Inactivation of Human Immunodeficiency Virus Type 1 by Ozone In Vitro

By Keith H. Wells, Joseph Latino, Jerrie Gavalchin, and Bernard J. Poiesz

A device was designed to deliver a constant source of given concentrations of ozone to fluids containing human immunodeficiency virus type 1 (HIV-1). Ozone was found to inactivate HIV-1 virions in a dose-dependent manner. Greater than 11 log inactivation was achieved within 2 hours at a concentration of 1,200 ppm ozone. Similar concentrations of ozone had minimal effect on factor VIII activity in both plasma and immunoaffinity-purified preparations of factor VIII treated for the same time period. The data indicate that the antiviral effects of ozone include viral particle disruption, reverse transcriptase inactivation, and/or a perturbation of the ability of the virus to bind to its receptor on target cells. Ozone treatment offers promise as a means to inactivate human retroviruses in human body fluids and blood product preparations.

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HUMAN immunodeficiency virus type 1 (HIV-1), the etiologic agent of acquired immunodeficiency syndrome (AIDS), is a lentivirus that completes its replicative cycle by budding through the host cell membrane, acquiring host-derived and virus-encoded components in the process. Subsequent rounds of replication require an intact lipid envelope containing the virally encoded envelope proteins necessary for receptor binding. It has been suggested that perturbation of the HIV-1 envelope may be a suitable approach to inactivating HIV-1. Compounds that fluidize membranes by removing cholesterol (AL721) or by complexing membrane cholesterol (amphotericin B methyl ester) can inhibit HIV-1 replication in vitro. Although their precise mode of action has yet to be defined, these compounds may reduce HIV-1 infectivity by perturbing the envelope of HIV-1. We therefore investigated the activity of another membrane active agent, ozone, on HIV-1 infectivity in vitro.

Ozone, the triatomic allotrope of oxygen, is an extremely potent oxidant that has been shown to possess broad spectrum antimicrobial activity. It has been widely used in sewage treatment, in water purification, and in medicine. In particular, ozone has been shown to be effective against a number of enveloped and nonenveloped viral species, with enveloped viruses being more susceptible to ozone inactivation than those lacking lipid envelopes. We report on the in vitro inactivation of HIV-1 in cell culture media and deliberately infected factor VIII preparations at concentrations of ozone that are not toxic to target cells, while maintaining the biologic activity of factor VIII. We also investigated the mechanism by which ozone mediates its antiviral effect.

MATERIALS AND METHODS

Cells and virus. The CD4+ T-cell line HUT 78 was maintained in RPMI 1640 + 10% heat-inactivated fetal bovine serum (FBS) + 1% penicillin/streptomycin (complete RPMI; all from GIBCO, Grand Island, NY) at 37°C, 5% CO2 humidified atmosphere. HUT 78 cells served as the target cells in the HIV-1 inactivation studies. HUT 78/HIV-1(ILAV) is a cell line stably infected with an isolate of HIV-1 from our laboratory (AAV). HUT 78/HIV-1(ILAV) served as the source of HIV-1 for the inactivation studies. Cell-free conditioned media from HUT 78/HIV-1(ILAV) (HIV-1(ILAV) CM) was harvested by centrifugation (1,200g) and filtered through a 0.45-μm filter (Nalgene, Rochester, NY). This virus preparation was titrated for production of progeny HIV-1 p24 in HUT 78 according to the infectivity assay described below.

Factor VIII. Factor VIII preparations used were either nonconcentrated, frozen, HIV–negative human plasma or immunoaffinity-purified human factor VIII (Monoclate; Armour Pharmaceuticals, Kankakee, IL). Factor VIII activity was determined in a one-stage assay.

