been undertaken to increase the yield of apheresis platelet product,\textsuperscript{2} and our studies have demonstrated that a recombinant thrombopoietin might also be of benefit.\textsuperscript{3,4}

Exposing healthy donors to hematopoietic growth factors has long been controversial, as has the concept of exposing donors to apheresis devices themselves. Donors have recently been asked to increase their donation of a variety of apheresis products (platelets, granulocytes, red cells, plasma) and peripheral blood stem cells. Shortages of apheresis platelets are not uncommon. To ask whether thrombopoietin (TPO) might join granulocyte colony-stimulating factor (G-CSF) and erythropoietin (EPO) as mobilizing agents was a valid clinical research issue that was approved by the appropriate institutional review boards and regulatory agencies, along with meticulous monitoring by the investigators to insure the safety of the donors. There were no adverse consequences to any of the donors.

In questioning the “ethics of TPO prescription in unrelated volunteer donors,” Verdijk should distinguish between a preliminary phase 1/2 clinical trial that we performed and its widespread use in the blood supply industry. Upon completion of our trial and before embarking on further platelet donor studies, a very large safety study of paid healthy volunteers was conducted, and it was here that some were found to develop autoantibodies to TPO after multiple injections.\textsuperscript{5} This finding resulted in the discontinuation of our studies of platelet donors with this recombinant thrombopoietin.

We believe that we have established the simple principle that thrombopoietin therapy can increase platelet apheresis yields. If apheresis platelet collection continues to be an important source of platelets and if a “nonimmunogenic” thrombopoietin is developed, further studies would be warranted to determine whether this approach would be of widespread use to increase the supply of platelets. A program involving HLA-compatible related donors would be an ideal area for investigation.

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References


To the editor:

Adenosine diphosphate (ADP) does not induce thromboxane A\textsubscript{2} generation in human platelets

Recently, Jin et al addressed the generally misunderstood problem of the factors responsible for adenosine diphosphate (ADP)–induced thromboxane A\textsubscript{2} (TxA\textsubscript{2}) production by human platelets.\textsuperscript{1} They used stirred, washed human platelet suspensions to which fibrinogen but no Ca\textsubscript{Cl\textsubscript{2}} had been added; as a consequence, the concentration of external Ca\textsuperscript{2+} ([Ca\textsuperscript{2+}]\textsubscript{o}) was much lower than physiological. The main results of their study can be summarized as follows: (i) the ADP receptor antagonists A2P5P (anti-P2Y\textsubscript{1}) or AR-C67085 (anti-P2Y\textsubscript{12}) inhibited platelet aggregation and TxA\textsubscript{2} production induced by ADP; (ii) the fibrinogen receptor antagonist SC49992 inhibited platelet aggregation and TxA\textsubscript{2} production induced by ADP; (iii) the Fab fragment of ligand-induced binding site 6 (LIBS6) antibody, which induces a fibrinogen binding site on \(\alpha_{\text{IIb}}\beta_{3}\), caused platelet aggregation and TxA\textsubscript{2} production when added to platelet suspensions with fibrinogen; (iv) A2P5P (P2Y\textsubscript{1} antagonist) or AR-C67085 (P2Y\textsubscript{12} antagonist), when added together or alone, inhibited the secondary wave of aggregation and TxA\textsubscript{2} production induced by LIBS6 plus fibrinogen; and (v) in the presence of physiological concentrations of Ca\textsuperscript{2+}, there was no production of TxA\textsubscript{2} by ADP-aggregated platelets. The authors concluded that ADP induces TxA\textsubscript{2} generation in human platelets, which requires coordinated signaling through the integrin \(\alpha_{\text{IIb}}\beta_{3}\) and ADP receptors.

We do not agree with the authors’ interpretation of their own results. Rather, we think that their results are in accord with well-established knowledge of the factors involved in ADP-induced TxA\textsubscript{2} generation: (1) ADP does not stimulate TxA\textsubscript{2} production directly; (2) it is the close platelet-to-platelet contact that is brought about by ADP-induced platelet aggregation that triggers the production of TxA\textsubscript{2}; and (3) this effect is greatly enhanced and can be seen in most healthy individuals when [Ca\textsuperscript{2+}]\textsubscript{o} is decreased to micromolar levels.\textsuperscript{2,5} Based on this interpretation, it is not surprising that the experiments performed by Jin et al showed that antagonists of P2Y\textsubscript{1}, P2Y\textsubscript{12}, or the fibrinogen receptor, which inhibit ADP-induced platelet aggregation, abolished the TxA\textsubscript{2} production. The dependency of TxA\textsubscript{2} production and the ensuing platelet secretion on platelet aggregation is demonstrated by the observation that no TxA\textsubscript{2} production or platelet secretion occurs from normal human platelets that are stimulated by ADP, even at high concentration, under conditions in which platelet aggregation does not occur: for instance, if the receptor function for adhesive proteins on platelets is decreased with inhibitors of monoclonal antibodies or Arg-Gly-Asp-containing peptides, or, more simply, if the platelet suspension is not stirred. The mechanism by which platelet aggregation triggers TxA\textsubscript{2} formation at low [Ca\textsuperscript{2+}]\textsubscript{o} is presently unknown. But the mechanism is by no means selective for ADP-induced platelet aggregation, because similar effects occur when platelets are aggregated by other weak agonists,\textsuperscript{6} and it can be observed also when close platelet-to-platelet contact is brought about by LIBS6 and fibrinogen,\textsuperscript{1} or the aggregating agents polylysine or ristocetin.\textsuperscript{7,8} The platelet-to-platelet contact caused by weak agonists, aggregating agents or LIBS6 and fibrinogen causes the formation of trace amounts of TxA\textsubscript{2} and the secretion of ADP, which supports the secondary full aggregation responsible for the production of large amounts of TxA\textsubscript{2}. None of these responses, including TxA\textsubscript{2} production, is observed when the same agonists or aggregating agents are added to the same platelet suspension under nonstirring conditions.\textsuperscript{6} This

