Antiviral Research, 16 (1991) 281 292

ADONIS 0166354291003426

AVR 00510

Ozone inactivates HIV at noncytotoxic concentrations

Michael T.F. Carpendale[1,2,3] and Joel K. Freeberg[1,3,*]

[1] *Rehabilitation Medicine and Research Services, Veterans Administration Hospital, San Francisco, CA 94121, U.S.A.,* [2] *Department of Orthopaedic Surgery, University of California School of Medicine, San Francisco, CA 94143, U.S.A. and* [3] *Center for Medical Ozone Studies, Bay Medical Research Foundation, San Francisco, CA 94117, U.S.A.*

(Received 8 October 1990; accepted 27 April 1991)

Summary

The inactivation of human immunodeficiency virus (HIV) and cytotoxic properties of ozone-treated serum and serum-supplemented media were examined. The titer of HIV suspensions in human serum was reduced in a dose-dependent manner when treated with total reacted ozone concentrations at a range of 0.5 to 3.5 $\mu g/ml^{-1}$. Complete inactivation of HIV suspensions was achieved by 4.0 $\mu g/ml^{-1}$ of ozone in the presence or absence of H-9 cells. In contrast, cellular metabolism, as measured by MTT dye cleavage, and DNA replication, as measured by BUdR incorporation, were enhanced in H-9 cells grown in media treated with quantities of ozone that completely inactivate HIV. The permissively HIV-infected cell line HXB/H-9 was cultured in ozone-treated media for six days with culture supernatants being sampled and assayed on alternate days for HIV p24 core protein. HIV p24 was reduced in all treated cultures compared to control cultures, with an average reduction of 46% [p24].

HIV; Ozone; Noncytotoxic; Virus inhibitor

Current address: Cutter/Miles, 4th and Parker St., Berkeley, CA 94701, U.S.A.

Correspondence to: M.T.F. Carpendale, Center for Medical Ozone Studies, Bay Medical Research Foundation, 425 Divisadero Street, 305, San Francisco, CA 94117, U.S.A.

Introduction

Ozone, the triatomic allotrope of oxygen with a high electrovoltaic potential, has multiple applications as a bactericidal and virucidal agent in sewage treatment, water purification and pharmaceutical manufacture. Ozone effectively inactivates both enveloped and nonenveloped viruses when introduced into suspensions in water (Akey and Walton, 1985; Roy et al., 1981), effluent (Harakeh and Butler, 1985; Katzenelson et al., 1976), and cell culture media (Bolton et al., 1982a; 1982b). Nonenveloped viruses, including poliovirus (Katzenelson et al., 1979), coxsackie virus (Emerson et al., 1982) bacteriophage f2 (Kim et al., 1982), canine hepatitis virus (Bolton et al., 1982b) and rotaviruses (Vaughn et al., 1987) have been substantially inactivated at moderate ozone concentrations, while enveloped viruses have been shown to be susceptible to degradation at lower ozone concentrations. Inactivation of enveloped viruses by ozone has been demonstrated for vesicular stomatitis virus (Bolton et al., 1982a), influenza type A (Bolton et al., 1982b), infectious bovine rhinotracheitis virus (Bolton et al., 1982b), and Venezuelan equine encephalomyelitis virus (Akey and Walton, 1985). The wealth of information demonstrating the effective inactivation of viruses by ozone in vitro led us to conduct a series of experiments to assess the ability of ozone to inactivate HIV relative to measurable cytotoxic effects, as well as examine the effect of ozone on HIV replication in lymphocytic cells.

Materials and Methods

Ozone generation and measurement

Ozone was generated from medical grade oxygen using high voltage corona discharge in an electric ozone generator (Model HC-1000, Griffin Technics, Lodi, NJ), which allows the voltage, flow rate and concentration to be controlled. Tygon® polymer tubing, Teflon® and glass tubing, and silicon stoppers were used throughout the reaction system to insure containment of the ozone and consistency in concentrations. Gaseous ozone concentrations were measured by an ultraviolet light absorption monitor (model EG-2001 AHC, Griffin Technics) capable of continuous measurement of ozone concentrations of 0.1–200 μg/ml.

