"Preliminary report of the effect of an oscillating electrical signal, on the CD4 and CD8 lymphocyte population and on HIV viral load, in HIV-1-infected adults".

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Abstract

In this study 26 patients infected with the HIV-1 virus were treated daily for two months with an electrotherapy device and their results compared with 27 non-treated patients matched for sex and age who were not participating in any anti-HIV treatment.

Over the 2 months of the trial, CD4 cell numbers reduced by 12% in the non-treated patients.
The treated group showed a 5% increase in CD4 cell numbers.
CD8 cell numbers dropped by 17% in the non-treated patients, and increased by 18% in the treated patients, over the same 2 months.
In addition HIV viral loads increased by 109% in the untreated patients, and decreased by 6% in treated patients.

In many parts of the world where HIV infection is particularly rife, there are insufficient financial resources to pay for treatment with anti-viral drugs. This electrotherapy technique appears to be a low cost method for treating HIV infected patients who cannot afford conventional anti-viral treatment, and so are currently receiving no treatment at all.

Further investigation of this electrotherapy methodology in large-scale multi-centre trials is warranted.

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Introduction

It is well known that human immunodeficiency virus (HIV) infection leads to depressed cellular immunity, which can result in serious opportunistic infections in acquired immune deficiency syndrome (AIDS) patients (Gottlieb et al. 1981; Gallo et al. 1984). At present, combination anti-retroviral drug regimens including protease inhibitors are used as a standard therapy for HIV-1 infection.

Access to antiretroviral drugs for HIV-infected patients in developing countries is a global public health priority. With the support of multilateral and bilateral programmes, non-governmental organisations, and national authorities, WHO has the ambitious objective to treat 3 million people with highly active antiretroviral therapy (HAART) by 2005 (WHO, UNAIDS 2003). WHO currently recommends first-line therapy with two

nucleoside reverse transcriptase inhibitors (NRTIs) and one non-NRTI (NNRTI), a combination with good efficacy, tolerability and simplicity, low cost, and good adherence to treatment (WHO 2003).

Generic fixed-dose combinations of such regimens are widely regarded as crucial for scaling-up AIDS treatment in developing countries. These treatments improve adherence owing to the fewer daily doses relative to individual formulations. Supply, storage, and distribution are also easier because the range of products is smaller. Generic drugs are generally much cheaper than brand-name formulations. Several generic fixed-dose combinations have been pre-qualified by WHO (WHO 2004) after assessment of manufacturers' product data (including data for purity of all ingredients, stability of the finished products, and results of in-vivo bioequivalence tests), actual pharmacological composition, and manufacturing practices. However, these formulations are not yet recommended by some of the major donor agencies, such as the US government's multi-billion dollar PEPFAR (President's emergency plan for AIDS relief funding) programme for developing countries (USAID 2004). In addition to political considerations, particularly on the legitimacy and consequences of using generic instead of brand-name drugs, this situation is partly explained by the absence of clinical studies showing the efficacy and tolerability of generic fixed-dose combinations. Quality control of different drug batches is also a difficulty in most developing countries.

The generic fixed-dose combination of nevirapine, stavudine, and lamivudine (Cipla, Mumbai Central, Mumbai, India) is one of the most frequently prescribed treatments in African countries. In Angola, a south west African country with more than 15 million inhabitants, the prevalence of HIV infection is increasing rapidly, with up to 8% of town-dwelling pregnant women infected (UNAIDS 2002). HIV-1 predominates, and HIV-1 groups M, N, and O and many subtypes and circulating recombinant forms co-circulate (Vergne at al 2003). A national antiretroviral access programme has started, most usually based on the generic combination of nevirapine, stavudine, and lamivudine owing to its low price. However the sad fact remains that many AIDS-infected patients in Angola go without any anti-viral treatment.

Use of these combination therapies has dramatically decreased morbidity and mortality rates in HIV-1 infected individuals (Hogg et al. 1997; Cameron et al. 1998; Palella et al. 1998). However, such intensive combination therapies have various drawbacks, such as drug side-effects, the complexity of the therapeutic regime, and the appearance of resistant HIV strains (Carr et al. 1998; Colgrove et al. 1998; Sarmati et al. 2002). So far, it appears that these combination anti-retroviral drug regimens must remain in use until a radical curative treatment and HIV-1 vaccine are established. Therefore, therapies based on new principles other than drug treatment would appear to be highly desirable.

Electrical stimulation effects on living cells have been extensively studied since the 1970s (Zimmerman et al. 1974). It has been reported that use of high d.c. voltage pulse application not only induces changes in cellular membrane structure and permeability, but also results in its breakdown (Berg et al. 1984; Powell et al. 1986). Moreover, cells suspended in solution were found to be easily fused by pulses of high d.c. voltage

(Zimmerman 1982). On the other hand, pulses of low d.c. voltage are known to regulate cellular proliferation and protein production together with induction of differentiation of various cell types (Kojima et al. 1992; Mie et al. 1996; Aizawa et al. 1999). Furthermore, the effects of electrical stimulation on HeLa cells chronically infected with HIV-1_{LAI} and on uninfected P6 HeLa cells (Tominaga et al. 2003) has been reported, and the possible causes for the significant damage that occurs to infected P6 HeLa/HIV-1_{LAI} cells compared with uninfected control cells has been considered.