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observation clearly indicates that a definite distinction should be made between platelet aggregation and fibrinogen receptor occupancy. When platelets are exposed to ADP under nonstirring conditions to prevent their aggregation, ADP elicits the inside-out signaling, which promotes the occupancy of the fibrinogen receptor on αIIbβ3, which in turn elicits the outside-in signaling. According to the model proposed by Jin et al, this should be enough to stimulate the generation of TxA2 by platelets; under these conditions, however, TxA2 production does not occur: it is only when platelets are stirred and, as a consequence, they are allowed to aggregate that platelet TxA2 production can be observed.

Finally, the fact that platelet secretion and the production of large amounts of TxA2 occur with platelets from most healthy individuals only when [Ca2+]o is at micromolar levels demonstrates that the whole process that can be observed in the turbidometric aggregometer is mostly an in vitro artifact.\(^1\)(The widespread use of citrate as an anticoagulant in the preparation of platelet-rich plasma introduces the same artifact as suspending media without added CaCl2.) Despite this, Jin et al suggest that ADP-induced TxA2 production may have physiological relevance in vivo, because [Ca2+]o might be “drastically lower”\(^1\)(p196) at sites of thrombus formation. We do not know of any support for this hypothesis, and we believe that, due to the secretion of Ca2+ from platelet granules, [Ca2+]o might even increase, rather than decrease. Needless to say, we agree with the authors’ statement that the results of clinical trials with aspirin indicate that TxA2 plays an important role in thrombus formation in vivo.\(^2\) But this is not a demonstration that, in vivo, ADP causes platelet TxA2 formation. Platelet thrombus formation in vivo depends on the synergistic interaction of several agonists, some of which, such as collagen and thrombin, directly cause the formation of TxA2. The recent trials combining drugs such as clopidogrel with aspirin\(^3\) add evidence to the fact that separate pathways contribute to thrombosis and that combined therapies are more effective than aspirin alone or clopidogrel alone.

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References


Response:

Signaling events regulating thromboxane A2 generation in platelets

Cattaneo et al argue that the results of our recently published paper are in accord with well-established knowledge of the factors involved in adenosine diphosphate (ADP)-induced thromboxane A2 generation, but they do not agree with the interpretation of our results. They state that (1) ADP does not stimulate thromboxane A2 production directly; (2) it is the close platelet-to-platelet contact that is brought about by ADP-induced platelet aggregation that triggers the production of thromboxane A2; and (3) this effect is greatly enhanced and can be seen in most healthy individuals when extracellular calcium is decreased to micromolar levels.

Our results have demonstrated that blockade of the P2Y1 receptor by A2PSP, of the P2Y12 receptor by AR-C67085, or of fibrinogen binding to its receptor by SC49992 inhibits both platelet aggregation and thromboxane A2 generation.\(^1\) These results are consistent with the argument of Cattaneo et al that ADP-induced thromboxane A2 generation is dependent on ADP-induced platelet aggregation. We addressed this issue and went further to investigate whether A2PSP and AR-C67085 block thromboxane A2 generation simply by inhibiting ADP-induced platelet aggregation, or whether ADP contributes beyond that to signaling events in thromboxane A2 generation. ADP under stirring conditions causes platelet aggregation, which results in close cell-to-cell contact. Signaling under these conditions is termed as “outside-in” signaling, which Shattil et al defined as “initiated at localized regions of cell matrix and cell-cell contact.”\(^2\)(p265) This outside-in signaling translates into several intracellular events including tyrosine phosphorylation of platelet proteins.\(^2\) Blockade of fibrinogen binding to activated integrin αIIbβ3 by Arg-Gly-Asp-Ser, EDTA (ethylenediaminetetraacetic acid), or mAb A2A9 abrogated tyrosine phosphorylations of platelet proteins, indicating the important role of fibrinogen binding.\(^3\) Our results\(^1\) with SC49992 are consistent with the importance of fibrinogen binding in outside-in signaling events. When LIBS6 Fab fragments and fibrinogen were used to induce platelet aggregation without adding any other agonist, thromboxane A2 was generated.\(^1\) But under these conditions, the ADP-receptor antagonists A2PSP or AR-C67085 inhibited thromboxane A2 generation.\(^1\) Furthermore, a combination of both A2PSP and AR-C67085 nearly abolished thromboxane A2 generation induced
by LIBS6 and fibrinogen, without affecting primary aggregation, although full irreversible aggregation was impaired. But inhibition of full, irreversible aggregation by wortmannin did not impair thromboxane A2 accumulation in response to LIBS6 plus fibrinogen. These data suggest that the close platelet-to-platelet contact that is brought about by platelet aggregation (primary or full) is not sufficient to generate thromboxane A2 and depends on signaling from the ADP receptors. Hence we disagree with the interpretation of Cattaneo et al of the previously published results from several laboratories. We stand by our interpretation of our results that outside-in signaling from both the integrin αIIbβ3 and the P2 receptor is required for ADP-induced thromboxane A2 generation in platelets. We agree with Cattaneo et al that ADP-induced thromboxane generation is greatly enhanced and can be seen in most healthy individuals when extracellular calcium is decreased to micromolar levels.