Ozone generation and delivery. Ozone was generated from electrical corona arc discharge in a commercial ozone generator (Hansler GmbH, Iffezheim-Baden, Germany). Ozone was delivered into cell culture medium or factor VIII preparations through a closed hollow fiber system (Fig 1A) as a stream of ozone/oxygen in carrier nitrogen. Ozone generation and delivery. Ozone was generated from electrical corona arc discharge in a commercial ozone generator (Hansler GmbH, Iffezheim-Baden, Germany). Ozone was delivered into cell culture medium or factor VIII preparations through a closed hollow fiber system (Fig 1A) as a stream of ozone/oxygen in carrier nitrogen. Ozone was generated from electrical corona arc discharge in a commercial ozone generator (Hansler GmbH, Iffezheim-Baden, Germany). Ozone was delivered into cell culture medium or factor VIII preparations through a closed hollow fiber system (Fig 1A) as a stream of ozone/oxygen in carrier nitrogen. Ozone was generated from electrical corona arc discharge in a commercial ozone generator (Hansler GmbH, Iffezheim-Baden, Germany). Ozone was delivered into cell culture medium or factor VIII preparations through a closed hollow fiber system (Fig 1A) as a stream of ozone/oxygen in carrier nitrogen.
A full infectious unit would be a dilution of 1:12. The CM (final protein concentration, 35 mg/mL) was then pumped through the system at a rate of 100 mL/min. Ozone flowed through the extracapillary space of the hollow fiber cartridge at the concentrations and times indicated. To find a threshold virucidal dose of ozone, ozone concentrations were sequentially elevated on an hourly basis (ie, 1 hour at 200 ppm, 1 hour at 400 ppm, etc) (Figs 3A and 4A). The time parameter (abscissa) is a composite of the total time of exposure. For N₂ only experiments, carrier N₂ alone flowed through the cartridge. For pump only experiments, no gas was introduced into the cartridge, although the cartridge ports were opened to filtered room air. At the times indicated, 1 mL samples of treated CM were removed for use in the virus infectivity assay described below.

**HIV-1 infectivity assay.** One million HUT 78 cells were pelleted in 15-cm³ sterile tubes and resuspended in 50 μL of HIV-1-containing CM. Cells were exposed to the CM for 1 hour at 37°C and 5% CO₂ in wells of a 24-well plate. Five days postinfection the CM were sampled and assayed for viral p24 using a commercial antigen capture assay (Cellular Products, Buffalo, NY).¹

In those experiments investigating the degree to which ozone inactivates HIV-1, the HIV-1 infectivity assay was modified.¹⁷ Serial 10-fold dilutions of nonozonated HIV-1 were introduced into the assay system while ozone-treated HIV-1-spiked CM or human plasma was used undiluted. The cultures were assayed for HIV-1 p24 twice a week up until day 28. Results are expressed as a mean of a minimum of six separate experiments, each consisting of triplicate runs. Results are expressed as picograms per milliliter of HIV-1 p24 or percent untreated control. The cutoff for positive is 30 pg/mL of HIV-1 p24.

**Ozone cytopathic effects.** To determine if ozone is causing the formation of products in cell-free CM that are deleterious to the target cells used in the infectivity assays, sham infections were performed. First, cell-free HUT 78 cell culture-CM was filtered and added to the cartridge. It was then treated with either room air, N₂, or 1,200 ppm ozone for 2 hours. Mock infections were then performed by placing 10⁶ HUT 78 cells in 50 μL of the above treated CM versus control for 1 hour at 37°C. Cells were then washed and placed into culture at a concentration of 5 x 10⁵/mL. Four days later, cells were pulsed with 1 μCi [³H]-thymidine. Eighteen hours later, cells were harvested, washed, and precipitated with 10% ice-cold trichloroacetic acid for 2 hours on ice. Acid-precipitable material was collected on Whatman GF/C filters (Whatman Intl Ltd, Maidstone, England), washed, and counted. Results are expressed as counts per minute incorporated or a percentage of untreated control. Similarly, cells were assayed for viability via trypan blue dye exclusion.

