Biocompatible Electric Current Attenuates HIV Infectivity

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The number of individuals infected by the human immunodeficiency virus type-1 (HIV) continues to increase on a worldwide basis.[1] A significant percentage, if not all, of these individuals will eventually develop the acquired immunodeficiency syndrome (AIDS).[2] While horizontal transmission in the homosexual population may be contained or decreasing,[3] heterosexual transmission and infection through contaminated blood supplies continues to increase.[4] Additionally, vertical transmission from infected females to their fetuses is also on the rise with a resultant increase in the number of children with AIDS.[5] New strategies, therefore, must be devised in order to limit more effectively the spread of this virus.

In this regard, three principal approaches are currently being investigated. In order to decrease susceptibility to the consequences of infection, vaccines are being sought which will induce the production of protective antibodies.[6] As treatment modalities, the use of soluble antagonists to block the receptor for HIV is being studied[7] as are pharmacologic agents such as nucleic acid analogues which can interfere with the transcription of viral genomic sequences.[8] Each of these systems has virtues and limitations, and to date none has proven completely effective.

Because heat or light in combination with drugs and dyes can inactivate viruses including HIV in vitro,[9] others have suggested the use of these forms of energy to treat AIDS patients. The results of studies using heat have not been peer-reviewed and are therefore impossible to evaluate. The use of light with drugs ("photopheresis")[10] appears to be efficacious, although this treatment may be limited by drug toxicity and the potential long-term effects of ultraviolet radiation on blood cell nucleic acids. Also, by its nature, this last system may not be suitable for the treatment of tissue-associated virus. As a result of our interest in the use of electric current to alter biological systems, we focused our investigations on the ability of direct electrical-current at biocompatible levels to

Table 1. Experimental paradigm

Current (μA)	Time of exposure (Minutes)			
0	1	4	8	12
25	2	4	8	12
50	3	4	6	12
75	2	4	8	12
100	1	3	4	6

alter the infectivity of HIV for susceptible CD4 positive cells in vitro.

MATERIALS AND METHODS

Electrical Treatment of HIV

The RF strain of HIV (AIDS Reagent Program) was cryopreserved prior to treatment at -70°C. For treatment, a sample of virus was thawed and maintained on ice at 4°C. Ten microliters (μL) of HIV at a concentration of 10^5 infectious particles per mL were placed into a chamber which included a pair of platinum electrodes 1 mm apart permanently mounted into a well 1.56 mm in length and 8.32 mm in depth equal to 12.9 μL volume capacity. The chamber was connected to a power supply capable of creating constant direct current. The viral aliquots were exposed to direct currents ranging from 0 microamperes (μA) for up to 12 minutes to 100 μA for up to 6 minutes. Intermediate currents of 25, 50, and 75 μA were used to expose similar viral aliquots. Under these conditions, for example, 0, 50, and 100 μA represent 0, 3.85, and 7.7 $\mu A/mm^2$ current densities respectively. The current was monitored throughout the experiment. A matrix of current and time employed is shown in Table 1.

After the exposure of virus to electric current, the contents of the chamber were removed and placed into sterile microtubes. Five μL of each sample were removed and diluted with 95 μL tissue culture medium supplemented with 10% fetal calf serum (FCS) for subsequent assays.

Syncytium-Formation Assay

This assay was performed as previously described by Nara et al.[11] Briefly, 10^5 CEM-SS cells were dispensed into poly-L-lysine coated microtiter wells. Thereafter, tenfold dilutions of H9 cells incubated with the treated HIV samples were co-cultured in triplicate for up to 4 days with the CEM-SS cells. Identical wells were prepared with control uninfected and infected cells. The wells were examined for syncytium formation at 2 and 3 days and quantified using an inverted microscope.

