Photochemical Inactivation of Pathogenic Bacteria in Human Platelet Concentrates

By Lily Lin, Helen Londe, J. Michael Janda, Carl V. Hanson, and Laurence Corash

Platelet concentrates (PC) may be infrequently contaminated with low levels of bacteria that can cause sepsis in patients receiving transfusion therapy. We evaluated the efficacy of a photochemical decontamination (PCD) technique using 8-methoxyxpsoralen (8-MOP) and long wavelength UV light (UVA) to inactivate bacteria in standard therapeutic PC. Twelve phylogenetically distinct pathogenic bacteria, 5 gram-positive and 7 gram-negative organisms, were seeded into PC to a final challenge dose ranging from 10^3 to 10^7 colony-forming units (CFU)/mL. Contaminated PC were treated with 8-MOP (5 μg/mL) and 5 J/cm² of UVA, a PCD treatment regimen found to adequately preserve in vitro platelet function. Greater than 10^6 CFU/mL of all 5 gram-positive (Staphylococcus aureus, Streptococcus epidemidis, Streptococcus pyogenes, Listeria monocytogenes, and Corynebacterium minutissimum) and 2 of the gram-negative (Escherichia coli and Yersinia enterocolitica) organisms were inactivated. The remaining 5 gram-negative organisms were more resistant, with less than 10³ to 10³ CFU/mL inactivated under these conditions. The inactivation efficiency for this resistant group of gram-negative organisms was improved when PC were resuspended in a synthetic storage medium with reduced plasma protein concentration (15%) and an increased 8-MOP concentration (23.4 μg/mL). Illumination with 3 J/cm² of UVA in this system inactivated greater than 10^6 CFU/mL of 4 resistant gram-negative organisms (Salmonella choleraesuis, Enterobacter cloacae, Serratia marcescens, and Klebsiella pneumoniae) and 10^4 CFU/mL of the most resistant gram-negative organism (Pseudomonas aeruginosa). This level of PCD treatment did not adversely affect in vitro platelet function. These results demonstrate that PCD using 8-MOP (5 to 23.4 μg/mL) effectively inactivated high levels of pathogenic bacteria in PC with adequate preservation of in vitro platelet properties.

© 1994 by The American Society of Hematology.

TRANSMISSION OF bacterial infections through transfusion of whole blood, packed red blood cells, and platelet concentrates is well documented.13 Although an infrequent complication, contamination of platelet concentrates with pathogenic bacteria has been persistently reported since the advent of platelet storage under room temperature conditions and extension of storage to more than 3 days. Several investigators have confirmed that pathogenic bacteria proliferate in platelet concentrates during room temperature storage.5-9 Transfusion of contaminated platelets results in significant morbidity and mortality.4,5,8-10 A phylogenetically diverse group of bacteria, encompassing gram-positive and gram-negative organisms, has been reported in association with platelet transfusion-related sepsis. The recent report by Muder et al further emphasizes that platelet transfusion-related bacteremia may be under reported because of a low index of suspicion. Other factors, such as neutropenia, may determine whether patients become bacteremic after receiving a contaminated transfusion product.10

Because transfusion-induced bacterial infection can lead to significant morbidity and mortality in immunocompromized individuals, blood banks have made efforts to reduce risks. Among the measures suggested are voluntary reduction of platelet concentrate storage times and pretransfusion gram-staining of all platelet units.10 Voluntary curtailment of storage time has a significant impact on platelet concentrate inventory management, and gram-staining is limited in sensitivity.1112 Recently, the sensitivity of other detection methods, including blood product pCO₂, pO₂, and a universal bacteria RNA probe, were evaluated.13 However, these methods may not be sensitive to bacterial levels less than 10³ bacteria/mL.14

Another approach to eliminate transfusion-associated sepsis is decontamination of platelet concentrates. We previously described a prototype technique for the inactivation of viruses and bacteria in human platelet concentrates using a naturally occurring psoralen, 8-methoxyxpsoralen (8-MOP), in conjunction with long wavelength UV (UVA) illumination. Utility of the prototype method was limited by requirements for control of ambient oxygen levels and the use of a high 8-MOP concentration (300 μg/mL). We have extended our studies with this technique to evaluate its efficacy to inactivate a broad spectrum of pathogenic bacteria in platelet concentrates using a simpler protocol using low concentrations of 8-MOP without control of ambient oxygen levels.