To further evaluate any potential effects of ozone-treated media on the target cells, which would influence their ability to be infected by HIV-1, we analyzed the effect of the various treated CM on CD4a levels on the HUT 78 cells. HUT 78 cells were exposed for 1 hour at 37°C to media treated as above and were then stained with fluorescein isothiocyanate (FITC)-conjugated OKT4a (Ortho Diagnostics, Raritan, NJ) and analyzed on a Coulter EPICS V Flow Cytometer (Coulter Electronics, Hialeah, FL). Results were expressed as percent cells positive and mean channel number of fluorescence.

**Effect of ozone on factor VIII levels and HIV-1 in factor VIII preparations.** Frozen factor VIII containing human plasma prepa-
Fig 3. Effect of ozone on HIV-1 infectivity in cell culture medium using an escalating dose regimen (A) and continuous high-dose (1,200 ppm) (B) ozone. Virus infectivity was assayed after treatment with room air (N), nitrogen atmosphere (Δ), or ozone in carrier gas (A). Residual infectious HIV-1 levels were assessed using the 5-day HIV-1 infectivity assay. CM from cultures 5 days postinfection were assayed for HIV-1 p24. Results are expressed as a percentage of the p24 produced by cultures infected with nonozonated HIV-1. The results shown are the mean values and ranges from three separate experiments. Each separate treatment (A: pump 1 to 6 hours, N, only 1 to 6 hours, escalating ozone; E: 1,200 ppm 1 to 6 hours) was tested in triplicate cultures within an individual experiment. Please note that the total concentration of ozone multiplied by time in (A) is 4,000 ppm hours and in (E) is 7,200 ppm hours.

Fig 4. Effect of ozone on HIV-1 infectivity (◇) and factor VIII activity (○) in factor VIII plasma using escalating dose (A) and continuous high-dose (1,200 ppm) (B) ozone. Residual infectious HIV-1 levels were assessed using the 5-day HIV-1 infectivity assay. CM from cultures 5 days postinfection were assayed for HIV-1 p24. Results are expressed as percentage of the p24 produced by cultures infected with nonozonated HIV-1. The values are taken from triplicate cultures and are expressed as mean values and ranges. Factor VIII activity was assessed by a one-stage assay and is an average of duplicate determinations. The ranges are so tight that they are not shown. Please note that the total concentration of ozone multiplied by time in (A) is 4,000 ppm hours and in (B) is 7,200 ppm hours.
Ozone Inactivation of HIV-1

The results indicate that ozone is a potent inactivator of HIV-1, with a threshold dose of 1,200 ppm achieving ≥2 log inactivation of virus within 1 hour. Ozone-treated preparations achieved 22 log viral inactivation within 45 minutes (900 ppm hours). Inactivation due to room temperature, mechanical shear, and nitrogen atmosphere is only approximately 25% within 1 hour. Hence, ozone is the major contributing factor to virus inactivation at both the higher dose and early time points.

Figure 3B shows the results of the time-dependent inactivation of HIV-1 in cell culture medium using 1,200 ppm ozone. As can be seen, 1,200 ppm achieves ≥2 log virus inactivation within 45 minutes (900 ppm hours). Inactivation due to room temperature, mechanical shear, and nitrogen atmosphere is only approximately 25% within 1 hour. Hence, ozone is the major contributing factor to virus inactivation at both the higher dose and early time points.

Figure 4A shows the results of the effect of a sequentially escalating dose of ozone on HIV-1 viability and factor VIII biologic activity in an HIV-1-spiked human plasma preparation. As can be seen, there is minimal loss of factor VIII biologic activity associated with ozone treatment at concentrations that eventually inactivate HIV-1. Treatment to a threshold dose of 1,200 ppm results in a 90% inactivation of HIV-1 with only a corresponding 25% loss of factor VIII biologic activity.

Figure 4B shows the results of the time-dependent inactivation of HIV-1 and factor VIII biologic activity in a plasma preparation using continuous 1,200 ppm ozone. As can be seen, 1,200 ppm ozone achieves ≥2 log inactivation of HIV-1 by 2 hours while the corresponding factor VIII activity remains high (90%).

