Enhancement of Platelet Aggregation by Tranylcypromine in Mouse Cerebral Microvessels

WILLIAM I. ROSENBLUM AND FAROUK EL-SABBAN

SUMMARY Tranylcypromine, given intraperitoneally at doses ≥ 10 mg/kg, enhanced platelet aggregation in the arterioles on the cerebral surface in mice. Tranylcypromine inhibits prostacyclin synthesis in vitro. Iproniazid, which inhibits monoamine oxidase but not prostacyclin synthesis, failed to enhance platelet aggregation. The failure of iproniazid to enhance aggregation in this study rules out an effect on monoamine oxidase as the cause of tranylcypromine's action. That iproniazid inhibited aggregation indicates it has an opposite effect to that of tranylcypromine. Imidazole, a drug known to inhibit synthesis of both prostacyclin and thromboxane, failed to affect platelet aggregation. All of our data are compatible with the hypothesis that prostacyclin is an inhibitor of platelet aggregation. This hypothesis has been based largely on in vitro data, to which we now add in vivo support.

RECENTLY it has been suggested that aggregation of circulating platelets is controlled by a balance of two factors derived from prostaglandin endoperoxides. These factors, thromboxane (TXA₂) and prostacyclin (PGI₂), have platelet-aggregating and platelet-aggregation-inhibiting effects, respectively. TXA₂ is synthesized by platelets, whereas PGI₂ is synthesized by vessel wall, perhaps from substrate supplied by platelets attempting to adhere to and/or aggregate adjacent to the vessel wall. This hypothesis concerning the physiological or pathological importance of a balance between TXA₂ and PGI₂ originally was based on in vitro data to which a brief report of in vivo work has been added recently. The following data come from a study employing a different in vivo model and a known inhibitor of PGI₂ synthesis. The data provide further support for the suggestion that PGI₂ inhibits platelet aggregation in vivo.

Methods

Production of Platelet Aggregation

Platelet aggregation was induced in microvessels on the surface of the brain using a model previously described in detail. Mice are anesthetized with urethane (2 mg/g, ip), a tracheotomy and craniotomy are performed, and the dura stripped. The exposed surface vessels (pial vessels) initially are viewed microscopically with tungsten epi-illumination. After selection of an appropriate field, sodium fluorescein (0.2 ml of a 2% solution) is injected via a tail vein, and illumination switched to a filtered UV light source. The combination of light and dye induces platelet aggregation which is not observed with dye alone, or with the filtered UV illumination in the absence of dye. The aggregating platelets fluoresce. A variety of parameters can be used to assess the tendency for aggregation. In this study we measured the time between onset of UV illumination and time to the first visible aggregate ("time to first aggregate"). We also observed aggregates enlarging until the lumen was totally occluded and determined the time elapsing between the onset of the noxious stimulus and occlusion ("time to stop flow"). In our earlier study, we pointed out the availability of this latter parameter, but elected not to use it because it seemed to be less sensitive than time to first

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aggregate, in detecting drug effects. Student's t-test was used to compare values from control mice with those from drug-treated mice. The arterioles studied were 20–50 μm in internal diameter.

Attempts to Influence Aggregation with Drugs

We already have shown that aspirin and indomethacin inhibit platelet aggregation in our model.7 Dipyridamole with or without aspirin has no effect.8 In the present study, we used three drugs. Tranylcypromine has been reported to inhibit synthesis of PGI2 from prostaglandin precursors.5–9 It was given in doses of 1, 10, and 50 mg/kg. Iproniazid is, like tranylcypromine, a monoamine oxidase inhibitor, but has no ability to inhibit PGI2 synthesis.2 This was given in doses of 50 and 100 mg/kg. Iproniazid is a weaker inhibitor of PGI2 synthesis than tranylcypromine2 but has stronger capacities to inhibit TXA2 synthesis.10 Imidazole was given in doses of 100 and 250 mg/kg. We administered these drugs intraperitoneally and observed the pial arterioles 60 minutes later. The anesthesia was administered and surgery was begun after drug injection and approximately 25 minutes before the intravenous administration of sodium fluorescein and the exposure of mice to UV light. In each study, control mice were observed on each day in which experimental mice were examined. The controls were injected with drug vehicle at the same pH as the drug plus vehicle. The controls were treated exactly like the experimental mice. A control was examined immediately after or immediately before each experimental mouse, the sequence being randomized. During the study, the control values obtained differed widely from day to day and, on the whole, differed greatly from some of those reported earlier7 but not from others.6 We do not know what factors determined the variability in response, but it appears to be a consistent finding in other in vivo models of platelet aggregation or clot formation12–14 for which standard deviations may even exceed the mean value of the parameter being tested. It was for this reason that we initially adopted7 and continue to follow the principle of performing control studies on the same day as the experimental studies, thereby assuring random distribution of uncontrolled factors between both groups. Our success in this respect was established by an unpublished study in which one set of controls was compared to another, tested on the same days. There were no differences between groups, with respect to platelet aggregation. We have also repeated certain studies from time to time and found no difference in their capacity to detect drug effects, in spite of different baseline or control values in the two studies. For example, aspirin significantly impairs platelet aggregation when used with mice showing control values like those in the present study or showing lower control values like those in our original report.7