MATERIALS AND METHODS

Selection, maintenance, and preparation of bacterial stocks. Twelve different species of bacteria (Staphylococcus aureus, Streptococcus epidermidis, Streptococcus pyogenes, Listeria monocytogenes, Corynebacterium minutissimum, Escherichia coli, Yersinia enterocolitica, Salmonella choleraesuis, Enterobacter cloacae, Serratia marcescens, Klebsiella pneumoniae, and Pseudomonas aeruginosa) were used in the present study. Species were selected based on reported association with platelet transfusion sepsis.1 All strains were recovered from cases of sepsis, with the exception of S. choleraesuis, which was isolated from feces. Bacterial strains were from the culture collection of the Microbial Diseases Laboratory of the State of California Department of Health Services (Berkeley, CA). Individual strains were maintained on extract agar slants (or blood slants for L. monocytogenes and S. pyogenes) at ambient temperatures during the course of the investigation. The relevant clinical, phenotypic and serologic characteristics of the 12 strains were determined (Table 1).
contaminated PC to a final concentration of 8-MOP (3 mg/mL) dissolved in ethanol was diluted in the remaining phosphate-buffered saline (PBS; pH 7.2) to an osmolality of 0.9 to 1.0, which yielded an approximate concentration of $5 \times 10^9$ colony-forming units (CFU/mL) by plate count.

**Inactivation of bacteria in PC.** PC (50 mL) in citrate-phosphate-dextrose-adenine (CPD-A) anticoagulant in CLX bags (Miles Inc, Cutter Biologicals, Elkhart, IN) were obtained from the Alameda-Contra Costa County Medical Association Blood Bank (Oakland, CA). For the majority of inactivation studies, units were recently outdated, ranging in age from 6 to 8 days after collection, with a pH range of 6.9 to 7.5 at time of study. A subset of inactivation experiments ($n=21$) were performed using freshly released PC (24 hours old). Before processing for use in bacterial inactivation experiments, a sample (1 mL) was taken from each PC unit to measure pH and test for pre-existing bacterial contamination. Contaminated units were discarded.

Inactivation kinetics for each bacterial strain were determined by diluting standardized bacteria stock suspensions 1:100 into PC to a final bacteria concentration of $10^8$ to $10^9$ CFU/mL. A 0.5-mL aliquot was withdrawn for baseline bacteria quantification. Two 3-mL aliquots were withdrawn to serve as controls treated with UVA only. 8-MOP (3 mg/mL) dissolved in ethanol was diluted in the remaining contaminated PC to a final concentration of 5 μg/mL followed by a 30-minute equilibration period. A 0.5-mL aliquot of contaminated PC containing 8-MOP was withdrawn and held in the dark to serve as a dark control. The two UVA-only control PC aliquots and three 3-mL aliquots of contaminated PC containing 8-MOP were transferred to 17-cm² Teflon bags (FL03; American Fluoroseal, Silver Spring, MD) for photochemical decontamination (PCD) treatment. These bags exhibit high UVA transmission (87%) and are compatible with platelets.32

For bacteria with which a low level of inactivation was achieved (less than 10³-fold reduction) in 100% plasma with 5 μg/mL 8-MOP, an alternative PCD inactivation protocol was evaluated. PC were centrifuged (4,000g for 6 minutes at 22°C) and 85% of the supernatant plasma volume was removed and replaced with an equal volume of a synthetic medium. Sterilite 3.0 (20 mmoVL Na acetate, 2 mmol/L MgCl₂, and 20 mmol/L Na₂HPo₄-NaH₂PO₄, pH 7.2). Sterilite 3.0 was formulated to contain 8-MOP at a final concentration of either 5 μg/mL or 23.4 μg/mL after addition to PC. 8-MOP was directly dissolved in Sterilite 3.0 without use of ethanol. After the addition of synthetic media, with or without 8-MOP, the platelet pellet was resuspended, bacteria were added as above, and handled similarly to PC suspended in 100% plasma. UVA-only control samples and dark control samples were prepared as for PC in 100% plasma.

