Human Thrombocytopenia Is Associated With Structural Abnormalities of the Endothelium That Are Ameliorated by Glucocorticosteroid Administration

By Craig S. Kitchens and J.F. Pendergast

Capillary fragility is characteristic of severe thrombocytopenia. This mechanical weakness may not be solely accounted for by decreased ability of platelets to repair endothelial breaks. Platelets may have a role in maintaining endothelial hemeostasis. This laboratory has demonstrated thinning of capillary endothelium in experimental thrombocytopenia. We now report similar findings in human thrombocytopenia. Capillary endothelium supplying either skin or skeletal muscle was found to have a mean thickness only half that of normal as well as frequent very thinned areas, including some fenestrations. All findings reverted toward normal after four days of prednisone administration at a time the degree of thrombocytopenia was equally severe. These findings are consistent with the hypothesis that platelets are necessary for normal structure and function of endothelial cells and that glucocorticosteroid administration may ameliorate the pathophysiology of thrombocytopenia.

ADEQUATE NUMBERS of functional platelets are necessary for normal hemostasis. Platelets seal endothelial breaks in the event of rupture. Claiming that lack of this sealing function alone does not account for capillary fragility seen in severe thrombocytopenia, it has been hypothesized that platelets additionally, in some manner, may play a role in maintaining endothelial integrity. This laboratory has published morphological data in experimental thrombocytopenia in rabbits supporting this hypothesis. We have additionally demonstrated that endothelial structural alterations in this experimental model were ameliorated by glucocorticosteroids. Herein are reported observations made on tissue obtained from humans with severe thrombocytopenia.

MATERIALS AND METHODS

Patients. Strict criteria for patient accession accounts for the few patients accrued over a five-year period. Patients had to have severe thrombocytopenia (≤15,000/μL) not due to a process (chemotherapy or radiation therapy) that conceivably could directly damage a variety of tissues, including endothelium. The platelet count had to remain ≤15,000/μL after four days of daily administration of prednisone, 1 mg/kg, in order to minimize endothelial structural changes related primarily to resolution of thrombocytopenia. Before their entry into the study, they could not have received glucocorticosteroids. Because of our strong feelings that glucocorticosteroids are effective for hemorraghe-associated with severe thrombocytopenia, we could not ethically accept transfer of thrombocytopenic patients from other cities without first recommending the administration of such agents. This greatly restricted the number of patients who were available to serve in this study comparing determinations made before and during prednisone administration. Finally, patients had to agree to serve in the experiment, all parts of which were approved by the Internal Review Board of the University of Florida.

Tissue. As rapidly as possible after initial evaluation and understanding of the protocol, biopsies were performed after which prednisone, 1 mg/kg/d, was begun. Biopsy material was also obtained on the fourth day of such treatment if the platelet count was ≤15,000/μL. If the platelet count was ≥15,000/μL, the original biopsy material was not further processed. Biopsy material was obtained from skin and muscle. Skin biopsies were taken from the leg or skeletal muscle. Skin or skeletal muscle data are complete for only three patients. Data regarding skin biopsy is complete for all five patients. Tissues were then immediately diced in cold 3% glutaraldehyde in phosphate buffer and postfixed in OsO4. It was then embedded, sectioned, and stained with lead citrate and uranyl acetate as previously described.

Electron microscopy. All material was examined using a Philips 300 electron microscope (Eindhoven, The Netherlands). All photomicrographs were made at a fixed magnification (50,000) without knowledge of patient or status of prednisone administration. It was impossible to be unaware of whether the biopsy source was skin or muscle. Several blocks from each patient sample were examined until approximately 100 capillaries from each biopsy sample had been photographed. All vessels encountered were photographed, regardless of photogenicity, in order to minimize bias in choosing which vessels to photograph.

Collection of data. Before breaking the patient and preparation code, each photomicrograph was examined for (1) “thin spots,” (2) fenestrations, and (3) mean thickness of the capillary endothelium. Figure 1 demonstrates these terms. A thin spot is defined as an area in which the endothelium attenuates to a thickness of only 700 to 800 Å, the thickness of an endothelial vessel. This degree of thickness is admittedly arbitrarily selected but being markedly thinner than normal endothelium (4,000 to 6,000 Å), such areas were readily identifiable. Fenestrations are not found in normal endothelium of capillaries supplying muscle or skin (except capillaries supplying sweat glands). Mean capillary thickness was determined using planometric methods previously described. In our experience, this method has a variability of ±2.7%. Because tissue from these patients before they developed thrombocytopenia was not available,
determinations from other studies this laboratory has performed using human skin and muscle capillaries were used to define normal.6,7 The methods were identical. To our knowledge, glucocorticosteroid administration does not affect normal mammalian capillary endothelial structure.4-8 The data were analyzed using the Statistical Analysis System (SAS)9 computer software package on an IBM 4341 machine in the Faculty Support Center at the University of Florida. Significance of changes in capillary thickness measures was determined using a one-sided paired t test on measures weighted to adjust for the variability within subjects. Comparisons between the study group and normal tissue were made using a two-sample t test, again after weighting the measures to adjust for within-subject variability.10

RESULTS

Five patients fulfilled all criteria and their results form the basis of this study (Table 1). Three were diagnosed as having immune thrombocytopenia purpura (ITP) resistant to prednisone administration. Two were initially diagnosed as having amegakaryocytic thrombocytopenia11,12; one subsequently developed aplastic anemia, whereas the other’s disease evolved into acute nonlymphocytic leukemia.

