Photochemical Inactivation of Cell-Associated Human Immunodeficiency Virus in Platelet Concentrates

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Photochemical decontamination (PCD) of platelet concentrates, with adequate preservation of platelet function, has been shown using 8-methoxypsoralen (8-MOP) and long wavelength UV light (UVA). To further evaluate this technique, models for the inactivation of pathogenic human cell-associated viruses and integrated proviral sequences are required. We have assessed the ability of the PCD technique to inactivate cell-associated human immunodeficiency virus 1 (HIV-1) in platelet concentrates. We correlated PCD inhibition of HIV-1 infectivity with 8-MOP-DNA adduct formation in contaminating nucleated cells, and measured the inhibition of polymerase chain reaction (PCR)-mediated amplification of cellular DNA sequences as a surrogate for inactivation of integrated proviral nucleic acid sequences. After PCD treatment (8-MOP 300 μg/mL, UVA 17 mW/cm²) for 60 minutes, 0.5 × 10⁶ plaque-forming units (PFU)/mL of cell-associated HIV-1 were inactivated and no virus was detectable by infectivity assay. After 60 minutes of PCD, 15 8-MOP-DNA adducts per 1,000 bp were formed, while in the absence of UVA, no adducts were formed. PCR-mediated amplification of a 242-bp cellular DNA sequence (HLA-DQα) was inhibited when greater than eight psoralen-DNA adducts per 1,000 bp were present. These studies indicate that high titers of cell-associated HIV-1 in platelet concentrates were inactivated by PCD, and the numbers of 8-MOP-DNA adducts in nucleated cells were sufficient to inhibit amplification of DNA segments that encode for as few as 80 amino acids. Based on the frequency of 8-MOP-DNA adducts, for the 10-kb HIV-1 genome, the probability of an integrated genome without at least one 8-MOP adduct after 60 minutes of PCD was 10⁻³².

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with 5% CO₂-95% N₂ for 12 to 30 minutes to reduce the generation of active oxygen species. After gassing, infected PC samples were treated with 8-methoxypsoralen (8-MOP) (300 μg/mL) and UVA (17 mW/cm²) using a psoralen ultraviolet A (PUVA) treatment light source equipped with HO PUVA bulbs (320 to 400 nm) (Dermacure, Frankfurt, IL) for up to 4 hours. PC samples were illuminated from both sides of the light source. UVA intensity was measured using a photometer (model IL1400A; International Light Inc, Newburyport, MA) equipped with a UVA-sensitive probe (model SEL 033, International Light Inc, Newburyport, MA). Control samples received either UVA exposure without 8-MOP, or were retained in the dark after addition of 8-MOP for up to 4 hours. During PCD, ambient temperature (25°C) was maintained through the use of a circulating water bath connected in series with the jacketed chambers. Samples for measurement of HIV-1 infectivity (2 mL) were periodically withdrawn for treated and control samples. Each sample was withdrawn from separate platelet-concentrate aliquots to maintain constant sample volume (5 mL) and path length. These samples were diluted to 10 mL with phosphate-buffered saline (PBS) (pH 7.5), the cells were pelleted (240g × 10 minutes), resuspended in 0.5 mL DMEM/15% FBS, and stored at −80°C until assayed for residual infectivity. Inactivation experiments were performed for 1 hour (n = 2) with eight time point samples and 4 hours (n = 1) with five time point samples. Each time point sample was assayed in quadruplicate for HIV-1 infectivity.

Measurement of psoralen-DNA adduct formation. The efficiency of 8-MOP-DNA adduct formation was determined using ³H-8-MOP added to PC contaminated with uninfected H9 cells (2.5 × 10⁹ per PC). Experimental samples and controls were handled identically to those used for the infectivity experiments. Radio-labeled 8-MOP was mixed with cold 8-MOP to bring the final concentration to 300 μg/mL with a specific activity of 118 mCi/mmol. After UVA irradiation for various times, cells were isolated as previously described, treated with extraction buffer containing Proteinase K, sodium dodecyl sulfate (SDS), and RNAse. After digestion, DNA (10 to 20 μg) was extracted using a phenol-chloroform process and ethanol precipitated three times. DNA concentration was determined by spectrophotometry at 260 nm, 1 od unit = 50 μg/mL. The numbers of 8-MOP adducts per 1,000 bp were determined using liquid scintillation counting.