**UVA illumination device and PCD treatment.** PC seeded with bacteria, with and without 8-MOP, were illuminated with UVA light using a custom device (Steritech, Concord, CA). The device contained two arrays of 8 to 10 P24T12-BL-HO fluorescent lamps (Voltaire, Fairfield, CT) filtered through 0.5-in BK7 glass to produce 320 to 400 nm UVA light at 17 to 20 mW/cm². UVA light intensity was measured at the sample surface with both light banks on, using a long wavelength UV meter (Model SEL033; International Light Inc, Newburyport, MA). The distance between the two light arrays was 4 in. Material to be illuminated was placed on a sheet of 0.5-in BK7 glass located halfway between the two banks of lights. The light pathlength was approximately 0.18 cm through the PC. The device was equipped with a circulating cooling unit to maintain temperature inside the samples at 22°C to 24°C. The light intensity of the device
was sufficient to use PCD treatment times ranging from 1 to 5 minutes to deliver a 1 to 5 J/cm² UVA dose.

Quantification of bacteria in platelet concentrates. After inactivation, samples were serially diluted (10-fold) in PBS, and 100-μL aliquots were plated in duplicate onto either HIA or HIA-BA plates and uniformly dispersed via a sterile spreader. Plates were incubated at 35°C for 18 hours, after which viable bacteria were enumerated by colony count and the average of duplicate plates determined. If the lower dilutions yielded no growth by plate counts, 1-mL samples of the inactivated suspensions, which had been simultaneously inoculated into brain heart infusion broth (BHI) and incubated as above, were plated and streaked onto appropriate culture media for identification. No growth of 1-mL samples in broth was considered to indicate an additional log₁₀ of bacterial inactivation. Inactivation results were expressed as the log₁₀ of the survival ratio (N/N₀), where N₀ was the number of organisms before treatment and N was the number detected in culture after PCD treatment.

Evaluation of platelet integrity. The quality of PC before and after treatment with 8-MOP and UVA was evaluated with a panel of in vitro assays. These experiments were performed in parallel with the inactivation experiments but without the addition of bacteria. Random donor PC, freshly prepared by centrifugation under routine blood bank conditions and obtained less than 24 hours post-phlebotomy, were used. After treatment with PCD, PC units were stored at 22°C in a metabolic cabinet on a horizontal shaker (Helmer Labs, Noblesville, IN). The duration of storage was calculated from the time of phlebotomy.

The quality of platelets suspended in autologous plasma was evaluated after treatment with 5 J/cm² of UVA illumination in the presence of 5 μg/mL 8-MOP. Because of limited supply of freshly prepared standard PC, 3-mL PC were prepared as described in the bacterial inactivation experiments. The 3-mL PC in Teflon bags had a volume-to-surface ratio within twofold of that of 50-mL PC in standard storage bags. Each standard unit was divided into two portions. 8-MOP was added to one portion to a final concentration of 5 μg/mL. Three-milliliter aliquots from each portion were transferred into FL03 Teflon cell culture bags (American Fluoroseal Corp, Silver Spring, MD). The 3-mL PC without 8-MOP were not illuminated and were used as paired controls for standard 50-mL PC stored under the same conditions. The 3-mL PC containing 5 μg/mL 8-MOP were illuminated with 5 J/cm² of UVA.

The quality of platelets resuspended in synthetic medium Sterilite 3.0 was also evaluated. For each unit of freshly prepared standard PC, two 3-mL aliquots were transferred into FL03 Teflon bags and used as standard plasma PC controls. The remaining platelets were pelleted and resuspended in Sterilite 3.0 with 15% plasma as described for bacterial inactivation studies. The final 8-MOP concentration was 23.4 μg/mL. Ten 3-mL aliquots were transferred into FL03 bags. Two 3-mL PC were illuminated with 1, 2, 3, or 4 J/cm² of UVA and two were reserved as untreated controls.

Extracellular pH of PC suspensions was measured immediately after sampling using a blood gas analyzer (CIBA Corning Diagnostics Corp, Pleasanton, CA) equilibrated at 37°C. After diluting an aliquot of the PC 1:3 by volume with saline, the platelet and leukocyte counts were determined using an electrical impedance counter (Sysmex 600; TOA Medical Electronics CO, Los Alamitos, CA). Platelet aggregation was determined by measuring expression of GMP-140 in conjunction with fluorescent isothiocyanate (FITC)-conjugated goat antimouse Ig (Caltag). The fraction of platelets expressing GMP-140 was quantified using a FACScan Analyzer (Becton Dickinson, Mountain View, CA) as described previously.