Aqueous solutions of ozone can be easily quantified by chemical titration (Shechter, 1973). Mixed organic solutions treated with ozone, however, react quickly to form secondary ozonides and peroxides requiring multiple and complex assays for quantitation (Lee et al., 1982). We therefore developed an apparatus for ozone titration employing three parallel gas lines, each receiving equal flow of oxygen/ozone mixture from the ozone generator and monitor, which introduces the gas by bubbling into flasks containing the solution to be exposed, and directs the overflow into flasks containing potassium iodide

solution (Fig. 1). This allows the total amount of ozone introduced into the solution, and the net amount of ozone reacted per unit volume of serum or media to be measured. A given net reacted ozone concentration was reproduced in each experiment by controlling the gaseous ozone concentration, flow rate, and exposure time for the 50 ml volumes of serum or media. Ozonized potassium iodine solution was measured spectrophotometrically using the methods of Shechter (1973) with the modifications of Emerson (1982). Human serum (HIV seronegative, heat inactivated) or RPMI-1640 medium supplemented with 20% human serum was treated in this apparatus with a gaseous ozone/oxygen mixture ranging from 5 to 20 mg/l for various time periods to produce a range of net reacted ozone concentrations. Control media received equal volumes of pure oxygen or were untreated. The pH of the treated media was checked after ozone treatment and was found not to be altered by treatment.

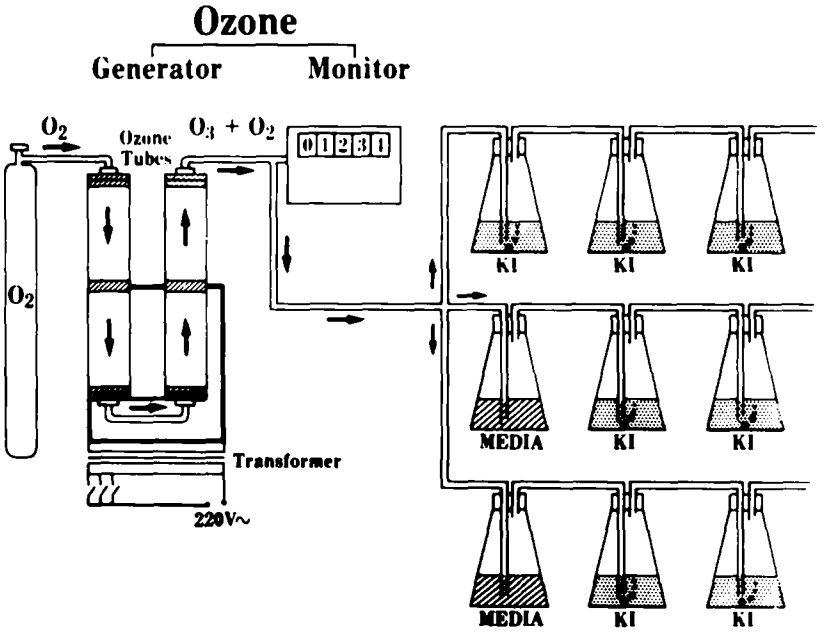

Fig. 1. Ozone generation, monitoring and measurement (reacted). Apparatus for measuring amount of ozone reacted with media and serum. The top line of flasks is used to measure the total amount of ozone introduced into the system. The middle and lower line of flasks are used to treat media or serum. The amount of ozone measured by the second and third flask in the middle or bottom line of flasks is subtracted from the ozone measurement made by the top line of flasks to calculate reacted ozone concentrations in media or serum.