To the editor:

Myelodysplastic syndromes: prognostic significance of multilineage dysplasia in patients with refractory anemia or refractory anemia with ringed sideroblasts

We read with interest the recent paper by Nosslinger et al comparing the French-American-British (FAB) and the World Health Organization (WHO) classifications on 431 unselected patients with myelodysplastic syndromes (MDS). We were particularly interested in their analysis of patients (n = 91) with refractory cytopenia with multilineage dysplasia (RC + Dys), which showed no significant difference in the median survival when compared to those (n = 47) with refractory anemia (RA) or refractory anemia with ringed sideroblasts (RARS). We wish to report our experience (in a tertiary referral center) in patients with RA or RARS, classified according to the WHO criteria for MDS.

We have studied 88 consecutive MDS patients with RA or RARS between January 1992 and January 1999. Clinical details and survival data were reviewed; bone marrow aspiration slides were examined and classified according to the WHO criteria for lineage dysplasia. Twenty-four patients were excluded from the study because of insufficient clinical information on survival (n = 20), death occurring within 4 weeks of diagnosis (n = 3), or treatment with bone marrow transplantation (n = 1).

The median age of patients with RA/RARS and single-lineage dysplasia was 74.7 years; median age of patients with bi- or trilineage dysplasia was 76 years. All but one patient (who had dysthrombopoiesis) in the single dysplasia groups had erythroid dysplasia.

In the RA group, patients with single-lineage dysplasia had a median survival of 32.8 months (range, 3-96 months) compared to 14.2 months (range, 3-53 months) in patients with multilineage dysplasia; the difference was significant (P < .05). Similarly, differences in survival were evident in the RARS groups with a median survival of 40.6 months (range, 2-85 months) in those with single-lineage dysplasia compared to 18.8 months (range, 3-69 months) in those with multilineage dysplasia. These results did not reach statistical significance. However, when we combined RA and RARS patients, we observed highly significant differences in survival times between those with single-lineage dysplasia (median survival [MS] 36.3 months) and those with multilineage dysplasia (MS 14.9 months). At the time of analysis, 59% (20/34) of patients with single-lineage dysplasia were alive, but only 10% (3/30) of patients with multilineage dysplasia were alive.

Cytogenetic studies showed abnormalities in 26% of all patients, but only 16% (8 patients) had results that conferred a worse prognosis (“intermediate risk” = 3, “poor risk” = 5). The majority of patients with adverse-risk cytogenetics had multilineage dysplasia (7/8). There was, as expected, a major difference in median survival between the 2 groups (30.5 months vs 7.9 months).

We did not find cytopenias predictive of the presence of multilineage dysplasia, nor did we find a difference in survival between those with 2 or 3 cytopenias (24.8 months) and those without (26.2 months).

The International Prognostic Scoring System (IPSS) stratification of the 64 patients were as follows: “low-risk” = 26; “intermediate 1 (INT-1) risk” = 20; and “INT-2 risk” = 3. The IPSS could not be applied to 15 patients because of a lack of cytogenetics.

Table 1 shows the relationship of median survival to various prognostic scoring systems in combined RA and RARS groups. Overall, combining the IPSS with lineage dysplasia had greater prognostic power than IPSS alone in these so-called good-risk FAB subtypes. Our findings are similar to those reported by Rosati et al.

Table 1. Relationship of median survival to various scoring systems in combined RA and RARS group

<table>
<thead>
<tr>
<th>Scoring system</th>
<th>Prognostic level</th>
<th>Median survival (months)</th>
<th>Statistical significance</th>
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<tr>
<td>Lineage dysplasia</td>
<td>Single</td>
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<td>INT-2</td>
<td>8.3</td>
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<td>IPSS and dysplasia</td>
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<td>P &lt; .05</td>
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<td>Low + multiple</td>
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References

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