Reverse Transcriptase Assay

Uninfected H9 cells were pelleted at 1,000 rpm for 5 minutes at room temperature, the supernatant was decanted, and the cells were resuspended in 100 μL treated viral sample. The cells were incubated for up to 6 hours with the viral samples. At the end of the incubation time, the viral/cell suspensions were centrifuged at 1,000 rpm for 5 minutes and the supernatant decanted. The cell pellet was then resuspended in 5 mL of RPMI, 10% FCS and placed into a T25 tissue culture flask and maintained at 37°C, 5% CO_2 in a humidified chamber. At 2 day intervals (beginning at day 2), 1 mL of the cell suspensions was removed from each sample and centrifuged at 1,000 rpm for 5 minutes in order to pellet the cells. The supernatant was subsequently centrifuged at 14,000 rpm for 15 minutes. The pellet was resuspended in suspension buffer and assayed using standard methodology employing Mg^{++} as the divalent cation, poly (rA) oligo d(T) 12-18 as template primer, and tritiated thymidine (^{3}H-TdR) which comprise the reaction mixture. Known HIV-positive and -negative control samples were included in each assay for reference. Thirty μL of the reaction mixture were added to each 10 μL viral sample and incubated at 37°C for 60 min. Samples were then incubated with 1 μL of cold quench solution on ice for 15 minutes and filtered through a Millipore manifold. Chimneys were rinsed first with wash solution and followed by cold 95% ethanol. The filters were dried by vacuum and counted in scintillation fluid. Reverse transcriptase activity is expressed as counts per minute (cpm) and is considered positive only if cpm are at least five times greater than the cpm obtained with HIV-negative control samples.

Biocompatibility of Electric Currents/Time

To determine if the electric currents used were in a biocompatible range of energy, uninfected H9 cells were exposed to distinct currents for different amounts of time. The H9 cells were washed two times in Hanks Balance Salt Solution (HBSS). Thereafter, the cells were resuspended in RPMI, 10% FCS at a concentration of 10^6 cells per mL. Ten μL of the cell samples were placed into the reaction chamber. The cell samples were then exposed to 0, 50, or 100 μA for 0, 3, or 6 minutes. At the end of each test, the cell sample was removed from the chamber and approximately 10 μL of the sample was mixed with 90 μL of trypan blue. The number of viable cells was determined by trypan blue exclusion using a hemocytometer and light microscope. Results are expressed as percentage of viable cells from the total of all cells. At least 200 cells per field were counted.

Statistical Analysis

Results of the syncytium-formation and reverse transcriptase assays were tested for statistical significance by the Student's t test and analyses of variance.

RESULTS

Syncytium-Formation Assay

Using this index of HIV infectivity, it was determined that exposing virus to direct electric current suppressed its capacity to induce the formation of syncytia. Figure 1 shows a representative experiment and Table 2 shows the group data for three separate experiments. As can be noted in Figure 1, a statistically significant ($p<0.001$) reduction in syncytium number was observed, and this reduction was dependent upon the current applied to the viral isolate. At three different viral dilutions, there were analogous results in that a total charge of 200 μA x min (25 μA for 8 minutes) reduced the number of syncytia from 50% to 65% while a charge of 300 μA x min (50 μA for 6 minutes, 75 μA for 4 minutes, or 100 μA for 3 minutes) resulted in 90% reduction.

Reverse Transcriptase Assays

The direct electric currents to which HIV was exposed also reduced reverse transcriptase activity. Five separate experiments were conducted; a representative

experiment is shown in Figure 2 and the group data are included in Table 3. As can be seen in Figure 3, there was a significant decrease in the amount of reverse transcriptase activity after exposure of the virus to either 50 μA for 3 or 6 minutes. An equivalent reduction in reverse transcriptase activity was also noted with exposure to 100 μA for 3 minutes, and near ablation of reverse transcriptase activity was seen with exposure of the viral isolate to 100 μA for 6 minutes. The group data (Table 3) show that after exposure to 50 μA for 6 minutes, there was a 44% reduction in activity and treatment of virus with 100 μA for 6 minutes resulted in a 94% reduction. An analysis of variance indicates that the decrease in reverse transcriptase activity was statistically significant ($p<0.0001$).

