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

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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 the administration of 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 hemorrhage 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 still ≤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 in areas near fresh petechiae but not of petechiae themselves. This area was chosen to ensure that capillary fragility was present. Our previous animal studies demonstrated that capillary thinning was not confined to actively hemorrhaging areas, being just as pronounced in normal-appearing tissue. An elliptical incision 6 × 2 mm was made with a surgical scalpel deep enough to reach subcutaneous tissue. The wound was closed with three sutures, and pressure dressings were applied. Tissue from the gluteal muscle was obtained using a Lee soft-tissue biopsy needle (Becton Dickinson, Atlanta), which was placed roughly along the axis of the indicated bone marrow aspirate and biopsy tract. Biopsy yielded muscle in both pretreatment and posttreatment attempts in only three patients; therefore, 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 A, the thickness of an endothelial vesicle. This degree of thickness is admittedly arbitrarily selected but being markedly thinner than normal endothelium (4,000 to 6,000 A), 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,

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determinations from other studies this laboratory has performed using human skin and muscle capillaries were used to define normal. The methods were identical. To our knowledge, glucocorticosteroid administration does not affect normal mammalian capillary endothelial structure. The data were analyzed using the Statistical Analysis System (SAS) 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.

**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 thrombocytopenia; 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. 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...
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 enfaceation 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 by glucocorticosteroid administration, this hypothesis is further strengthened.

The mechanism by which either platelets or glucocorticosteroids may support normal endothelial structure, and hence strength, is not known at this time, but several possibilities exist. Endothelium could appear thinner if fewer endothelial cells were available to cover a fixed luminal surface area. It is unlikely that the number of endothelial cells changes as rapidly as necessary to explain the changes observed in either this clinical study or the previous experimental studies.

Endothelium could appear thinner if the mean circumference of the microcirculation increased. We do not observe any differences among groups of patients (or animals in experimental work) with respect to diameter of capillaries or to how many capillaries were "open" or "closed" as determined by relaxation or constriction of arterioles proximal to the microcirculation. Thinned or fenestrated endothelium was just as likely to be found in a small, flat (ie, "closed") capillary as in a nearly perfectly round capillary. As capillaries themselves lack smooth-muscle cell investiture, their "openness" is determined by proximal blood flow. The law of Laplace determines that the thin-walled capillary, because of its overall small diameter compared with the rest of the vascular tree, is most resistant to passive engorgement or overinflation due to proximal pressure.

Most likely these alterations are caused by the metabolic effects that platelets and glucocorticosteroids have on endothelial cells. These effects are imperfectly understood and are now being discovered. Platelets can provide prostaglandin endoperoxides to endothelial cells for metabolism. King and Buchwald have demonstrated a factor in platelets that promotes endothelial cell growth and is distinct from platelet-derived growth factor. Blachman et al have demonstrated that hydrocortisone administration decreases vessel wall prostacyclin production in vivo. Profound alterations in protein synthesis by endothelial cells studies in vitro are induced by glucocorticosteroids. Any or all of these observations may in part play a role in endothelial cell homeostasis.

Shepro et al were unable to find similar capillary ultrastructural changes in nonquantified observations in experimental thrombocytopenia. The wide variation in endothelial thickness observed in the present study indicates that many vessels in thrombocytopenic preparations may appear normal and many capillaries in normal rabbits or humans may appear thinner than the mean. Only by observing and quantifying many vessels in a blinded morphometric analysis will such changes be statistically apparent.

REFERENCES
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