The response of each patient to prednisone administration was noteworthy. Whereas each patient manifested fresh petechial formation and epistaxis on admission, such hemorrhagic phenomena resolved with the commencement of glucocorticosteroid administration, although the patient’s thrombocytopenia did not substantially improve. Likewise, the initial biopsy sites bled significantly, whereas those made on the fourth day bled only little more than similar biopsies performed on patients having normal platelet counts.13 These events correlated with their template bleeding times, all of which were >20 minutes before prednisone administration and all of which were <20 minutes (mean, 16.5 ± 2.2 minutes; normal, <9.0 minutes) during prednisone administration.

Table 2 gives our morphological data. Data from the five patients (five pairs of skin biopsies and three pairs of muscle biopsies) were pooled for statistical analysis. Despite the small number of subjects, the capillary endothelium of both skin and muscle is thinner (P = .001 and P = .013, respectively) and shows more thin spots and fenestrations than normal human endothelium. Additionally, these changes tend to revert toward normal after the administration of glucocorticosteroids (P = .001 for skin and P = .055 for muscle) at a time when the patients’ bleeding was clinically markedly abating. Skin and muscle capillary endothelium in thrombocytopenia after prednisone administration is still thinner than normal endothelium (P = .001 for skin and P = .008 for muscle). All interendothelial junctions observed were normal.

Data from each individual were also analyzed. Mean capillary thickness was thinner (P < .05) in all three muscle and five skin biopsies during thrombocytopenia compared with normal. Mean capillary thickness increased significantly (P < .05) after glucocorticosteroid administration in two of the three muscle samples and in all five of the skin specimens. There was no difference in the data whether the thrombocytopenia was due to decreased production (amegakaryocytic thrombocytopenia) or increased destruction (immune thrombocytopenia).

DISCUSSION

The results of this study are remarkably similar to those obtained in our studies of experimental thrombocytopenia

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Base Line</th>
<th>Prednisone x Four d</th>
</tr>
</thead>
<tbody>
<tr>
<td>58</td>
<td>F</td>
<td>AMT</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>73</td>
<td>M</td>
<td>AMT</td>
<td>3</td>
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<tr>
<td>88</td>
<td>M</td>
<td>ITP</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>71</td>
<td>M</td>
<td>ITP</td>
<td>11</td>
<td>15</td>
</tr>
</tbody>
</table>

AMT, amegakaryocytic thrombocytopenia; ITP, immune thrombocytopenic purpura.
produced in the rabbit. In each situation, the endothelium during thrombocytopenia becomes roughly half the original thickness, and thin spots and fenestrations are encountered in 43% and 6%, respectively, of the capillaries encountered. After prednisone administration, the endothelial thickness reverts toward normal, assuming approximately 75% of original thickness, while thin spots and fenestrations largely, but not totally, disappeared. Using scanning electron microscopy, we demonstrated enfacement of the capillary endothelial surface in experimental thrombocytopenia. These changes also were partially reversed by prednisone administration. Endothelial alterations similar to those found in humans were also found in dogs rendered severely thrombocytopenic by infusion of thrombin.

As these results during thrombocytopenia are consistent with decreased mechanical strength of the capillary endothelium at a time when capillary fragility and hemorrhage are manifest, it is attractive to hypothesize a casual relationship. As both structural alterations and bleeding are ameliorated and prednisone (n = 100)

Table 2. Observation of Human Microvascular Endothelium

<table>
<thead>
<tr>
<th>Material</th>
<th>Thickness + SD</th>
<th>Thin Spots*</th>
<th>Fenestrations*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin: normal (n = 36)</td>
<td>5,881 ± 2,399 Å</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Skin: thrombocytopenia (n = 120)</td>
<td>2,832 ± 933 Å</td>
<td>43.3</td>
<td>6.7</td>
</tr>
<tr>
<td>Skin: thrombocytopenia and prednisone (n = 100)</td>
<td>4,040 ± 1,702 Å</td>
<td>5.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Muscle: normal (n = 100)</td>
<td>4,180 ± 1,321 Å</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Muscle: thrombocytopenia (n = 109)</td>
<td>2,074 ± 737 Å</td>
<td>44.9</td>
<td>5.5</td>
</tr>
<tr>
<td>Muscle: thrombocytopenia and prednisone (n = 147)</td>
<td>2,552 ± 1,013 Å</td>
<td>20.4</td>
<td>1.3</td>
</tr>
</tbody>
</table>

*Per 100 capillaries.

REFERENCES

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