Platelet activation was determined by measuring expression of GMP-140 (p-selectin) on the plasma membrane of platelets. GMP-140 is a unique α-granule membrane glycoprotein that is expressed only on the surface of activated platelets. A mouse monoclonal antibody, CD62 (Caltag, South San Francisco, CA), specific for p-selectin, was detected in platelet concentrates even with culture of 1 mL.

Table 2. Inactivation of Pathogenic Bacteria in Platelet Concentrates Under Varying PCD Conditions

<table>
<thead>
<tr>
<th>Organism</th>
<th>Condition A</th>
<th>Condition B</th>
<th>Condition C</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>&gt;6.0 ± 0.3 (3)*</td>
<td>&gt;6.3</td>
<td>&gt;6.3</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>&gt;5.1</td>
<td>&gt;6.3</td>
<td>&gt;6.3</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>&gt;5.9</td>
<td>&gt;6.3</td>
<td>&gt;6.3</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>&gt;6.7</td>
<td>&gt;6.3</td>
<td>&gt;6.3</td>
</tr>
<tr>
<td>C. minuta</td>
<td>&gt;5.3</td>
<td>&gt;6.3</td>
<td>&gt;6.3</td>
</tr>
<tr>
<td>E. coli</td>
<td>&gt;5.7 ± 0.3 (3)</td>
<td>&gt;6.3</td>
<td>&gt;6.3</td>
</tr>
<tr>
<td>Y. enterococitica</td>
<td>6.2 ± 0.7 (3)</td>
<td>&gt;6.3</td>
<td>&gt;6.3</td>
</tr>
<tr>
<td>S. cholerae</td>
<td>3.7</td>
<td>&gt;6.3</td>
<td>&gt;6.3</td>
</tr>
<tr>
<td>E. cloacae</td>
<td>2.2</td>
<td>4.8</td>
<td>5.6</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>0.9</td>
<td>5.1</td>
<td>5.8</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>0.7 ± 0.3 (4)</td>
<td>4.0</td>
<td>5.9 ± 0.1 (4)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>0.6 (2)</td>
<td>1.6</td>
<td>4.1 ± 0.5 (9)</td>
</tr>
</tbody>
</table>

Condition A consists of 5 J/cm² UVA, 5 μg/mL 8-MOP, 100% plasma. Condition B consists of 3 J/cm² UVA, 5 μg/mL 8-MOP, 15% plasma/85% Sterilite 3.0. Condition C consists of 3 J/cm² UVA, 23.4 μg/mL 8-MOP, 15% plasma/85% Sterilite 3.0. *For inactivation experiments in which more than two platelet concentrates were used, the number of individual PC is indicated (n) with the SD of the average log reduction. The greater than symbol indicates that the maximal number of bacteria added to the PC were inactivated and none were detectable after PCD treatment even with culture of 1 mL.

RESULTS

Bacterial inactivation. Bacterial challenges ranged from 10³ to 10⁵ CFU/mL of PC. None of the strains studied were significantly inactivated by 8-MOP in the absence of UVA or by UVA without 8-MOP. During the initial phase of the study, the inactivation kinetics of each organism were determined in standard PC containing 100% autologous plasma and 5 μg/mL 8-MOP. Under these conditions, an exposure of 5 J/cm² of UVA inactivated greater than 10⁴ CFU/mL of all 5 gram-positive and 2 gram-negative organisms (E. coli and Y. enterococitica). The remaining 5 gram-negative strains were either not inactivated (P. aeruginosa and K. pneumoniae) or were inactivated to a lesser degree than the highly susceptible bacteria; for example, 10³ CFU/mL S. cholerae was inactivated under these PCD treatment conditions (Table 2).

The 12 strains appear to fall into three groups according to rates of inactivation. Four gram-positive bacteria (S. aureus, S. epidermidis, S. pyogenes, and L. monocytogenes) demonstrated rapid inactivation rates, more than a 10⁴-fold decrease in CFU per milliliter after 1.25 or 2.5 J/cm² UVA with 5
PHOTOCHEMICAL INACTIVATION OF BACTERIA

The mean leukocyte concentration in 63 freshly prepared units used for inactivation studies and assessment of platelet quality was 3.9 × 10^9/L ± 2.8 × 10^9/L (SD), with a range of 0.9 to 12.0 × 10^9/L, and the mean platelet concentration was 1,246 × 10^9/L ± 376 × 10^9/L (SD), with a range of 612 to 2,384 × 10^9/L. The platelet count in the subset of PC used for platelet studies was similar to that of the entire PC set (Fig 6).