Similar results were seen when immunoaffinity-purified factor VIII preparations were inoculated with HIV-1 and treated continuously with 1,200 ppm ozone (Fig 5). In this instance, ≥2 log viral inactivation was achieved within 1
Table 1. Comparison of Ozone-Treated HIV-1 With a Titration of Untreated HIV-1 in a 28-Day HUT 78 Cell Culture System

<table>
<thead>
<tr>
<th>HIV-1 Viral Input*</th>
<th>HIV-1 p24 Assay Result for Each Biweekly 28-Day Culture Sample (day in culture)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>3.5 × 10⁷ ng HIV-1 in CM treated 2 h, 1,200 ppm ozone</td>
<td>−</td>
</tr>
<tr>
<td>3.5 × 10⁷ ng HIV-1 in human plasma treated 2 h,</td>
<td>−</td>
</tr>
<tr>
<td>1,200 ppm ozone</td>
<td>−</td>
</tr>
<tr>
<td>3.5 × 10⁷ ng HIV-1 no ozone</td>
<td>−</td>
</tr>
<tr>
<td>3.5 × 10⁷ ng HIV-1 no ozone</td>
<td>−</td>
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<tr>
<td>3.5 × 10⁷ ng HIV-1 no ozone</td>
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<td>3.5 × 10⁷ ng HIV-1 no ozone</td>
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</tr>
<tr>
<td>HIV-1 Viral Input*</td>
<td>3.5 × 10⁷ ng HIV-1 no ozone</td>
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<tr>
<td>3.5 × 10⁷ ng HIV-1 no ozone</td>
<td>−</td>
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<tr>
<td>3.5 × 10⁷ ng HIV-1 no ozone</td>
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<td>3.5 × 10⁷ ng HIV-1 no ozone</td>
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<td>3.5 × 10⁷ ng HIV-1 no ozone</td>
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<td>3.5 × 10⁷ ng HIV-1 no ozone</td>
<td>−</td>
</tr>
<tr>
<td>3.5 × 10⁷ ng HIV-1 no ozone</td>
<td>−</td>
</tr>
<tr>
<td>0 ng HIV-1 p24 no ozone</td>
<td>−</td>
</tr>
</tbody>
</table>

*Total HIV-1 viral input as measured by p24 antigen capture of 50 μL of cell-free HUT 78 HIV-1, cell culture CM either undiluted (3.5 × 10⁷ ng) or serially diluted in virus-negative culture media.

†The mean of six separate experiments each run in triplicate being ≥ 30 pg/mL of HIV-1 p24. All 18 replicates of data points listed as negative were negative, and all replicates of data points listed as positive were positive save for day 24 of the 3.5 × 10⁴ cultures, in which the mean was 28 pg/mL and some replicates were positive and some negative. A culture was deemed positive if two successive samples were positive with the second being either four times the first or ≥ 250 pg/mL of HIV-1 p24. All cultures save for the ozone-treated HIV-1, 0 pg HIV-1 p24, and 3.5 × 10⁴ ng HIV-1 p24 were positive.

Table 2. Effects of Ozone-Treated CM on HUT 78 Cells in a Mock Infection

| Condition of CM | CD4a Levels | Cytotoxic Effect | | | |
|-----------------|-------------|-----------------|---|---|---|---|
|                 | % Cells Positive | Channel No. | Cell Viability (×10⁹) | % Control | [³H] Thymidine Incorporation (cpm) | % Control |
| No treatment    | 57 ± 2      | 84 ± 2         | 2.89 ± 0.33           | 100        | 12,850 ± 1,340           | 100  |
| Room air (2 h)  | 59 ± 2      | 77 ± 2         | 2.73 ± 0.24           | 94         | 10,280 ± 985            | 89   |
| N₂ (2 h)        | 58 ± 2      | 86 ± 2         | 2.64 ± 0.38           | 91         | 10,993 ± 1,002          | 85   |
| 1,200 ppm ozone (2 h) | 61 ± 2 | 89 ± 2 | 2.73 ± 0.18           | 94         | 9,124 ± 878            | 71   |

All assays were run six times and results equal mean ± 3 SD.