Inhibition of amplification of leukocyte nucleic acid sequences. The efficiency of 8-MOP-DNA adduct formation also was assessed by measuring the inhibition of PCR-mediated amplification of a 242-bp sequence in the HLA-DQα locus using the following conditions. Uninfected H9 cells (2.5 × 10⁹ per PC) were added to PC and treated with 8-MOP and UVA as described. After treatment, leukocyte DNA was isolated as described in the preceding section, and 1 μg was added to each PCR reaction mixture. PCR reactions were set up for samples that had received PCD and for control samples. For each level of adduct formation, PCR products were analyzed after 20, 25, and 30 PCR amplification cycles. The PCR reaction buffer consisted of 10 mmol/L Tris-HCl, pH 8.4, containing 50 mmol/L KCl, 2.5 mmol/L MgCl₂, 0.02% gelatin, and deoxynucleotide triphosphates (dNTPs), each at a final concentration of 200 μmol/L. The final concentration of GH 26/GH 27 primers was 0.10 pmol/L. The final concentration of GH 26/GH 27 primers was 0.10 pmol/L. Taq polymerase 2.5 U, was used per 100-μL reaction. The nucleic acid tracer was α-32P-dCTP (3,000 Ci/mmol). A thermal profile of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 60 seconds was used. Detection of the amplification was performed by electrophoresis in a 12.5% denaturing polyacrylamide gel electrophoresis (PAGE) followed by autoradiography.

RESULTS

Photochemical inactivation of cell-associated HIV-1. After 60 minutes of PCD treatment (Fig 1) no viable HIV-1 was detectable; and further sampling, up to 4 hours of PCD treatment, showed consistently undetectable infectious virus. In contrast, the control sample, incubated under the same conditions with the exception of 8-MOP or UVA, showed viable virus over the entire 4-hour period. 8-MOP in the absence of UVA had no effect on HIV-1 titer, but after 4 hours of UVA without 8-MOP, there was a small reduction (0.7 log) in HIV-1 titer. PCD treatment for 60 minutes with more frequent sampling (Fig 2) showed log-
linear kinetics of viral inactivation with no detectable infectious virus after 60 minutes of treatment. In contrast, 60 minutes of UVA irradiation without 8-MOP (Fig 2), did not result in significant reduction of infectious virus.

**Photochemical addition of 8-MOP to cellular DNA during HIV-1 inactivation.** 8-MOP–DNA adduct formation was measured in parallel with HIV-1 inactivation over a 4-hour time course (Fig 3). To insure complete removal of unincorporated \([-8\text{MOP}]\), DNA samples were analyzed after 2 and 3 ethanol precipitations. After 60 minutes of photocatalyst treatment, approximately 15 8-MOP–DNA adducts formed per 1,000 bp, and the numbers of 8-MOP–DNA adducts increased slowly to a maximal level of 22 per 1,000 bp over 4 hour of PCD treatment. In the absence of UVA irradiation, no adducts were formed.

**Correlation of 8-MOP–DNA adduct formation and inhibition of amplification of cellular nucleic acid sequences.** PCR analysis of amplification of the HLA-DQ-\(\alpha\) locus was performed for 20, 25, and 30 cycles (Fig 4). After 20 amplification cycles no amplicon was detectable when 8, 15, 17, or 22 adducts per 1,000 bp were formed (Fig 4: lanes 4 through 7). In contrast, in the absence of adduct formation, amplification of the 242-bp sequence was detected (Fig 4: lanes 2 and 3). A control lane containing no DNA extract (Fig 4: lane 1) showed no reaction product. After 25 PCR cycles, small amounts of the 242-bp reaction product were observed at all levels of adduct formation. After 30 PCR cycles, more reaction product was detected. Signal intensity correlated inversely with the number of 8-MOP–DNA adducts. While no virus was detectable by infectivity assay after formation of 15 or more 8-MOP–DNA adducts, inhibition of amplification of the 242-bp cellular DNA sequence was not complete (Fig 4: lanes 4 through 6).

![Fig 3](image-url)  
**Fig 3.** Formation of 8-MOP–DNA photoadducts in nucleated cells \((2.5 \times 10^7 \text{ cells per PC})\) during PCD treatment. Decontamination was performed with radiolabeled 8-MOP. After PCD, nucleated cellular DNA was extracted and the incorporation of 8-MOP into DNA was measured. The ordinate indicates the numbers of 8-MOP adducts formed per 1,000 bp. The abscissa indicates UVA irradiation time in hours. Similar results were obtained after two and three ethanol precipitations of DNA.

**DISCUSSION**

We have previously described the inactivation of cell-free and cell-associated viruses in platelet concentrates treated with 8-MOP and UVA irradiation. These studies used model viruses that are not human pathogens. In addition, the UVA light source was composed of low energy black light bulbs (BLB) lights compared with the current more intense light source. Under the present conditions, \(0.5 \times 10^6\) cell-associated HIV-1/mL were inactivated in 60 minutes with log-linear kinetics over the first hour, after which time further infectious virus was undetectable. Thus, PCD with 8-MOP is capable of inactivating high concentrations of cell-associated HIV-1 in human platelet concentrates.