In order for our data to support the hypothesis being tested, namely, that PGI2 inhibits platelet aggregation in vivo, we would have to observe the following results: facilitation of aggregation by tranylcypromine, the inhibitor of PGI2 synthesis; failure of iproniazid to inhibit aggregation, thus establishing the tranylcypromine effect as being unrelated to monoamine oxidase inhibition; and a lesser, absent, or opposite effect of imidazole as compared with tranylcypromine, since the former is a weaker inhibitor of PGI2 synthesis and an inhibitor of TXA2 synthesis.

Results

Platelet Aggregation

Preliminary experiments* were performed on 40 mice given either tranylcypromine (TCP), 50 mg/kg, or vehicle, 1 hour before examination. Aggregation in arterioles was significantly facilitated (P < 0.05). These experiments were followed by two others reported in Table 1. In each study, two doses of TCP were compared. The table shows that doses of 50 and 10 mg/kg effectively enhanced aggregation.

The doses we selected were based on our knowledge that a concentration of TCP of 500 μg/ml is required in vitro for 100% inhibition of PGI2 synthesis.2 Simple calculations showed that the maximal dose we used (50 mg/kg) would be expected to produce blood levels of TCP somewhat lower than 500 μg/ml. However, higher doses were toxic, and moreover, it was not thought necessary to inhibit 100% of enzyme activity in order to produce a biological effect.

Iproniazid Effect on Platelet Aggregation

Iproniazid, an MAO inhibitor without effect on PGI2 synthesis,7 failed to facilitate aggregation as shown in Table 2. The doses of iproniazid selected in these studies were two and four times greater than the ED50 reported for rats and mice,15 and thus effective inhibition of MAO was assured.

As Table 2 indicates, iproniazid not only failed to facilitate platelet aggregation, but actually inhibited aggregation, as did aspirin and indomethacin in a previous study.7 Thus, both time to first aggregate and time to stop flow were significantly prolonged.

Imidazole Effect on Platelet Aggregation

This drug failed to have a consistent effect on platelet aggregation, as shown in Table 3.

Discussion

The data presented here offer in vivo evidence supporting the hypothesis that PGI2 synthesis is a significant factor inhibiting platelet aggregation in vivo.1–5 When we began these studies, no other in vivo evidence existed. A recent abstract by Higgs et al.5 reports that local irrigation with PGI2 inhibits platelet aggregation induced in hamster cheek pouch by regionally applied ADP. Because our study used a different species, a different vascular bed, and a different means of initiating aggregation, we believe the compatibility of our data with that of Higgs et al. is especially important. We do not suggest that our data

our data suggest that this will be a fruitful area of investigation, in which one may use available data to be sure that MAO inhibitors lack the confounding capacity to inhibit PGI2 synthesis.

**Table 1** Tranlycypromine Facilitates Platelet Aggregation in Cerebral Arterioles

<table>
<thead>
<tr>
<th></th>
<th>Time to first aggregate (sec)</th>
<th>Time to stop flow (sec)</th>
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<tbody>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tranlycypromine (n = 5)</td>
<td>42 ± 10*</td>
<td>125 ± 21†</td>
</tr>
<tr>
<td>Tranlycypromine (n = 10)</td>
<td>53 ± 18†</td>
<td>146 ± 69</td>
</tr>
<tr>
<td>Control  (n = 10)</td>
<td>98 ± 36</td>
<td>197 ± 74</td>
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</tbody>
</table>