To determine the quality of standard PC after treatment with 5 µg/mL of 8-MOP and 5 J/cm^2 of UVA, 10 freshly prepared standard PC units were used. Each in vitro parameter to be evaluated was followed by either 15% plasma and 85% synthetic media or 4 J/cm^2 UVA.

Because 8-MOP is highly bound (91%) to plasma albumin,^2^ and only free 8-MOP binds to nucleic acid, we sought to increase the concentration of free 8-MOP by reducing the plasma protein concentration to improve the inactivation of PCD-resistant pathogens. Plasma protein concentration was reduced in PC by replacing 85% of the plasma with a synthetic storage buffer, Sterilite 3.0. Using 5 µg/mL 8-MOP in synthetic media with 3 J/cm^2 of UVA, the level of inactivation ranged from greater than 10^4-fold reduction (S choler aeruis) to only a 10^4-fold reduction (P aeruginos) (Table 2). When the level of 8-MOP was increased to 23.4 µg/mL, a greater than 10^6-fold CFU/mL reduction was achieved for all the resistant bacteria except for P aeruginos, which was reduced by 10^6-fold (Table 2). Under the three conditions of inactivation used for these resistant organisms, the relative susceptibility to inactivation was consistent. S choler aeruis, which showed the greatest sensitivity at 5 µg/mL 8-MOP in 100% plasma, was also the most rapidly inactivated pathogen at higher 8-MOP levels and lower protein concentration (Table 2 and Fig 4). P aeruginos was the most resistant organism to inactivation under all PCD conditions (Table 2 and Fig 5).

Because the majority of bacterial inactivation experiments were performed using outdated PC, a subset of experiments with the most resistant bacteria (K pneumoniae and P aeruginos) were performed using 24-hour-old PC. Inoculation of 10^2, 10^3, or 10^4 CFU/mL of P aeruginos into fresh PC (n = 11) containing 15% plasma and 85% synthetic media with 8-MOP (24.3 µg/mL) followed by either 3 or 4 J/cm^2 UVA resulted in complete inactivation. Under similar conditions, 10^6 CFU/mL of K pneumoniae was inactivated in fresh PC (n = 10). In another series of experiments modeled to mimic routine blood banking procedures, 10^1, 10^2, 10^3, and 10^4 CFU/mL of P aeruginos were added to fresh platelet-rich plasma (n = 4). PC were prepared by centrifugation with resuspension in 15% plasma and 85% synthetic media and stored overnight at 22°C. The final titer on the following day was less than 10^3 CFU/mL in each PC. After overnight storage, these PC were treated with 8-MOP (24.3 µg/mL) and 3 J/cm^2 UVA. At each bacterial concentration, complete inactivation was achieved.

Platelet quality after treatment with 8-MOP and UVA. The mean leukocyte concentration in 63 freshly prepared units used for inactivation studies and assessment of platelet quality was 3.9 × 10^9/L ± 2.8 × 10^9/L (SD), with a range of 0.9 to 12.0 × 10^9/L, and the mean platelet concentration was 1,246 × 10^9/L ± 376 × 10^9/L (SD), with a range of 612 to 2,384 × 10^9/L. The platelet count in the subset of PC used for platelet studies was similar to that of the entire PC set (Fig 6).

To determine the quality of standard PC after treatment with 5 µg/mL of 8-MOP and 5 J/cm^2 of UVA, 10 freshly prepared standard PC units were used. Each in vitro parameter to be evaluated was followed by either 15% plasma and 85% synthetic media or 4 J/cm^2 UVA.