Cells and viruses

HIV-DV, a primary isolate from the peripheral mononuclear cells of a homosexual male with Kaposi's sarcoma, has been described previously (Crowe et al., 1982). HIV was purified from cell-free culture supernatant ($TCID_{50}$ = $10^{5.5}$/ml) of infected VB T lymphoma cells (Lifson et al., 1986) by ultracentrifugation at 140000 × *g* for 90 min at 4°C. HIV-DV, H-9 T lymphoma cells, HXB/H-9 described previously (Crowe et al., 1982) and VB T lymphoma cells were generous gifts from Dr. Michael McGrath (San Francisco General Hospital Medical Center, San Francisco). All cell cultures were maintained in RPMI-1640 supplemented with 10% fetal bovine serum, 1 mM L-glutamine, 2 mM sodium pyruvate, and 0.5 μg/ml gentamycin (U.C. San Francisco Cell Culture Facility). Peripheral blood mononuclear cells (PBM) were isolated by the Ficoll-hypaque method from a healthy donor negative for HIV antibodies by ELISA, and were cultured under identical conditions with the addition of 10 μg/ml phytohemaglutinin (Sigma, St Louis, MO). Cell lines were maintained in a humidified incubator at 37°C with 5% CO_2.

HIV titer and ozone exposure

Heat inactivated, HIV antibody-negative human serum was treated with ozone to obtain a range of concentrations. Aliquots of purified HIV were added to treated or control solutions (final density of 10^6 infectious units/ml) and exposed for 30 min. Samples from the exposed virus suspension were removed, serially diluted in quadruplicate and titered by addition to microtiter plates containing 10^4 VB cells/well. The presence of infectious HIV was scored by cell fusion and subsequent multinucleated giant cell formation after incubation for seven days.

Ozone cytotoxicity

In these experiments RPMI-1640 medium supplemented with 20% human serum was treated with ozone in the apparatus described while control media received either equal volumes of oxygen for equal time periods or were untreated. Thrice washed H-9 T lymphoma cells or freshly isolated normal peripheral blood mononuclear cells were added at equal volumes (10^5 cells/well) to ozone-treated or control media in 96-well tissue culture plates and incubated at 37°C plus 5% CO_2 for four days, with daily replacement of ozonized or control media. The cells were then resuspended in RPMI-1640 plus 10% fetal calf serum supplemented with 10% (v/v) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma), and assayed as described previously (Mossman, 1983). Samples of the control cell suspensions were serially diluted and added to each microtiter plate in order to correlate absorbance values with cell density, and a standard curve was generated for each trial.

DNA replication in identically prepared cultures was quantified by incorporation of 5-bromo-2-deoxyuridine (BUdR, Sigma) using the method of Porstmann et al. (1985). H-9 cells (10^5 cells/well in 96-well microtiter plates) were incubated in ozonized, oxygen-treated, or untreated media supplemented with BUdR at 10^{-5} molar final concentration for 6 h. After centrifugation and cell disruption, BUdR-DNA was quantified by antigen-capture immunoassay utilizing anti-BUdR monoclonal antibody (Chemicon Inc., El Segundo, CA). BUdR-BSA conjugate was quantified at various concentrations in each plate as standards.

Infectious HIV yield

Cocultures were prepared containing 10^6 VB and 5×10^4 HXB/H-9 cells per ml in 5 ml total volume of ozone-treated, oxygen-treated, or untreated RPMI-1640 media supplemented with 20% human serum. After incubation for four days, cells were removed by centrifugation at $1200 \times g$ for 10 min, and virus was concentrated by ultracentrifugation at $140\,000 \times g$ for 90 min at 4°C. The virus pellet was resuspended and titered in VB cell cultures as described above.

p24 Antigen expression in HXB/H-9 cells

The permissively HIV-infected cell line HXB/H-9 was cultured (5×10^5 cells/ml) in ozone-treated, oxygen-treated, or untreated control RPMI-1640 media supplemented with 20% human serum in six-well culture plates. Of the culture supernatant volume 25% was sampled and replaced daily with treated or control media. Samples taken on days 2, 4 and 6 were assayed in triplicate for p24 core protein by antigen-capture assay (Abbot Laboratories) following the manufacturer's instructions. The p24 protein solution supplied with the Abbot assay kit was diluted in ozone-treated media without cells and incubated in parallel with each culture to verify recovery of p24 protein in media.

