Effect of Albumin on the Inhibition of Platelet Aggregation by β-Lactam Antibiotics

By E.M. Sloand, H.G. Klein, K.B. Pastakia, P. Pierce, and K.N. Prodouz

Platelet aggregation and bleeding time abnormalities are reported in patients receiving β-lactam antibiotics (βLAs), although clinical bleeding most frequently occurs in chronically ill, malnourished patients. Although most βLAs bind to serum albumin, the relative influence of bound versus unbound βLAs on platelet function is unknown. We examined the effect of βLAs on the aggregation of gel-filtered platelets from normal subjects and on platelet-rich plasma (PRP) from hypoalbuminemic patients. Therapeutic concentrations of five βLAs were added to normal platelets at different albumin concentrations (1.5 to 4.5 g/dL). Inhibition of aggregation by the βLAs was inversely proportional to the albumin concentration, and most antibiotic-treated samples showed more than 50% inhibition at albumin levels below 2.0 g/dL. When platelet agonists with their receptor^.

P LAs was inversely proportional to the albumin concentration, and this decreased platelet aggregation was partially restored (25% to 75%) by increasing the albumin concentration above 4.0 g/dL. Specific binding of [35S]-benzylpenicillin to normal platelets decreased proportionately as the albumin concentration increased in the range of 1.0 to 5.0 g/dL. The inhibitory effects of βLAs on platelets in vitro appear to be influenced by albumin concentration. Plasma albumin concentration may influence bleeding in patients receiving βLAs.

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METHODS AND MATERIALS

Platelet sources. Platelets were obtained from three groups of individuals. Group 1 consisted of platelet concentrates from normal blood donors obtained by automated platelethepresis (Fenwal CS-3000; Baxter Health Care, Deerfield, IL). Group 2 consisted of platelet-rich plasma (PRP) samples from selected oncology patients, six with normal serum albumin (range, 3.0 to 4.6 g/dL) and four with low serum albumin levels (range, 2.5 to 2.8 g/dL; mean, 2.6 g/dL). Samples from patients with normal albumin levels were incubated with cephalothin, aggregation was completely inhibited, in contrast to samples from patients with normal albumin levels, and this decreased platelet aggregation was partially restored (25% to 75%) by increasing the albumin concentration above 4.0 g/dL. Specific binding of [35S]-benzylpenicillin to normal platelets decreased proportionately as the albumin concentration increased in the range of 1.0 to 5.0 g/dL. Inhibition of aggregation by the βLAs was inversely proportional to the albumin concentration, and most antibiotic-treated samples showed more than 50% inhibition at albumin levels below 2.0 g/dL. When platelet agonists with their receptor^.

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From the National Heart, Lung, and Blood Institute, and the Department of Transfusion Medicine, National Institutes of Health, the Warren G. Magnuson Clinical Center, the Center for Biologies Evaluation and Research, Food and Drug Administration, Bethesda, MD; and the Georgetown University Medical Center, Washington, DC.


Address reprint requests to E.M. Sloand, MD, NIH Building 31, Room 5A21, 9000 Rockville Pike, Bethesda, MD 20892.

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Platelet inhibition was measured by platelet aggregation 15 minutes after βLA addition. Each sample (0.45 mL) was incubated at 37°C for 10 minutes and aggregation was induced by adding 50 μL of 80 μmol/L adenosine diphosphate (ADP; Sigma) and stirring. The maximum slope of aggregation was determined for duplicate samples and the results were averaged. Inhibition for each albumin level was calculated as the percent of maximum slope of aggregation of the control sample at the same albumin level, but without βLA.

**Aggregation response of platelets from hypoalbuminemic patients.** Thirty milliliters of PRP from nine oncology patients (six with normal albumin and three hypoalbuminemic) was prepared from venous blood collected into a solution of acid-citrate dextrose (15% [vol/vol]) and centrifuged at 160g for 15 minutes at room temperature. Platelet counts ranged from 1.4 to 2.0 × 10^10/mL. Cephalothin (in phosphate-buffered saline [PBS], pH 7.4) was added to a sample of PRP at a concentration of 250 μg/mL and an equal volume of PBS was added to a control sample. The βLA-treated and untreated samples were stored overnight in modified PL 732 platelet storage bags (Fenwal; Baxter Health Care) at 22°C with constant agitation. Platelet aggregation was measured as described above. The maximum slope of aggregation in response to 8 μmol/L ADP was calculated for samples tested in duplicate.

In separate experiments, PRP from 10 hypoalbuminemic patients (four of whom were receiving cephalexin) was divided into two aliquots. Fatty acid-free human albumin (25 g/dL in 0.9% NaCl; Sigma) was added to one aliquot to adjust the albumin concentration to approximately 4.0 g/dL. An equal volume of 0.9% NaCl was added to the control aliquot. Platelet aggregation in response to 0.5 μmol/L ADP was measured as described above.