**Table 2** Iproniazid Inhibits Platelet Aggregation in Cerebral Arterioles

<table>
<thead>
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<th>Time to first aggregate (sec)</th>
<th>Time to stop flow (sec)</th>
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<tbody>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
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<tr>
<td>Iproniazid (n = 10)</td>
<td>141 ± 81*</td>
<td>230 ± 75*</td>
</tr>
<tr>
<td>Iproniazid (n = 10)</td>
<td>210 ± 63†</td>
<td>329 ± 87†</td>
</tr>
<tr>
<td>Control (n = 10)</td>
<td>78 ± 44</td>
<td>143 ± 58</td>
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**Table 3** Imidazole Has No Effect on Platelet Aggregation in Arterioles

<table>
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<th>Time to first aggregate (sec)</th>
<th>Time to stop flow (sec)</th>
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<tr>
<td>Study 2</td>
<td></td>
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<tr>
<td>Iproniazid (n = 10)</td>
<td>250 mg/kg</td>
<td>75 ± 10</td>
</tr>
<tr>
<td>Iproniazid (n = 10)</td>
<td>100 mg/kg</td>
<td>148 ± 52</td>
</tr>
<tr>
<td>Control (n = 10)</td>
<td>98 ± 36</td>
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Values are mean ± standard deviation. Imidazole failed to influence platelet aggregation in cerebral arterioles.

Imidazole had no consistent effect on platelet aggregation. This drug was selected for test because Gryglewski et al.2 presented data showing that it was a weak inhibitor of PGI2 synthesis. Therefore it is to be expected that it would have a lesser effect than TCP, if PGI2 synthesis is modifying platelet aggregation in our system. We should note, however, that the imidazole effect of PGI2 synthesis may be overlooked on review of the literature, because the pertinent data reported in the text of Gryglewski et al.2 are not mentioned in their abstract or summary. Moreover, subsequent reports state that the same agent, imidazole, is a more potent inhibitor of TXA2 synthesis.10,11 Our results require further discussion in light of this recent information.

The present experiments were designed to test only the latter half of the hypothesis stating that TXA2 and PGI2 formed an important system of counterbalancing aggregation producing and inhibiting factors.2-3 However, since imidazole is now known to have a relatively greater capacity to inhibit the synthesis of TXA2 than the synthesis of PGI2, one must wonder why the stronger effect did not prevail in our study. In other words, why wasn’t platelet aggregation inhibited by the effect of imidazole on TXA2 synthesis? Perhaps the strong inhibition of TXA2 synthesis was balanced by the weak inhibition of PGI2 synthesis. Such a balance would occur if more TXA2 were available to the platelet than PGI2, or if TXA2 were much more potent than PGI2. If either of these suggestions is correct, then a given dose of imidazole could result in equipotent amounts of TXA2 and PGI2 being available to the platelet, in spite of a much greater inhibition of TX synthesis than of PGI synthesis. This may be the case, since the TXA2 is synthesized in the platelet4,5 and the PGI2 is synthesized in vessel wall from prostaglandin endoperoxides generated by the platelet.2 Thus PGI2 reaches the platelet only after bidirectional movement, first of endoperoxide from platelet to wall, and then of PGI2 from wall back to platelet. Indeed, Gryglewski et al.7 suggest that plasma endoperoxide levels are rate limiting for PGI2 synthesis, a concept in keeping with the suggestion that TXA2 normally may be more available to the platelet than PGI2. It is also possible that imidazole failed to inhibit aggregation because thromboxane synthesis is not causally related to aggregation, a possibility suggested by Needleman et al.15

The present data strongly suggest that PGI2 and tranlycypromine act in vivo as they do in vitro, with the former inhibiting platelet aggregation and the latter inhibiting synthesis of the inhibitor. Although we appear to
have ruled out an effect of tranylcypromine on MAO as a cause of our results, it is possible that some other effect of tranylcypromine could account for our data. This possibility would exist even if one were to demonstrate with biochemical techniques an effect of tranylcypromine on the PGI₂ synthesis of the cerebral arterioles observed in the study. Moreover, such a biochemical study would depend on one’s ability to harvest a sufficient number of microsomes from microvessels on the cerebral surface, a formidable undertaking. In any case, one possible effect of any drug, which must be considered in a model such as ours, is an anti-inflammatory effect of the drug on the vessel wall. However, we have shown that anti-inflammatory properties do not, by themselves, inhibit platelet aggregation in our model (unpublished observations). In summary, our data certainly seems to strengthen the hypothesis implicating PGI₂ as a physiological inhibitor of platelet aggregation.

Acknowledgments
Ann Litten provided essential technical assistance.

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