Statistical calculations

Standard curves of cell number vs. absorbance, or ng/ml of BUdR-BSA vs absorbance, were generated for each MTT or BUdR ELISA and analyzed by linear regression. Only assays with standards yielding r values of greater than 0.994 were included in the final data. Treatment and control populations were compared for significant difference in cytotoxicity and p24 antigen expression by Student's t test.

Results

Inactivation of HIV by ozone-treated human serum

The first experiments conducted were designed to determine if human serum reacted with ozone possesses antiviral properties. The total amount of ozone reacted with a given volume of human serum was measured in the test apparatus. The treated or control serum was placed in microtiter plates, and a concentrated HIV suspension was added to the serum, exposed and titered. Previously published kinetic studies had indicated that 30 min would be a sufficient exposure time for virus inactivation (Katzenelson, 1979). No significant reduction in HIV titers was measured in oxygen-treated or untreated serum. The infectious titer of HIV is reduced 99% in serum reacted with 0.5 μg O_3 per ml of serum, and a six-log reduction is achieved by reacted ozone concentrations of 4 μg per ml of serum (Fig. 2). The logarithmic inactivation of HIV in ozone-treated media is presented as a function of reacted ozone concentration. No detectable infectious units remained at total reacted ozone concentrations of greater than 4.0 μg/ml.

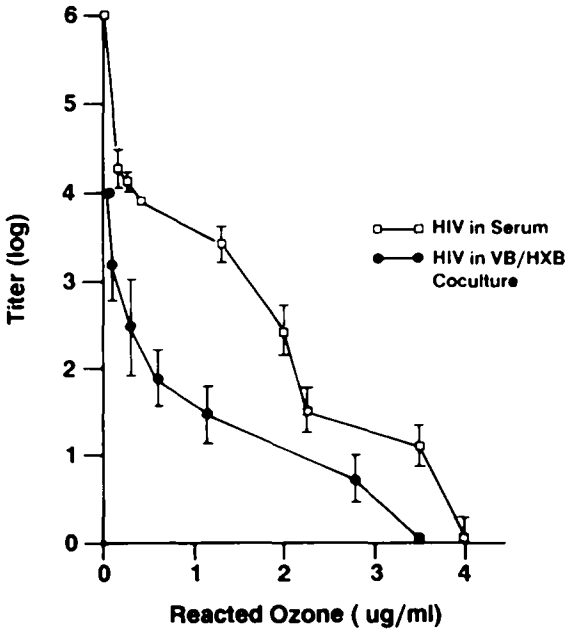

Fig. 2. HIV inactivation in ozone-treated serum and media. Titer of HIV in ozone treated human serum, and infectious HIV titer in VB/HXB cocultures grown in ozone-treated media as a function of the quantity of reacted ozone. Zero reacted ozone concentration is oxygenated and untreated controls. (Error bars represent 99% confidence interval, $n = 3$ replicate experiments.)

HIV infectious yield

In order to determine if HIV is inactivated by ozone-treated media in the presence of cells as well as in purified suspensions in serum, mixed cultures were prepared containing the $CD4^+$ cell line VB and permissively HIV/III_B infected cell line HXB/H9. Mixed cultures of HXB/H9 and VB cells resulted in multiple syncytia, a high degree of cytopathology and rapid increase in viral replication and release (Lifson et al., 1986). These culture conditions were chosen as a maximum challenge to the virus inactivation capabilities of ozone. Supernatants from these cocultures grown in ozone-treated or control media were titered by syncytium formation assay after four days. Infectious titers were reduced in relation to ozone dosage to a similar degree as in purified suspensions, with a complete loss of four-log titer infectivity resulting from pretreatment of media with 3.5 μg/ml of ozone (Fig. 2).

HIV titers in oxygen-treated media were not significantly different from those in untreated media.