Biocompatibility of Electric Currents/Time

The results of a viability analysis using trypan blue exclusion criteria applied to uninfected cells exposed to the different currents and times used for these studies are shown in Table 4. The viability of H9 cells, after exposure to 100 μA for either 3 or 6 minutes, did not show a significant decrease when compared to the 0 current control. After maximum treatment at 100 μA for 6 minutes, cell viability was 93%. Interestingly, in other preliminary experiments in which HIV-infected H9 cells were used, the results show that at 100 μA there may have been a significant decrease in the number of viable cells. That is, while an instantaneous pulse of 100 μA did not affect the viability of infected cells, a decrease in viability was noted at 3 and 6 minutes of exposure to 100 μA. This decrease was time-dependent in that exposure to 100 μA for 3 minutes resulted in a viability of 83% while 100 μA for 6 minutes resulted in a viability of 80%. Although these data are provocative, they only represent a preliminary experiment and require further investigation.

With respect to the possibility that the electric current was transduced into heat, the calculated rise in temperature within the chamber was determined to be less than 1°C. In order to verify this, a temperature microprobe was introduced into the chamber containing tissue culture medium alone. Results of these studies are shown in Table 5. Similar results were obtained when H9 cell-containing medium was placed in the reaction chamber. The data indicate that for the currents and times used for these experiments, there was no alteration in the temperature of the chamber.

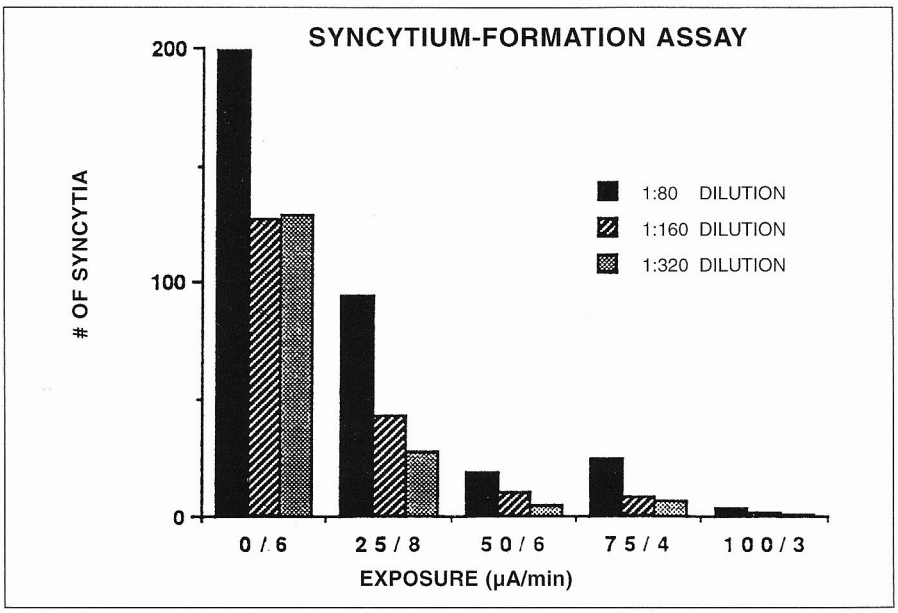

Figure 1. Results of a representative syncytium-formation assay. Five aliquots of the RF strain of HIV were exposed to direct electric current for up to 8 minutes. Three of the samples were exposed to a total electric charge of 300 μA x min (50/6, 75/4, and 100/3). At all the dilutions tested (three shown here), electrical treatment of the virus aliquots resulted in a significant decrease in syncytium formation.

Table 2. Effect of electric current on syncytium formation[a]

Current (μA)	% of 0 Current Control (%)[b] Six Minute Exposure	
0	100	(0)
50	50	(-50)
100	35	(-65)

a = Value at 1:160 dilution of virus.

b = Value equals the mean of 3 experiments.

DISCUSSION

The results reported here demonstrate that HIV treated with direct electric currents from 50 to 100 μA has a significantly reduced infectivity for susceptible cells in vitro. This reduction of infectivity correlates with the total electric charge passing through the chamber. Although extrapolation of these data predicts that ablation of HIV infectivity may be possible, and additional preliminary data support this prediction, the expectation that some virions may still escape the electrical effect cannot be discounted. Nevertheless, the therapeutic potential of electric current may reside in its ability to lower the viral titer to subclinical significance or in its incorporation into a strategy analogous to that of other therapies in which repeated cycles of treatment eventually achieve remission or cure.