*Channels are grouped from 1 to 267 and are on a log scale.

hour, whereas factor VIII activity after 2 hours was approximately 90%.

As can be ascertained from Figs 3, 4, and 5, increasing amounts of protein and possibly other plasma components decreased the virucidal effect of ozone. The HIV-1-spiked material in Figs 3 and 5 has a protein concentration of 35 mg/mL, while the human plasma in Fig 4 has a concentration of 400 (A) or 435 (B) mg/mL.

In an effort to determine the degree of inactivation of HIV-1 by ozone, HIV-1-spiked CM (35 mg protein/mL) and HIV-1-spiked plasma (435 mg protein/mL) were each treated with 1,200 ppm ozone for 2 hours. Fifty microliters of each of these treated materials (initially containing 35 ng of HIV-1 p24) were placed in a 28-day HIV-1 culture assay and compared with a serial dilution of untreated HIV-1 virus. In this experiment the transmission of an 11 log dilution of untreated virus could still be detected, whereas the transmission of ozonated, undiluted HIV-1 in CM or human plasma could not be discerned, suggesting that 1,200 ppm of ozone for 2 hours achieved a minimum of an 11 log inactivation (Table 1).

Studies were performed to evaluate the mechanisms of antiviral action of ozone treatment. There were no appreciable effects of ozone-treated CM on the viability, rates of DNA synthesis, and CD4a levels of HUT 78 target cells exposed in mock infections (Table 2). However, treatment of HIV-1-infected CM with 1,200 ppm ozone did result in a marked decrease in recoverable sucrose-banded virions as measured by reverse transcriptase and HIV-1 p24 analyses (Fig 6). In six separate experiments comparing the surface area under the curve for reverse transcriptase activity and p24 antigen content between media treated and untreated with ozone, the loss of recoverable virions ranged from 75% to 93% with a mean of 85%. Western blot analysis of the fractions in Fig 6 for HIV-1 gp41 and gp120 envelope protein content showed a loss of antigen comparable with that seen with reverse transcriptase and p24 gag protein (data not shown). Hence, there was no preferential loss in residual intact virions of envelope antigen or functional reverse transcriptase activity secondary to ozone.

As shown in Fig 6, there was no appreciable accumulation of viral cores in the ozone-treated CM. All of the
Fig 6. Analysis of fractions from sucrose density gradients of HIV-1-positive cell-free CM untreated (A) or treated with 1,200 ppm ozone (B) for 2 hours. Specific gravity, HIV-p24 (•••••), and reverse transcriptase (○○○○) levels are shown. Specific gravity was determined by refractive index. HIV-1 p24 was determined by HIV-1 p24 antigen capture immunoassay and is expressed as micrograms per milliliter with mean values and ranges of triplicate determinations shown. Reverse transcriptase activity was determined using the template: primer, poly rC:oligo dG, 10 mm Mg++, and [3H]-GTP. Results are expressed as picomoles per liter of GMP incorporated per hour and are the mean values and ranges from triplicate samples.

missing HIV-1 p24 antigen, but not the reverse transcriptase activity, was recovered in the supernatant after the original high speed pelleting step before sucrose banding (data not shown). This result would indicate that ozone treatment led to complete solubilization of most of the treated HIV-1 particles and that, while key epitopes of solubilized HIV-1 p24 could still be detected in the antigen capture assay, functional reverse transcriptase activity was lost secondary to the oxidation of solubilized enzyme.

Further evaluation of ozone-treated and untreated HIV-1 sucrose-banded virions for their ability to compete against rhodamine-labeled HIV-1 for cell surface receptor binding has shown an impaired ability of ozone-treated virions to displace HIV-1 (Table 3). This experiment was performed twice with similar results. Although further investigation is necessary, the implication is that ozone-treated virions have a diminished capacity to bind to their natural cell surface receptor, CD4.