In addition to documenting the inactivation of cell-associated HIV-1 by a biologic assay, the present study used a novel PCR assay to assess the effects of cellular 8-MOP–DNA adducts on amplification of a cellular genomic DNA sequence, and correlated the results with quantitative measurements of radiolabeled psoralen-DNA adduct formation. In previous studies, we have shown that PCD treatment of PC containing \(0.8 \times 10^8\) lymphocytes resulted in a similar degree of inhibition of PCR amplification of the HLA-DQ-\(\alpha\) locus when a similar level of DNA-8-MOP adduct formation was achieved. Earlier studies have shown that both bifunctional and monofunctional psoralen-nucleic acid adducts block the replication and expression of DNA sequences. More importantly, others have shown that a single psoralen-DNA adduct is lethal to a viral genome without repair processes. At the level of DNA modification achieved in the present study, cellular repair mechanisms are overwhelmed. In other investigations, we have observed that HIV-1 reverse transcriptase activity is completely inhibited by PCD conditions similar to those used in this study. The current results indicate that the PCR inhibition assay can be a surrogate test to measure the efficiency of inactivation conditions with greater sensitivity than the biologic assay. Therefore, it can be used to model the efficiency of adduct formation to inactivate integrated proviral nucleic acid sequences. For a specified nucleic acid sequence, as more 8-MOP–DNA adducts are formed the ability to generate amplicons from that sequence will decrease (Fig 5). Longer sequences will be more efficiently inactivated than small sequences.

Based on the size of a target sequence and measurement of the numbers of adducts formed, the probability of a sequence with no adducts can be estimated according to the Poisson distribution using the formula: 
\[ f(n) = \frac{a^n e^{-a}}{n!} \]
where
\[ a = \text{the average number of adducts per genome} \]
\[ f(n) = \text{the fraction of genomes with n adducts when the average number of adducts per strand is a}. \]

Because a can be measured using the radiolabeled 8-MOP incorporation assay (Fig 3) and the size of an integrated proviral sequence is known, then a family of probability curves can be calculated to estimate the likelihood of an unmodified genome surviving PCD treatment (Fig 6). Using this model for the 10-kb HIV-1 genome, with the following assumptions: the entire genome is integrated, adduct formation is random along the length of the genome, and the average number of adducts
PHOTOCHEMICAL DECONTAMINATION OF PLATELETS

Fig 4. Inhibition of amplification of cellular nucleic acid by PCD treatment. After PCD of PC containing $2.5 \times 10^7$ uninfected H9 cells, nucleated cellular DNA was extracted and analyzed (1 µg per reaction) using the PCR reaction with primers for the 242-bp HLA DQ-α sequence. The PCR reaction was performed for 20, 25, and 30 cycles. The UVA treatment time in hours is indicated and the number of 8-MOP-DNA adducts, as previously determined (Fig 3), is indicated. The HIV-1 titer is indicated at the bottom of the gel. N.D. indicates not detected. The PCR reaction products (242-bp sequence) were analyzed by denaturing PAGE analysis. The reagent control (lane 1) in which no DNA was added to a PCR reaction was negative. Positive controls (lanes 2 and 3) showed a strong band at 242 bp.

formed in the DNA extract (Fig 3) is representative of those formed in the HIV-1 genome, then at a modification density of 15 adducts per 1,000 bp the likelihood of an unmodified surviving genome is $10^{-33}$. For small nucleic acid sequences, with increasing amplification cycles (Fig 4), unmodified segments will be amplified, ultimately. The PCR inhibition assay can be used to estimate the numbers of unmodified copies. In addition, because small sequences will be amplified as the number of PCR cycles increases, this assay provides an internal control for each inactivation experiment. Thus, this assay has great utility to evaluate the efficiency of psoralen-based inactivation protocols. More importantly, previous studies have shown that when psoralen-DNA adduct modification density approaches 1 adduct per 100 bp, the expression of a single gene becomes impossible.

The PCD protocol and psoralen compound described in this report are not ideal because of the requirement for long

![Diagram](http://www.bloodjournal.org)

**Fig 5.** Model for inhibition of amplification of nucleated cell nucleic acid sequences. The biological assay indicating infectivity, PCR signal, target strands, and photochemical modification density are shown for a theoretical nucleic acid sequence and primer pair. With increasing photochemical modification density, caused by psoralen adduct formation, there is progressive diminution of the corresponding PCR signal in the treated sample (S) while the control (C) sequence remains unmodified. Biologic activity (infectivity) expressed as the titer of viable organisms detected was less sensitive than PCR for detection of unmodified nucleic acid sequences. N.D. indicates not detectable.
treatment times and the necessity to reduce ambient O₂ levels to prevent platelet injury. Design of novel psoralens with greater nucleic acid binding affinity and reduced generation of free oxygen species should result in a more pragmatic PCD protocol. Despite these limitations, our study shows the utility of the basic principal of psoralen-based PCD for the inactivation of cell-associated viruses and cellular genomes in platelet concentrates, and establishes assay systems to evaluate new PCD compounds and treatment protocols using target pathogens known to cause disease.

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