The data presented in this report are based on both quantitative and quantal determinations of viral infectivity. Although the syncytium-formation assay can be used to quantify the number of infectious viral particles, this use with respect to HIV may be abridged because of the ability of free fusigenic peptide (gp41) to induce syncytia by itself. Therefore, while syncytia were observed at some dilutions of electrically treated virus, this may simply represent the presence of soluble gp41 in the tissue culture medium. We believe that the correlation between total charge and reduction in syncytium number more adequately reflects the ability of direct electric current to reduce HIV infectivity.

This belief is also supported by the results of the reverse transcriptase assays. Although a decrease in HIV reverse transcriptase does not assure reduced

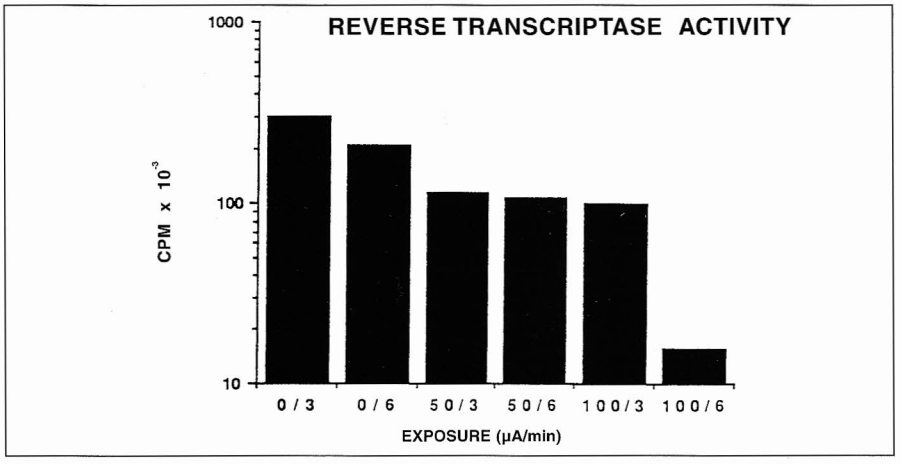

Figure 2. Results of a representative reverse transcriptase assay. Six aliquots of the RF strain of HIV were exposed to different amounts of current for 3 or 6 minutes. A significant decrease ($p<0.005$) from 0 current levels (0/3 and 0/6) in reverse transcriptase activity is noted. However, the decrease is more significant ($p<0.0001$) when virus is exposed to 100 μA for 6 minutes.

Table 3. Effect of electric current on reverse transcriptase activity[a]

% of 0 current control (%)

Current (μA)	Six minute exposure	
0	100	(0)
50	56	(44)
100	6	(-94)

a = Value equals the mean of 5 experiments. The standard error of the mean in each case was less than 10% of the mean value.

Table 4. Effect of electric current on viability of uninfected H9 cells

(% viable cells[a])

	Length of exposure (minutes)		
Current (μA)	0	3	6
0	96	94	96
50	98	95	98
100	96	97	93

a = At least 200 cells counted in hemocytometer field

Table 5. Effect of electric current on temperature of tissue culture medium[a]

(°C)

	Length of exposure (minutes)		
Current (μA)	0	3	6
0	19	19	19
50	19	19	19
100	19	19	19

a = The temperature was monitored before, during, and after exposure.

infectiousness of this virus for susceptible cells, we feel that, taken together with the syncytium-formation data, the results indicate that significant attenuation of HIV infectivity is achieved by treatment with direct electric currents.