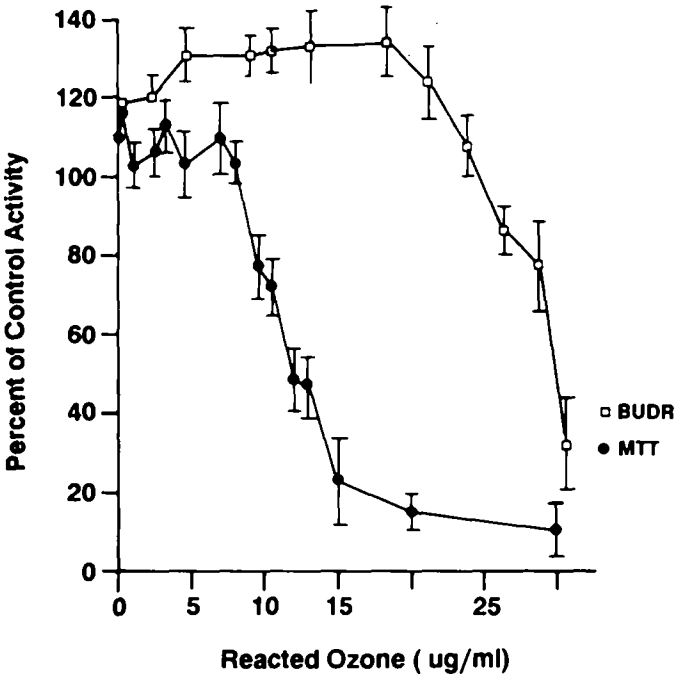

Fig. 3. H-9 cell cytotoxicity in ozone-treated media. Percent of control MTT dye conversion, and percent of control BUdR incorporation as a function of reacted ozone concentration in human serum supplemented media. Zero reacted ozone concentration is oxygenated and untreated controls. (Error bars represent 99% confidence interval of $n = 4$ replicate experiments.)

Ozone cytotoxicity

The ability of RPMI-1640 medium supplemented with 20% human serum to support the growth of H-9 cells and PBM cells when treated with various quantities of ozone was tested in the third set of experiments. In all cytotoxicity tests, ozonized media was replaced daily to assure that the maximum cytotoxicity for a given concentration of ozonized media was insured. The viability of the cells was assessed by the cellular cleavage of MTT dye, an objective method of determining cell viability. BUdR incorporation in H-9 cells was measured in response to ozone-treated media as a non-radioisotopic alternative to the [^{3}H]thymidine incorporation assay for DNA replication (Porstmann et al., 1985). Neither MTT dye conversion or BUdR incorporation were effected by oxygenation of culture media when compared to untreated control cultures. The mean percent of cell survival and BUdR incorporation, relative to controls, is shown for H-9 cells at each ozone concentration tested (Fig. 3). No significant difference was found in the measured MTT dye conversion by H9 cells and PBM cells in response to ozone-treated media (data not shown). MTT vital dye conversion is significantly enhanced in ozone-treated media relative to controls at ozone concentrations that completely inactivate purified HIV suspensions (1-8 μg/ml, $P < 0.01$). BUdR incorporation was enhanced in ozone-treated media over a range of 1-21 μg/ml. Significant reduction of H9 or PBM cell viability is observed at reacted ozone concentrations above 9.5 μg/ml of media, while significant inhibition of DNA replication in H-9 cells is observed at reacted ozone concentrations above 25 μg/ml of media ($P < 0.01$).

HIV replication

The intracellular antiviral properties of ozone-treated medium were investigated by cultivation of permissively infected HXB/H-9 cells for a period of six days with daily replacement of 25% of the treated or control media. Samples of media removed on alternate days were assayed for p24 viral core protein by antigen-capture ELISA. A statistically significant reduction ($P < 0.01$) in p24 antigen expression was measured in supernatants from all treated cultures in proportion to reacted ozone concentration (Fig. 4). Viral core protein antigen levels were not changed in cultures treated by oxygen alone. Cell viability, tested by trypan blue exclusion, was $\pm 10\%$ of control cell viability in all cultures except the maximum treatment dosage (10.2 μg/ml^{-1}), which showed 87% of control viability. Solutions containing known concentrations of p24 antigen, diluted in ozone-treated media, were measured in this assay system and yielded expected values, excluding the possibility that the p24 antigen was degraded in treated media.