**Penicillin binding experiments.** A 15-μL suspension of Apiezon oil A (Biddle Co, Bluebell, PA) and n-butyl phthalate (1:9 vol/vol) together with 4 μL of ^[35]S]-benzylpenicillin (0.28 to 4.0 nmol/μL; 27 to 162 nmol/10 platelets; Du Pont Co, Wilmington, DE) in ethanol (final concentration, <0.5%) was added to 200 μL samples of gel-filtered albumin-free platelets obtained from normal individuals. Samples were inverted several times, incubated at 37°C for 15 minutes, and then centrifuged at 1,200g for 15 minutes. The supernatant was removed and the tip of the tube containing the pellet was excised. The pellet was solubilized in 500 μL of 1% sodium dodecyl sulfate. Radioactivity was measured after addition of Aquasol (Du Pont Co). Binding specificity was assessed by measuring the displacement of ^[35]S]-benzylpenicillin by 1,000-fold excess nonradioactive penicillin. Binding experiments were also performed on three gel-filtered platelet concentrates at different albumin levels. All samples were tested in quadruplicate.

**RESULTS**

**Aggregation response of normal platelets treated with βLA.** Aggregation was decreased significantly in gel-filtered platelets treated with any of the five βLAs at all albumin concentrations less than 2.5 g/dL in the medium. An example of the relationship of platelet inhibition to albumin level is seen in Fig 1 when 8 μmol ADP was used as an agonist. The mean inhibition of aggregation for cephalothin in the five different platelet samples was 64% ± 35% for an albumin of 2 g/dL and 0% for an albumin of 4.0 g/dL. For most βLAs tested, platelet aggregation was either slightly affected or similar to the control sample (at the same albumin concentration but without βLA) when albumin levels exceeded 3.0 to 3.5 g/dL. Below this level, the inhibitory effect of βLAs was inversely related to albumin level in all samples. Similar effects were observed in washed platelets when 8 μmol/L ADP or thrombin (0.1 to 1.0 U/mL) were used as agonists and in gel-filtered platelets when collagen and epinephrine were used (Table 1). When

![Graph](link)
doses of cephalothin, 10-fold in excess of those used therapeutically, were added to gel filtered platelets, all samples showed inhibition of function regardless of albumin level (when ADP [8 μmol/L], collagen [200 μg/mL], or epinephrine were used as agonists).

Aggregation response of platelets from hypoalbuminemic patients. Complete inhibition of platelet aggregation in response to 8 μmol/L ADP was achieved when platelets from four hypoalbuminemic individuals were incubated overnight with 200 μg/mL cephalothin (Fig 2B). Little or no inhibition of ADP aggregation (8 μmol/L) occurred in platelets from six patients with albumin levels greater than 3.0 g/dL (Fig 2A). The inhibition exhibited by the platelets in hypoalbuminemic plasma was partially reversible (25% to 75%) when the albumin levels were increased to 4.0 to 4.8 g/dL by the addition of 25 g/dL human albumin.

Representative aggregation curves from a single subject are shown in Fig 3.

When the albumin concentration of PRP from hypoalbuminemic patients (albumin range, 2.6 to 3.0 g/dL; mean, 2.8 g/dL) receiving cephalothin (1 g every 4 hours) was increased to 4.0 to 4.5 g/dL by the addition of human albumin, the platelet aggregation response to 0.5 μmol/L ADP increased (Fig 4A). The maximum slope of platelet aggregation increased from a mean of 0.72 to 1.6 in patients receiving cephalothin when the albumin level was raised from an average of 2.8 g/dL to 4.2 g/dL. In contrast, the slope of aggregation decreased in PRP samples from patients with hypoalbuminemia who were not receiving βLA (Fig 4B). The maximum slope of aggregation decreased from a mean of 1.7 to 1.2 in samples from those individuals not receiving cephalothin. This latter observation is consistent with previous reports of the effect of albumin concentration on platelet aggregation."Similar effects were obtained by using albumin prepared for human use.

Penicillin binding. The binding of [35S]-labeled benzylpenicillin to albumin-free (< 0.04 g/dL) gel-filtered platelets is shown on the Scatchard plot in Fig 5. Binding was specific and reversible, as indicated by displacement of radiolabeled penicillin by unlabeled penicillin. Saturation was achieved in albumin-free platelets at 130 pmol/10⁸ platelets. Scatchard plot analysis of these data indicates, under these conditions, approximately 4,800 binding sites per platelet with an apparent dissociation constant of 200 nmol/L and an affinity constant of 5 × 10⁶ L/mol. At a concentration of 54 pmol of benzylpenicillin/10⁸ platelets, binding of penicillin to platelets was increased in samples with lower albumin concentrations, and platelets showed a decrease in binding with increasing albumin concentrations (Fig 6). Similar results were obtained when stabilized albumin prepared for transfusion was substituted for fatty acid-free albumin. Platelets exposed overnight to radiolabeled penicillin continued to show decreased binding of penicillin at higher albumin levels. However, platelets incubated overnight bound less penicillin than those incubated for 15 minutes. This is most likely due to a loss of platelet integrity that occurs after gel filtration and storage in a non-gas-permeable container.