Because 8-MOP is highly bound (91%) to plasma albumin,^2^ and only free 8-MOP binds to nucleic acid, we sought to increase the concentration of free 8-MOP by reducing the plasma protein concentration to improve the inactivation of PCD-resistant pathogens. Plasma protein concentration was reduced in PC by replacing 85% of the plasma with a synthetic storage buffer, Sterilite 3.0. Using 5 µg/mL 8-MOP in synthetic media with 3 J/cm^2 of UVA, the level of inactivation ranged from greater than 10^4-fold reduction (S choler aeruis) to only a 10^4-fold reduction (P aeruginos) (Table 2). When the level of 8-MOP was increased to 23.4 µg/mL, a greater than 10^6-fold CFU/mL reduction was achieved for all the resistant bacteria except for P aeruginos, which was reduced by 10^6-fold (Table 2). Under the three conditions of inactivation used for these resistant organisms, the relative susceptibility to inactivation was consistent. S choler aeruis, which showed the greatest sensitivity at 5 µg/mL 8-MOP in 100% plasma, was also the most rapidly inactivated pathogen at higher 8-MOP levels and lower protein concentration (Table 2 and Fig 4). P aeruginos was the most resistant organism to inactivation under all PCD conditions (Table 2 and Fig 5).

Because the majority of bacterial inactivation experiments were performed using outdated PC, a subset of experiments with the most resistant bacteria (K pneumoniae and P aeruginos) were performed using 24-hour-old PC. Inoculation of 10^2, 10^3, or 10^4 CFU/mL of P aeruginos into fresh PC (n = 11) containing 15% plasma and 85% synthetic media with 8-MOP (24.3 µg/mL) followed by either 3 or 4 J/cm^2 UVA resulted in complete inactivation. Under similar conditions, 10^6 CFU/mL of K pneumoniae was inactivated in fresh PC (n = 10). In another series of experiments modeled to mimic routine blood banking procedures, 10^1, 10^2, 10^3, and 10^4 CFU/mL of P aeruginos were added to fresh platelet-rich plasma (n = 4). PC were prepared by centrifugation with resuspension in 15% plasma and 85% synthetic media and stored overnight at 22°C. The final titer on the following day was less than 10^3 CFU/mL in each PC. After overnight storage, these PC were treated with 8-MOP (24.3 µg/mL) and 3 J/cm^2 UVA. At each bacterial concentration, complete inactivation was achieved.

Platelet quality after treatment with 8-MOP and UVA. The mean leukocyte concentration in 63 freshly prepared units used for inactivation studies and assessment of platelet quality was 3.9 × 10^9/L ± 2.8 × 10^9/L (SD), with a range of 0.9 to 12.0 × 10^9/L, and the mean platelet concentration was 1,246 × 10^9/L ± 376 × 10^9/L (SD), with a range of 612 to 2,384 × 10^9/L. The platelet count in the subset of PC used for platelet studies was similar to that of the entire PC set (Fig 6).

To determine the quality of standard PC after treatment with 5 µg/mL of 8-MOP and 5 J/cm^2 of UVA, 10 freshly prepared standard PC units were used. Each in vitro parameter to be evaluated was followed by either 15% plasma and 85% synthetic media or 4 J/cm^2 UVA.
The quality of 5-day-old PC suspended in synthetic medium and platelet quality with storage time. The pH and the extent those of the untreated standard PC in 100% plasma. The content were equivalent for controls and PCD-treated PC. GMP-140 expression, platelet count, and secretable ATP concentrations of 8-MOP demonstrated the following significant differences when compared with standard control PC. The pH of PCD-treated PC was significantly lower than that of the control PC, but no significant difference \( P > .05 \) at any UVA dose. These results suggest that, under reduced plasma concentration, platelets were exposed to more UVA light, resulting in more UVA-related damage. However, comparing these data with the paired untreated standard PC, which also deteriorated because of normal storage lesions, 3 J/cm² of UVA illumination in Sterilite 3.0 produced minimal adverse effects on platelet function.

**DISCUSSION**

Several studies have been undertaken to survey the frequency of bacterial contamination of platelet concentrates. Goldman and Blajchman recently reviewed this literature and reported rates of bacterial contamination of random platelet concentrates ranging from 0% to 10% of units cul-

---

Fig 3. Inactivation of resistant gram-negative bacteria in plasma. PC suspended in plasma were seeded with 10⁶ to 10⁷ CFU/mL of the following gram-negative bacteria: S. choleraesuis (S chol.), E. cloaceae (E cloa.), S. marcescens (S mar.), K. pneumoniae (K pneu.), and P. aerugi-nose (P aer.). PCD inactivation was performed using 5 \( \mu \)g/mL 8-MOP at varying UVA dose (abscissa) ranging from 1.25 to 5.0 J/cm². The log₁₀ reduction of viable bacteria is expressed on the ordinate.