With respect to the biocompatibility of the electric currents and total charges reported here, two separate sets of evidence are applicable. The first has to do with the results showing that, by trypan blue exclusion, no significant cytotoxicity was induced in H9 cells by any total charge tested. The other evidence is obtained from reports which clearly indicate that the amount of electricity used for these experiments is significantly below presently used therapeutic electric currents which are in the milliampere range.[12-16]

Rather than negative effects, exposure of cells to electric current may actually have positive consequences for resistance to infection in that important cellular electrochemical changes correlate with enhancement of specific enzymatic activities. In particular, a facilitation of succinate dehydrogenase (SDH) and ATPase activity has been observed.[12,15] Both of these enzymes are associated with the oxidative capacity of the cell. Specifically, it has been suggested that an electrochemical reaction occurs between mitochondrial membrane-bound H^+ ATPase and ADP leading to the formation of ATP. Therefore, exposure of cells to direct electric current may directly or indirectly increase energy resources within a cell and facilitate cell metabolism. This, in turn, may actually render a cell less susceptible to the effects of viral infection.

In summary, the data presented here indicate that biocompatible direct electric current significantly reduces the infectivity of HIV. Continuing investigations are exploring the mechanisms through which this effect is mediated. The initial focus of these experiments is centered on the potential role which ionic and molecular species generated by electrolysis may have on the virus. However, the complete mechanism by which direct electric current attenuates HIV infectivity is undoubtedly far more complex than simple electrolysis. Nonetheless, and independent of a complete understanding of all of the mechanisms involved in the attenuation of HIV infectivity, the present observations may serve as an initial step for the development of new strategies to treat infection or prevent transmission of HIV through the treatment of the blood supply.

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REFERENCES

1. Sato PA, Chin J, Mann JM. Review of AIDS and HIV infection: global epidemiology and statistics. AIDS 1989;3 Suppl 1:S301-7.
2. Centers for Disease Control. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. MMWR 1987;1 Suppl 36:S1-15.
3. Thacker SB, Berkelman RL. Public health surveillance in the United States. Epidemiol Rev 1988;10:164-90.
4. Klein RS, Friedland GH. Transmission of human immunodeficiency virus type 1 (HIV) by exposure to blood: defining the risk. Ann Int Med 1990;113:729-30.
5. Oxtoby MJ. Epidemiology of pediatric AIDS in the United States. In: Kozlowski PB, Snider DA, Vietze PM, et al, eds. Brain in pediatric AIDS; 1990. p 1-8.
6. Broder S, Mitsuya H, Yarchoan R, et al. Antiretroviral therapy in AIDS. Ann Int Med 1990;113:604-18.
7. Perno CF, Baseler MW, Broder S, et al. Infection of monocytes by human immunodeficiency virus I blocked by inhibitors of CD4-gp120 binding, even in the presence of enhancing antibodies. J Exp Med 1990;171:1043-56.
8. Mitsuya H, Weinhold KJ, Furman FA, et al. 3'-Azido-3'-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus in vitro. Proc Natl Acad Sci USA 1985;82:7096-100.
9. Quinnan GV, Wells MA, Wittek AE, et al. Inactivation of human T-cell virus, type III by heat, chemicals and irradiation. Transfusion 1986;26:481-3.
10. Bisaccia E, Berger C, Kainer AS. Extracorporeal photopheresis in the treatment of AIDS-related complex: a pilot study. Ann Int Med 1990;113:270-75.
11. Nara PL, Hatch WC, Dunlop NM, et al. Simple, rapid quantitative, syncytiumforming microassay for the detection of human immunodeficiency virus neutralizing antibody. Aids Res Hum Retrovirus 1987;3:283-302.
12. Cheng N, Van Hoof H, Bockx E, et al. The effects of electric currents on ATP generation, protein synthesis, and membrane transport in rat skin. Clin Ortho Rel Res 1982;171:264-72.
13. Frank C, Schachar N, Dittrich D, et al. Electromagnetic stimulation of ligament healing in rabbits. Clin Ortho Rel Res 1983;175: 263-72.
14. Eriksson E, Haggmark T. Comparison of isometric muscle training and electrical stimulation supplementing isometric muscle training in the recovery after major knee ligament surgery. Amer J Sports Med 1979;7:169-71.
15. Stanish WD, Valiant GA, Bonen A, et al. The effects of immobilization and of electrical stimulation on muscle glycogen and myofibrillar ATPase. Can J Appl Sport Sc 1982;7:267-71.
16. Pilla AA. Electrochemical information transfer at living cell membranes. Ann NY Acad Sci 1974;205:148-70.