Discussion

In vitro inactivation of HIV has been demonstrated for a variety of agents, including sodium hypochlorite, ethanol, paraformaldehyde, glutaraldehyde, isopropanol, hydrogen peroxide, phenol and nonoxynol-9 (Martin et al., 1985; Spire et al., 1984; Hicks et al., 1985). The addition of ozone to this list is consistent with the oxidative and/or surfactant properties of these agents. Normal human serum did not inactivate HIV in these experiments, in agreement with previously reported results (Banapour et al., 1986), and treatment of serum or culture media with oxygen produced no significant effect on HIV inactivation or cell viability.

In all experiments reported here, treatment of serum or culture media with ozone was completed before the addition of virus or cells. Ozone is a highly reactive oxidizing agent, and these results support the hypothesis that HIV inactivation is effected by secondary reaction products of ozone in serum and media containing 20% serum. The ozonide reaction products of fatty acids have been shown to mimic the direct cellular effects of ozone at concentrations of 10^{-5} to 10^{-4} molar (Rietjens et al., 1987; Cortesi and Privett, 1972). Thin films of virus suspensions in media have been successfully inactivated by less than one ppm of gaseous ozone in roller bottle cultures (Bolton et al., 1982a; 1982b), but it is unclear if direct exposure of virus to gaseous ozone occurred under these conditions. Emerson et al. (1982) found that significantly higher

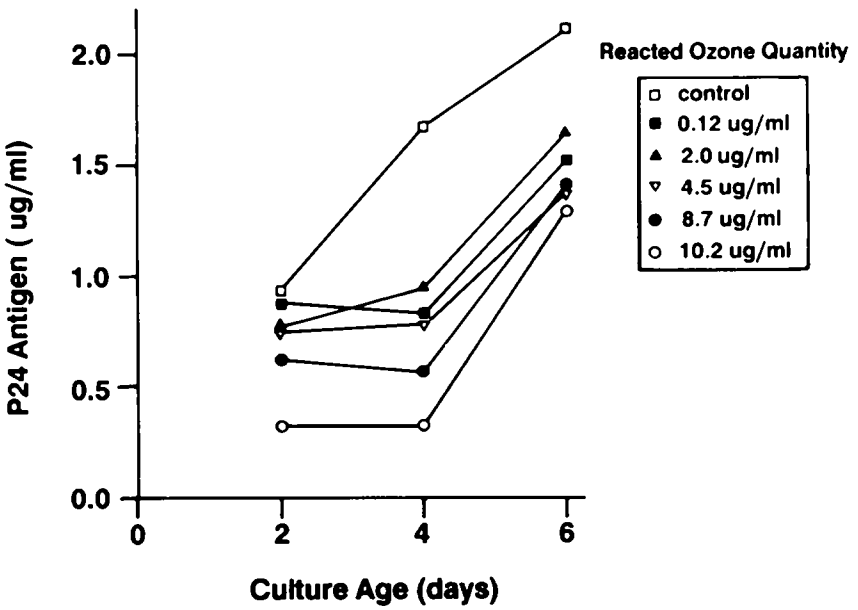

Fig. 4. P24 antigen expression in ozone-treated media. P24 antigen concentration in HXB/H-9 culture supernatants for five ozone treatment quantities and oxygen treated controls. (Values are the mean of duplicate measurements of three cultures for each data point, with a coefficient of variation of $\leq 26\%$.)

dissolved ozone concentrations were required in aqueous systems to inactivate cell-associated poliovirus and coxsackievirus A9 compared to purified virus suspensions. This does not appear to be true for HIV in the HXB-VB cocultures we tested, perhaps due to the high degree of cytolysis in these cultures which allowed greater direct exposure of virus to ozone and/or ozonides.