DISCUSSION

Reducing the infectious risk of human blood products is an area of intense research. Inactivation of infectious agents in blood products under conditions that retain their intrinsic protein biologic activities has undergone extensive study. Thermal inactivation of viruses in blood derivatives has been marginally successful, due in part to the thermolability of these components. Methods ranging from gamma-irradiation, porous membrane filtration, and solvent/detergent mixtures have been investigated. To date, tri(n-butyl)phosphate (TNBP) detergent mixtures have demonstrated a minimum 4 to 5 log 10 reduction in a
work in chimpanzees with TNBP-treated, exogenously viral-spiked blood samples with hepatitis B and non-A, variety of lipid-enveloped viruses, including HIV-1, vesicular stomatitis virus, and Sindbis virus, with a concomitant high recovery rate of protein biologic activity. Analogous work in chimpanzees with TNBP-treated, exogenously viral-spiked blood samples with hepatitis B and non-A, non-B (Hutchinson strain) viruses has shown similar log reductions. However, methods eclipsing a 6 log viral reduction with a similar protein recovery are still being explored. In addition, methods that obviate the necessity for removal of the inactivating agent (ie, Sephadex G-25) and/or that are compatible with cellular systems would ultimately be preferable.

Ozone has previously been shown to possess potent antiviral activity, especially when used against lipid-enveloped viruses. For this reason we investigated the use of ozone as a potential anti–HIV-1 agent. We used a hollow fiber delivery system that maximizes the surface area available for ozone to interact with the fluid material of interest. The system also allows for the precise regulation of concentrations of ozone to be delivered into the hollow fiber cartridge. Afferent as well as efferent concentrations of ozone can also be monitored to determine if saturating levels of ozone are achieved in the treated material. With regard to laboratory safety concerns, this closed system has proven to be safe and leak-proof, thereby reducing possible exposure of laboratory personnel to ozone and human retroviruses to an absolute minimum.

We first examined the ability of ozone to inactivate cell-free HIV-1 in cell-free CM. These results indicate that ozone has potent anti–HIV-1 activity. Preliminary experiments using an escalating dose regimen indicated that a 1,200 ppm dose of ozone achieved a ≥ 2 log inactivation of virus. The data show that neither incubation of the virus preparation at room temperature and at atmospheric conditions for the duration of the experiment nor mechanical shear created by pumping the virus through the system inactivate the virus to any great extent. However, there was a significant inactivation of the virus due to exposure of virus-containing CM to the stream of carrier nitrogen. Exposure of the virus to pure nitrogen for 6 hours results in an almost 85% inactivation of virus.

Table 3. Effect of Ozone on the Binding of HIV-1 Virions as Measured In a Competition Assay

<table>
<thead>
<tr>
<th>Condition</th>
<th>Rhodamine-Labeled HIV-1</th>
<th>( \text{N}^\text{a} ) Channel No.*</th>
<th>( \text{N}^\text{b} ) Channel No. Minus Autofluorescence of HUT 78 Cells</th>
<th>% of Control*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HUT 78 cells</td>
<td>No</td>
<td>81</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HUT 78 cells</td>
<td>Yes</td>
<td>155</td>
<td>74</td>
<td>100</td>
</tr>
<tr>
<td>HUT 78 cells preincubated with unlabeled HIV-1 not treated with ozone</td>
<td>Yes</td>
<td>122</td>
<td>41</td>
<td>34</td>
</tr>
<tr>
<td>HUT 78 cells preincubated with unlabeled HIV-1 treated with ozone</td>
<td>Yes</td>
<td>143</td>
<td>62</td>
<td>67</td>
</tr>
</tbody>
</table>

*Channels are grouped from 1 to 256 and are on a log scale.

Calculation of percentage of control is performed by first converting \( \text{N}^\text{a} \) channel number to an arithmetic value, subtracting autofluorescence of HUT 78 cells, and dividing by the value obtained with HUT 78 cells plus rhodamine-labeled HIV-1.