Fig 4. Inactivation of S. choleraesuis under varying PCD conditions. PC suspended in plasma or mixtures of plasma and synthetic media (Sterilite 3.0) were seeded with 10⁶ to 10⁷ CFU/mL of S. choleraesuis. PCD inactivation was performed under the following conditions: (A) (·), 5 \( \mu \)g/mL 8-MOP with 100% plasma; (B) (△), 5 \( \mu \)g/mL 8-MOP with 15% plasma-85% Sterilite 3.0; and (C) (○), 23.4 \( \mu \)g/mL 8-MOP with 15% plasma-85% Sterilite 3.0. Inactivation was performed with 1.25 to 5.0 J/cm² UVA under each condition. The log₁₀ reduction in viable bacteria is indicated on the ordinate. Arrows indicate points at which no viable bacteria were detected after culture of 1 mL PC suspension.
tured. Survey of bacterial cultures from single donor apheresis platelet concentrates demonstrated rates of contamination ranging from 0% to 4.9%. The results of these studies are widely variable, and suggest that some of the variability may be caused by the volume of platelet concentrate cultured, ranging from one drop to 1 mL. Buchholz et al noted that selection of culture medium, temperature of culture, and volume of platelet concentrate cultured may account for the low frequency of contamination observed in the early studies.

Many investigators have consistently observed that the level of bacterial contamination is low, in the range of 10^3 to 10^5 CFU/mL of platelet concentrate. In addition, the time after processing at which platelet concentrates are tested for bacterial contamination may have a significant effect on the frequency of positive cultures. For example, Arnow et al reported that the rate of positive cultures from platelet concentrates was higher (13%) in concentrates stored more than 3 days compared with concentrates stored less than 3 days (4%). Analysis of all data from the Goldman and Blajchman review, without selection for culture technique, yields a frequency of bacterial contamination among 10,396 random donor units of 2.85%, and 1.07% from 3,346 single donor apheresis units. The most common source of bacterial contamination appears to be gram-positive microbial flora associated with skin that cannot be completely eliminated by surface decontamination techniques. Other sources of contamination have been shown to come from occult bacteremia that did not cause clinically significant illness detected on routine donor screening. Based on this survey of the literature, we studied the inactivation of clinically significant bacterial pathogens associated with platelet transfusion related sepsis using strains obtained from documented clinical isolates.

PCD treatment was efficacious in inactivating more than 10^6 CFU/mL of E. coli, S. aureus, S. epidermidis, Y. enterocolitica, L. monocytogenes, S. pyogenes, and C. minutissimum in plasma PC containing 5 μg/mL 8-MOP. When PC were suspended in 15% plasma with 85% synthetic medium containing 23.4 μg/mL 8-MOP, more than 10^6 CFU/mL of the gram-negative bacteria (S. choleraeuis, E. cloacae, S. marcescens, and K. pneumoniae) resistant to inactivation in 100% plasma were inactivated. In addition, use of synthetic medium permitted inactivation of 10^4 CFU/mL of P. aeruginosa, the most resistant pathogen in plasma PC. High concentrations of all gram-positive bacteria were rapidly inactivated in synthetic medium.

Bacterial inactivation experiments were performed using a large number (n = 43) of PC prepared under routine blood bank conditions from different donors containing widely variable leukocyte and platelet counts. We did not observe any correlation between inactivation efficiency and either of these parameters. Based on prior studies that indicated that the initial concentration of bacteria in contaminated PC ranged from 10^3 to 10^6 CFU/mL, we sought to develop an inactivation method that would permit at least a 10-fold margin of safety. To further examine the effect of bacteria concentration, a series of experiments were performed using low concentrations (10^2 to 10^5 CFU/mL) of the most PCD-resistant organism (P. aeruginosa). These experiments confirmed that reduced plasma protein levels and increased free 8-MOP concentrations were required to inactivate, consistently and completely, low concentrations of this pathogen in human PC. With respect to PCD-resistant bacteria, at no time did we observe enhanced inactivation efficiency from selected donors that would have been explained by the presence of preformed antibody. The highly resistant gram-negative bacteria could only be consistently inactivated using increased 8-MOP levels with reduced plasma concentrations (condition C) to increase the free 8-MOP concentration. A separate series of experiments with these PCD-resistant pathogens were conducted using fresh (24-hour-old) PC to insure that aged PC did not inhibit nor enhance inactivation efficiency. Using fresh PC, no significant differences in inactivation efficiency were observed compared with outdated PC.