Ozone had a hormetic effect, i.e., a stimulating effect at low concentrations, on H9 and PBM cells in these experiments comparable to that reported by other agents (Wolff, 1989). The growth of normal PBM cells and H9 lymphoma cells was equally susceptible to inhibition by ozone-treated media as defined by the MTT dye conversion assay. In contrast, Sweet et al. (1980) found that lung, breast, and uterine tumor cells were more susceptible to growth inhibition than lung diploid fibroblasts when exposed to 0.3–0.8 ppm of gaseous ozone. DNA synthesis was unimpaired in H9 cells when grown in less than 25 μg/ml of ozone-treated media in our experiments. This is consistent with the findings of Bolton et al., that labeled thymidine incorporation was not decreased in Madin-Darby bovine kidney cells when exposed to 0.64 ppm of gaseous ozone in roller bottle cultures.

The p24 antigen assay has been demonstrated to be 100-fold more sensitive than reverse transcriptase assays in detecting HIV (Feorin et al., 1987). The p24 antigen in the media supernatant of persistently HIV-infected H9 cell cultures was reduced an average of 46% when grown in ozone-treated medium (range 6–81%). Azidothymidine has been reported to reduce p24 levels in culture supernatants of PBM cells 50% to 95% at drug concentrations of 0.02–0.08 μM (Hartshorn et al., 1987). These ozone-mediated reductions in p24 antigen expression of HXB/H-9 cultures, as well as the reduction of infectious virus titer of mixed VB/HXB cultures described above, were accomplished at ozone concentrations shown here to be nontoxic in uninfected cells. Current models of HIV pathogenesis hypothesize dissemination by both free virus and direct cell cell transmission (Levy, 1988). An agent or combination of agents which act at multiple sites of viral replication has obvious advantages.

We have demonstrated here an agent which, in vitro, acts to both inactivate cell-free HIV and suppress intracellular replication without apparent cytotoxicity. These results indicate a potential HIV inactivation treatment for blood and blood products. Future research will attempt to elucidate the molecular mechanisms of this phenomenon.

Acknowledgements

This study was partially funded by a grant from the Griffin Foundation to Bay Medical Research Foundation, Center for Medical Ozone Studies. Grateful appreciation is extended to David Griffin and Joseph Giannone. The authors also wish to thank Dr. Michael McGrath, Dr. Lynn Pulliam, Dr. Raxit Jariwalla, and Dr. Steven Harakeh for their helpful suggestions and contributions to this research.

References

Akey, D.H. and Walton, T.E. (1985) Liquid-phase study of ozone inactivation of Venezuelan equine encephalomyelitis virus. Appl. Environ. Microbiol. 50, 882–886.

Banapour, B., Sernatinger, J. and Levy, J.A. (1986) The AIDS-associated retrovirus is not sensitive to lysis or inactivation by human serum. Virology 152, 268–271.

Bolton, D.C., Tarkington, B.K., Zee, Y.C. and Osebold, J.W. (1982a) An in vitro system for studying the effects of ozone on mammalian cell cultures and viruses. Eviron. Res. 27, 466–475.

Bolton, D.C., Zee, Y.C. and Osebold, J.W. (1982b) The biological effects of ozone on representative members of five groups of animal viruses. Environ. Res. 27, 476–484.

Cortesi, R. and Privett, O.S. (1972) Toxicity of fatty ozonides and peroxides. Lipids 7, 715–721.

Crowe, S., Mills, J., McGrath, M.S., Lekas, P. and McManus, N. (1987) Quantitative immunocytofluorographic analysis of CD4 surface antigen expression and HIV infection of human peripheral blood monocyte/macrophages. AIDS Res. Human Retro. 3,135 145.

Emerson, M.A., Sproul, O.J. and Buck, C.E. (1982) Ozone inactivation of cell-associated viruses. Appl. Environ. Microbiol. 43, 603 608.RR

Feorin, P., Forrester, B., Schabele, C., Warfield, D. and Schochetman, G. (1987) Comparison of antigen assay and reverse transcriptase assay for detecting human immunodeficiency virus in culture. J. Clin. Microbiol. 25, 2344-2346.