Ozone has potent antiviral activity, especially when used against lipid-enveloped viruses. For this reason we investigated the use of ozone as a potential anti–HIV-1 agent. We used a hollow fiber delivery system that maximizes the surface area available for ozone to interact with the fluid material of interest. The system also allows for the precise regulation of concentrations of ozone to be delivered into the hollow fiber cartridge. Afferent as well as efferent concentrations of ozone can also be monitored to determine if saturating levels of ozone are achieved in the treated material. With regard to laboratory safety concerns, this closed system has proven to be safe and leak-proof, thereby reducing possible exposure of laboratory personnel to ozone and human retroviruses to an absolute minimum.

Investigation of the degree of inactivation of HIV-1 in CM or human plasma by 1,200 ppm ozone for 2 hours indicates a minimum 11 log reduction in viral infectivity, as determined by a 28-day culture assay. Therefore, the effects of temperature, \( \text{N}_0 \), and mechanical shear, which yield an approximate 0.22 log reduction in HIV-1 infectivity, are certainly eclipsed by the 11 log reduction in infectivity achieved by ozone.

We examined the ability of ozone to render blood products, such as factor VIII plasma preparations, free of HIV-1 without seriously damaging their biologic activity. The escalating dose study indicated that, at 1,200 ppm, ozone can achieve a 90% inactivation of HIV-1 in human plasma with only a corresponding 25% decrease in factor VIII biologic activity. Measurement of exhaust ozone levels indicated that saturating levels of ozone in the factor VIII preparations were not achieved in this experiment (data not shown) and, thus, longer exposures of HIV-1-containing factor VIII preparations at the higher ozone concentrations were used to eliminate the virus entirely. Use of 1,200 ppm ozone for a period of 2 hours achieved saturation and resulted in an 11 log inactivation of HIV-1 in the human plasma preparation. Under the same conditions, factor VIII biologic activity was diminished only 10%. Similarly, ≥ 2 log HIV-1 inactivation in immunoaffinity-purified factor VIII preparations occurred within 1 hour of exposure to 1,200 ppm of ozone with an approximately 10% loss of factor VIII.

Comparison of the results of inactivation of HIV-1 by high-dose ozone (1,200 ppm) in cell culture media, monocyte, and factor VIII plasma preparations indicated that a longer exposure time was necessary to achieve total virus inactivation in the plasma factor VIII preparation. The reason for the apparent protection of HIV-1 from the
effects of ozone in this experiment is most likely the increased protein levels in the factor VIII preparation. Such increased protein levels and other plasma components may act to protect the virus from the effects of ozone.32

The exact mechanism by which ozone mediates the inactivation of HIV-1 remains to be elucidated. Our preliminary data indicate that, at the concentrations tested, ozone results in the solubilization of virions. However, the reduction in infectious virus (about 103) was far greater than the observed reduction in detectable virions (about 85%). This finding suggested that, in addition to the physical destruction of HIV-1 virions, there was a far greater functional impairment of these virions in the infectivity assay. Our data do not suggest that there was any obvious effect of ozone-treated CM on the viability and CD4a levels of the HUT 78 target cells used in the assay. Nor do our results indicate any preferential quantitative effect on HIV-1 envelope proteins and reverse transcriptase levels in the virions. However, there was some suggestion of a deleterious effect on virus binding to cell surface receptors.

Ozone may react with the unsaturated fatty acids in the lipid envelope of the virus, thereby increasing membrane fluidity and resulting in viral inactivation. Alternatively, ozone may react with other components in the virus preparation (serum proteins, fatty acids, etc) to produce secondary reaction products, such as hydroxyperoxides, that in turn mediate the actual viral inactivation. Far more extensive analyses of all components of the HIV-1 life cycle will be required to fully elucidate ozone’s antiretroviral activity.

Ozone has potent anti–HIV-1 activity in cell culture media and factor VIII preparations. Although the exact mechanism by which ozone mediates its effect remains unclear at this time, it is readily apparent that ozone may be of use in rendering factor VIII and possibly other blood products, both proteinaceous and cellular, free of HIV-1 and other infectious agents.

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