**DISCUSSION**

Bleeding episodes observed in patients who receive βLAs have been attributed to platelet dysfunction, hypoprothrombinemia, and the inhibition of fibrin polymerization. Factors that have been implicated in the increased risk of bleeding include thrombocytopenia, uremia, chemother-
Fig 4. Platelet aggregation responses to 0.5 μmol/L ADP were tested using PRP obtained from four hypoalbuminemic patients (mean albumin, 2.9 g/dL) receiving cephalothin (1 g every 4 hours) (A) and six hypoalbuminemic patients receiving no antibiotics (B). Samples were split and the albumin levels of one of the pair were adjusted to approximately 4 g/dL; a similar volume of PBS was added to the control sample. Samples of PRP from patients not receiving antibiotics showed decreased aggregation after addition of albumin, a well-described phenomenon, while the aggregation response of PRP from patients receiving PIs increased.

Fig 5. The binding of [14C]-benzylpenicillin to samples of gel-filtered, albumin-free platelets from two donors was determined as previously described for concentrations of benzylpenicillin of 27 pmol through 162 pmol/10^8 platelets. Saturation was previously determined to have been achieved at 130 pmol/10^8 platelets. A Scatchard analysis is presented. Nonspecific binding was determined by the addition of a 1,000-fold excess unlabeled benzylpenicillin. A total of 4,800 sites per platelet were seen with an apparent dissociation constant of 200 nM and an affinity constant of 5 x 10^4 M⁻¹/mol.

Fig 6. The effect of albumin concentration on the binding of [14C]-benzylpenicillin was measured for three different gel-filtered plateletpheresis concentrates. Binding of benzylpenicillin at each albumin level was measured in quadruplicate for each sample using a concentration of 54 pmol of benzylpenicillin/10^8 platelets. Results were averaged and plotted using linear regression analysis. The error bars represent the standard error of the mean.
exhibit significant binding to protein,\textsuperscript{25,26} and the inhibition of platelet function by each βLA was blocked by albumin in a similar manner.

It is likely that βLAs exert their inhibitory effect by interfering with agonist interaction with platelet surface receptors.\textsuperscript{1,12-15} This mechanism is supported by evidence that binding of an α-adrenergic antagonist, \((3H)\) dihydroergocryptine, to platelet adrenergic receptors is reduced twofold, and \((13C)\) serotonin release is completely blocked after incubation of platelets with βLAs.\textsuperscript{12} Evidence supporting reversible βLA binding to the platelet surface can be found in reports that platelet function returns to normal after exposure to βLA-free plasma.\textsuperscript{13-15}

In our storage studies, in which platelets were stored under standard blood banking conditions, but in the presence of a therapeutic concentration of cephalothin, antibiotic-mediated platelet dysfunction was at least partially reversed by the addition of albumin. However, irreversible binding of radiolabeled penicillin to platelets and irreversible inhibition of platelet function after prolonged exposure to very high doses of penicillin (10 to 20 mmol/L) have been reported.\textsuperscript{15} Using similar conditions, we found that 24 hours of exposure to a high dose of penicillin G (5.4 mmol/L) in the presence of lowered albumin levels (1.27 to 1.45 g/dL; average, 1.36 g/L) impaired the aggregation response of platelets to thrombin even after removal of the antibiotic by washing, but no structural changes were observed in glycoprotein Ib or IIb by polyacrylamide gel electrophoresis and immunoblotting (data not shown). This finding is consistent with the report that in rat platelets treated with a variety of βLAs, the loss of platelet function does not correlate with structural changes in surface glycoproteins.\textsuperscript{21} This irreversible inhibition of platelet function seen at higher doses of penicillin probably is caused by a different mechanism of platelet membrane damage from the one responsible for the reversible inhibition seen at lower doses.

Our observation that inhibition of platelet aggregation by βLAs is inversely related to the albumin level suggests that albumin-bound antibiotic is unable to bind to platelets, or does so with less affinity than does unbound antibiotic. While our experiments do not directly address βLA-induced bleeding, we suspect that the increased frequency of bleeding reported in individuals with chronic illness who are treated with βLAs may be related to low albumin concentration. Our observations may further explain why βLA administration to normal subjects is rarely associated with platelet dysfunction or bleeding.

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