Harakeh, M.S. and Butler, M. (1985) Factors influencing the ozone inactivation of entericviruses in effluent. Ozone: Sci. Eng. 6, 235 243.

Hartshorn, K.L., Vogt, M.W., Chou, C., Blumberg, R.S., Byington, R.R., Schooley, R.T. and Hirsch, M.S. (1987) Synergistic inhibition of human immunodeficiency virus in vitro by azidothymidine and recombinant alpha a interferon. Antimicrob. Agents. Chemother. 31, 168 172.

Hicks, D.R., Martin, L.S., Getchell, J.P., Heath, L., Francis, P., McDougal, J.S., Curran, J.W. and Voeller, B. (1985) Inactivation of HTLV III/LAV-infected cultures of normal human lymphocytes by nonoxynol-9 in vitro. Lancet 1, 1422 1423.

Katzenelson, E. and Biedermann, N. (1976) Disinfection of viruses in sewage by ozone. Water Res. 10, 629–631.

Katzenelson, E., Koerner, G., Biedermann, N., Peleg, M. and Shuval, H.I. (1979) Measurement of the inactivation kinetics of poliovirus by ozone in a fast-flow mixer. Appl. Environ. Microbiol. 37, 715 718.

Kim, C.K., Gentile, D.M. and Sproul, O.J. (1982) Mechanism of ozone inactivation of bacteriophage f2. Appl. Eviron. Microbiol. 39, 210 218.

Lee, S.D., Mustafa, M.G. and Mehlman, M.A. (Eds) (1982) International symposium on the biomedical effects of ozone and related photochemical oxidants. Vol V: Princeton Scientific Publishers, Princeton, N.J.

Levy, A. (1988) Mysteries of HIV: challenges for therapy and prevention. Nature 333, 519 522.

Lifson, J.D., Reyes, G.R., McGrath, M.S., Stein, B.S. and Engleman, E.G. (1986) AIDS retrovirus induced cytopathology: giant cell formation and involvement of CD4 antigen. Science 232, 1123 1127.

Martin, L.S., McDougal, J.S. and Loskaski, S.L. (1985) Disinfection and inactivation of the human T lymphotropic virus type III/lymphadenopathy-associated virus. J. Inf. Dis. 152, 400 403.

Mossman, T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assays. J. Immunol. Methods 65, 55 63.

Porstmann, T., Ternynck, T. and Avrameas, S. (1985) Quantitation of 5-bromo-2-deoxyuridine incorporation into DNA: an enzyme immunoassay for the assessment of the lymphoid cell proliferative response. J. Immunol. Methods 82, 169 179.

Rietjens, I.M.C.M., Lemmink, H.H., Alink, G.M. and Van Bladeren, P.J. (1987) The role of glutathione S-transferases in fatty acid ozonide detoxification. Chem. Biol. Interactions 62, 3–14.

Roy, D., Wong, P.K.Y., Englebrecht, R.S. and Chian, S.K. (1981) Mechanism of enteroviral inactivation by ozone. Appl. Environ. Microbiol. 41, 718–723.

Shechter, H. (1973) Spectrophotometric method for determination of ozone in aqueous solutions. Water Res. 7, 729–739.

Spire, B., Barre-Sinoussi, F., Montagnier, L. and Chermann, J.C. (1984) Inactivation of lymphadenopathy-associated virus by chemical disinfectants. Lancet 1, 899–901.

Sweet, F., Kao, M.S., Lee, S.D., Hagar, W.L. and Sweet, W.E. (1980) Ozone selectively inhibits growth of human cancer cells. Science 209, 931–933.

Vaughn, J.M., Chen, Y.S., Lindburg, K. and Morales, D. (1987) Inactivation of human and simian rotaviruses by ozone. Appl. Environ. Microbiol. 53, 2218–2221.

Wolff, S. (1989) Are radiation-induced effects hormetic? Science 245, 575.