. Our initial hypothesis was that these factors would be related, and would also be covariant with depressive symptoms. Although there was evidence supporting this hypothesis among the nonsmokers, B_max and depressive symptoms were unrelated to PA among smokers, suggesting that other mechanisms of GPIIb/IIIa receptor binding (such as adrenergic activation) are operative among smokers.

This study is the first, to our knowledge, that has examined platelet serotonin receptor density among smokers, and this is the major new finding of the study. Receptor density was greater among smokers who reported smoking 1.5 packs/d usage than among smokers reporting 1 pack/d, indicating a dose-response relationship. Other studies have found greater serotonin metabolite excretion among smokers, and greater platelet serotonin content has also been found among smokers. Taken together, these studies indicate serotonergic dysfunction among smokers, which could be related to the increased incidence of coronary heart disease among smokers. Although the present study suggests that serotonergic mechanisms do not operate through increased PA among smokers, local vascular mechanisms may come into play, such as vasoconstriction in response to serotonin release, and smooth muscle cell proliferation. Such mechanisms could be the focus of future research in this area.

We also found increased GPIIb/IIIa receptor binding among smokers, although platelet secretion was not elevated. In addition, there were no changes in PA after smoking, in

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**Table 2. Platelet Serotonin Receptor Binding (B_max) and Saturation (K_d) in Smokers and Nonsmokers**

<table>
<thead>
<tr>
<th>Variable</th>
<th>smokers (n=38)</th>
<th>nonsmokers (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B_max, fmol/mg protein*</td>
<td>82.7 (67.7)</td>
<td>40.0 (20.2)</td>
</tr>
<tr>
<td>K_d, nmol/L</td>
<td>0.75 (0.31)</td>
<td>0.70 (0.40)</td>
</tr>
</tbody>
</table>

Values are mean (SD).

*P < 0.005.

**Table 3. Resting Platelet Activation in Smokers and Nonsmokers**

<table>
<thead>
<tr>
<th>Platelet Activation Measure</th>
<th>smokers</th>
<th>nonsmokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPIIb/IIIa receptor binding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vitro—collagen*</td>
<td>8.4 (14.3)</td>
<td>-0.8 (3.7)</td>
</tr>
<tr>
<td>Wound-induced—1 minute*</td>
<td>34.0 (51.8)</td>
<td>13.7 (29.5)</td>
</tr>
<tr>
<td>Wound-induced—2 minutes</td>
<td>19.0 (27.5)</td>
<td>19.4 (31.2)</td>
</tr>
<tr>
<td>P-selectin expression (secretion)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vitro—collagen</td>
<td>80.2 (64.7)</td>
<td>72.0 (44.3)</td>
</tr>
<tr>
<td>Wound-induced—1 minute</td>
<td>47.0 (28.6)</td>
<td>39.4 (22.9)</td>
</tr>
<tr>
<td>Wound-induced—2 minutes</td>
<td>50.0 (26.3)</td>
<td>55.9 (40.0)</td>
</tr>
</tbody>
</table>

Values are mean (SD) fluorescence intensity units (activation minus unstimulated venous sample).

*Smokers vs nonsmokers, P < 0.05 by Wilcoxon two-sample test.
agreement with some studies, but not others; this finding, however, may be limited by the lack of assessment of smoking abstinence during the morning hours before the study (see below). The present study used flow cytometric detection of PA, which has been cited as the best available method of assessing platelet function; hence, the results may differ from earlier studies using other methods of assessing PA. In addition, there may have been other factors, such as differences in deprivation of smoking or other procedural details, that could account for results differing from earlier studies.

Platelet secretion was not related to smoking, consistent with our previous work in restenosis after coronary stenting. The general lack of a relationship between smoking and platelet secretion is not entirely unexpected, as adrenergic mechanisms, which are likely responsible for increased PA in smokers, may affect fibrinogen receptor binding without affecting platelet secretion. Taken together with our previous work, the present study indicates that not all measures of PA are the same, and that assessment of the activation of GPIIb/IIIa, the final common pathway to platelet aggregation, may generally prove to be more worthwhile than other measures.

Although the present findings indicate a strong relationship between subclinical depressive symptoms and Bmax in non-smokers, they also indicate that smoking may be an important confounding variable in studies of platelet receptor density among patients with clinical depression. None of these studies reported the smoking habits of either the patients or the control subjects. Smokers have an increased incidence of major depression over their life span and the present study indicates that smokers have increased receptor density that is independent of depressive symptoms (although clinical depression could not be determined using the Beck Depression Inventory alone). Future work in this area would benefit from taking smoking into consideration.

The present study is limited by a lack of more objective measures of smoking, such as exhaled carbon monoxide, to assess morning abstinence from smoking and self-dosing behavior; hence our ability to assess the acute effects of smoking may have been diminished. In addition, alcohol intake (chronic and recent) was not measured, and alcohol is known to inhibit PA. Given the higher amount of alcohol consumption among smokers, this factor may have accounted for lower PA in some smokers in the present study, which may have attenuated the effect of smoking on PA. A number of outlying values (>3 standard deviations from the mean) were present and were eliminated from the analysis to avoid potential artifact error, but most of these values (9 of 11) were found among the smokers; therefore, if any bias occurred in eliminating these values, it was toward the null hypothesis. Finally, although no subject had clinical cardiovascular disease, it is possible that subclinical disease, including endothelial dysfunction commonly found among smokers, may have played a role in the increased PA and platelet serotonin receptor density. Specificaly, endothelial production of nitric oxide, a potent platelet inhibitor, may have been decreased among smokers. Hence, the increases in PA among smokers in this study may have been related to endothelial dysfunction caused by smoking, rather than the direct effect of smoking per se. However, smoking also diminishes release of nitric oxide from platelets. Further studies of smokers with overt or subclinical cardiovascular disease may be useful to further assess the mechanisms responsible for increased PA among smokers.

In summary, smoking is associated with increased platelet serotonin receptor density and with increased GPIIb/IIIa receptor binding. The relationship between serotonin receptor density and smoking appears to be dose-related, but increased PA among smokers does not appear to be related to increased serotonin receptor density. Serotonergic dysfunction among smokers may be an important mediator linking cardiovascular disease to smoking, and further research in this area is indicated.

Acknowledgments

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References

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