Viable leukocytes in blood products may phagocyte contaminating bacteria within 24 hours of phlebotomy, thus preventing proliferation. However, the properties of different bacterial species may vary widely in this respect, and some strains are resistant to phagocytosis. The present study was designed to address the safety margin of the PCD technique and to demonstrate that, in the absence of functioning leukocytes or preformed antibody, at least 10^4 CFU/mL of pathogenic bacteria is inactivated by the PCD method.

We are presently unaware of any other studies that have shown inactivation of such a phylogenetically diverse group of bacteria, with a broad array of pathogenic mechanisms involved in human disease, with the simultaneous preserva-
tion of platelet function. Bacteria chosen for these studies included neurovirulent E. coli possessing polysialic acid (K1) capsules involved in neonatal meningitis, clonally restricted invasive group A streptococci causing toxic shock syndrome-like disease and possessing M1 antigen,33 serogroup-restricted enteropathogenic Y. enterocolitica possessing the virulence plasmid and invasion loci, and invasive S. cholerasuis. By using such a group of defined bacteria based on molecular, serologic, and pathogenic analysis, we provided a rigorous challenge to 8-MOP-mediated bacterial inactivation.

The PCD treatment did not induce additional injury to platelets other than the storage lesion observed with standard PC. Granna and Kao34 have shown that mouse platelets treated with 8-MOP and UVA exhibit normal posttransfusion recovery, and we have recently observed that murine platelets treated with our bacterial PCD conditions exhibit normal posttransfusion recovery, half-life, and mean life-span (data not shown). Other decontamination systems using other psoralens35,36 or photosensitizers have not shown bacterial inactivation with preservation of blood cell function.37,38

Photochemical bacterial inactivation offers several potential improvements for platelet concentrate processing. Using this methodology, with synthetic media, additional donor plasma can be salvaged and platelet function may be better preserved than with plasma as the suspension medium.40 In 1986, the time for storage of PC was reduced from 7 to 5 days because of concerns about bacterial contamination.1 However, since that time, investigation using synthetic media for improved preservation of platelet function during prolonged storage has evolved, and there is potential for prolonging platelet storage provided that bacterial growth can be prevented.41 Bacterial decontamination in combination with sterile docking techniques might also allow more efficient pooling and storage of PC in a closed, sterile system before transfusion.

The present technique results in the transfusion of 8-MOP along with the platelet concentrate. Using an 8-MOP concentration of 25 μg/mL, a 6-U random donor pool will contain 7.5 mg of 8-MOP, approximately 20% of the oral 8-MOP dose used for psoralen UVA (PUVA) treatment of psoriasis.42,43 Based on the established gastrointestinal absorption kinetics of oral 8-MOP, we estimate that posttransfusion peak plasma levels will be less than the peak plasma levels (290 to 400 ng/mL) reported with oral PUVA regimens.42 In view of the long-term safety of 8-MOP PUVA therapy...
with respect to noncutaneous neoplasia and reproductive toxicity.\textsuperscript{19,21} 8-MOP–based PCD treatment of PC may be a feasible method to reduce bacterial contamination of PC.

**ACKNOWLEDGMENT**

The authors acknowledge the cooperation of Dr Cheri Evans and the staff of the Alameda-Contra Costa County Blood Bank in supplying platelet concentrates. We also acknowledge the excellent technical assistance of Ryan Alfonso, Chu Lin, Margaret Rheinschmidt, Ken Metchte, Lainie Corten, Bill Behman, and Deborah Hanson in the performance of bacterial assays and platelet function studies. Aaron Reames assisted with the statistical analysis.

**REFERENCES**

40. Shamizu T, Murphy S: Roles of acidit and phosphate in the successful storage of platelet concentrates prepared with an acetate-containing additive solution. Transfusion 33:304, 1993
Photochemical inactivation of pathogenic bacteria in human platelet concentrates

L Lin, H Londe, JM Janda, CV Hanson and L Corash