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.

From the Division of Neuropathology, Medical College of Virginia, Richmond, Virginia.


Address for reprints: Dr. William I. Rosenblum, Division of Neuropathology, Medical College of Virginia, Box 17, MCV Station, Richmond, Virginia 23298.

Received December 7, 1977; accepted for publication March 30, 1978.

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

---

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. Tranylcypromine with or without aspirin has no effect. In the present study, we used three drugs. Tranylcypromine has been reported to inhibit synthesis of PGI$_2$ from prostaglandin precursors. 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 PGI$_2$ synthesis. This was given in doses of 50 and 100 mg/kg. Imidazole is a weaker inhibitor of PGI$_2$ synthesis than tranylcypromine but has stronger capacities to inhibit TXA$_2$ synthesis.

In order for our data to support the hypothesis being examined, our report of 1978 stated that the hypothesis that PGI$_2$ is the major factor regulating platelet aggregation in vivo is supported by the fact that PGI$_2$ syntheses is inhibited in vivo.

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 PGI$_2$ synthesis. 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 PGI$_2$ synthesis, 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 ED$_{50}$ reported for rats and mice, 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. 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 PGI$_2$ synthesis is a significant factor inhibiting platelet aggregation in vivo. When we began these studies, no other in vivo evidence existed. A recent abstract by Higgs et al. reports that local irrigation with PGI$_2$ 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...
must necessarily be interpreted as an indicator of the importance of PGI₂, but this certainly is the simplest interpretation. Any other would require ascribing to tranylcypromine properties as yet undiscovered.

The facilitation of aggregation by TCP is consonant with the known capacity of TCP to inhibit PGI₂ synthesis² and the known capacity of PGI₂ to inhibit platelet aggregation in vitro. The inhibition of inhibitor synthesis certainly should lead to facilitation of aggregation.

The failure of iproniazid to mimic TCP is consonant with the failure of iproniazid to inhibit PGI₂ synthetase,³ and also rules out an effect on MAO as the explanation for the action of TCP. In fact, iproniazid had the opposite action, and significantly reduced platelet aggregation in a dose-dependent manner. We are unaware of other studies of platelet aggregation either in vitro, and certainly in vivo, in which an effect of MAO inhibition was tested. Our data suggest that this will be a fruitful area of investigation, in which one may use available data to be sure that tested MAO inhibitors lack the confounding capacity to inhibit PGI₂ synthesis.

### Table 1  Tranylcypromine Facilitates Platelet Aggregation in Cerebral Arterioles

<table>
<thead>
<tr>
<th>Study</th>
<th>Tranylcypromine</th>
<th>Time to first aggregate (sec)</th>
<th>Time to stop flow (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 mg/kg (n = 5)</td>
<td>42 ± 10*</td>
<td>125 ± 21†</td>
</tr>
<tr>
<td></td>
<td>10 mg/kg (n = 5)</td>
<td>53 ± 18†</td>
<td>146 ± 69</td>
</tr>
<tr>
<td></td>
<td>Control (n = 10)</td>
<td>98 ± 36</td>
<td>197 ± 74</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation. The time required to produce platelet aggregation and the time required for aggregates to build up and occlude the lumen are significantly shortened in tranylcypromine-treated mice at doses greater than 1 mg/kg. ip.

* P < 0.01 compared with control.
† P < 0.05 compared with control.

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

<table>
<thead>
<tr>
<th>Study</th>
<th>Iproniazid</th>
<th>Time to first aggregate (sec)</th>
<th>Time to stop flow (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 mg/kg (n = 10)</td>
<td>141 ± 81*</td>
<td>230 ± 75*</td>
</tr>
<tr>
<td></td>
<td>Control (n = 10)</td>
<td>78 ± 44</td>
<td>143 ± 58</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation. The time required to produce platelet aggregates and the time required for aggregates to build up and occlude the lumen are significantly lengthened in iproniazid-treated mice.

* P < 0.05 compared with control.
† P < 0.01 compared with control.

### Table 3  Imidazole Has No Effect on Platelet Aggregation in Arterioles

<table>
<thead>
<tr>
<th>Study</th>
<th>Iproniazid</th>
<th>Time to first aggregate (sec)</th>
<th>Time to stop flow (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 mg/kg (n = 10)</td>
<td>210 ± 63†</td>
<td>329 ± 87†</td>
</tr>
<tr>
<td></td>
<td>Iproniazid</td>
<td>145 ± 76*</td>
<td>243 ± 87*</td>
</tr>
<tr>
<td></td>
<td>Control (n = 10)</td>
<td>69 ± 28</td>
<td>158 ± 44</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation. Imidazole failed to influence platelet aggregation in cerebral arterioles.

* P < 0.05 compared with control.
† P < 0.01 compared with control.

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.¹⁻³ 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.² 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 Needelman et al.¹⁵

The present data strongly suggest that PGI₂ and tranylcypromine 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 PGI$_2$ 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 PGI$_2$ as a physiological inhibitor of platelet aggregation.

Acknowledgments
Ann Litten provided essential technical assistance.

References
1. Moncada S, Gryglewski RJ, Bunting S, Vane JR: A lipid peroxide inhibits the enzyme in blood vessel microsomes that generates from prostaglandin endoperoxides the substance (prostaglandin X) which prevents platelet aggregation. Prostaglandins 12: 715-737, 1976
2. Gryglewski RJ, Bunting S, Moncada S, Flower RJ, Vane JR: Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin X) which they make from prostaglandin endoperoxides. Prostaglandins 12: 685-713, 1976
Enhancement of platelet aggregation by tranylcypromine in mouse cerebral microvessels.
W I Rosenblum and F El-Sabban

Circ Res. 1978;43:238-241
doi: 10.1161/01.RES.43.2.238

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1978 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/43/2/238