In this study we tested the effectiveness of an electrotherapy apparatus (The Bio-Lyfe machine), that delivered an oscillating electrical signal via hand-held probes, on the immunological parameters and viral load in HIV infected patients.

Methods

All participants in the trial were periodically monitored with respect to their clinical features; any side effects were noted. Blood was withdrawn and placed into tubes with ethylenediaminetetraacetic acid (EDTA) as anticoagulant; the plasma was used in tests and some was also stored at -70°C. Laboratory tests, including routine blood tests, and CD4 and CD8 counts, were performed at the Military Hospital. The HIV viral load was determined in the HIV Laboratory, Laboratory Services Branch, of the Military Hospital which used the sensitivity cut-off value of 10 000 HIV copies of RNA per millilitre) (Chiron Diagnostics 1997). All data and clinical records were kept confidential.

In this study, 37 patients with HIV infection were treated with The Bio-Lyfe machine. The treatment period was at least 2 months. Patients were exposed to hand held electrode probes daily for 20 minutes at the highest tolerable current. There were no obvious side effects reported throughout the two months. Compliance was difficult to maintain as it involved patients coming to a medical centre daily. Seven patients were discontinued from the trial due to poor compliance. Thirty HIV infected patients who were not participating in any anti-HIV treatment were randomly chosen from the hospital database and matched with trial patients who were observed for two months.

The CD4 and CD8 counts and HIV viral loads of patients were measured before the trial, at 1 month and at 2 months of treatment with the Bio-Lyfe machine (Tables 1 and 2). These results were compared to test results for the untreated patients. All patients gave their written informed consent.

The Bio-Lyfe Unit – in its container:

Results

Raw Data

Table 1 Treated Patients:

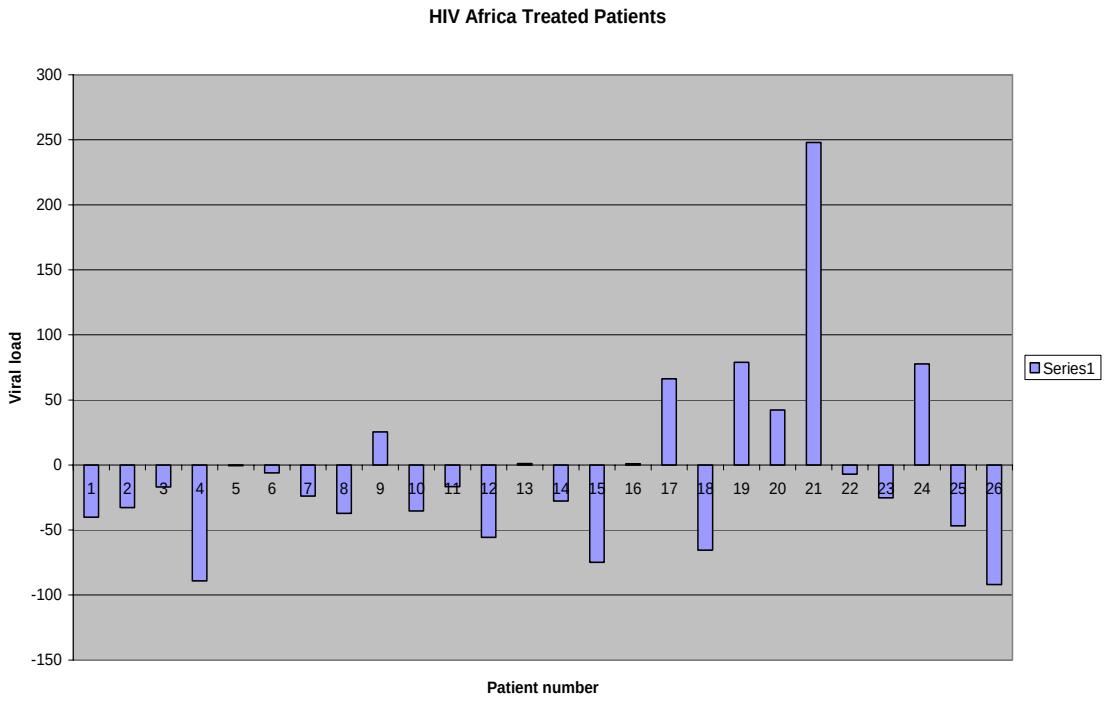
			Month 0			Month 1			Month 2		
ID	Sex	Age	CD4	CD8	Viral Load	CD4	CD8	Viral Load	CD4	CD8	Viral Load
1	M	23	293	417	41475	313	676	31888	304	711	24852
3	M	27	290	377	131802	330	612	114223	409	785	88574
4	M	25	349	724	86182	312	715	78208	339	730	71556
5	M	32	484	853	276163	445	798	278917	422	751	29885
6	M	41	754	1046	229558	688	943	249003	678	936	228557
7	M	23	453	801	208412	445	815	210234	478	866	195633
8	M	27	670	1040	223524	585	927	150749	526	839	169857
9	M	46	707	958	249882	611	978	176912	587	888	156985
10	M	32	369	1002	203176	452	989	207899	352	785	254877
12	M	25	337	871	138702	415	878	87269	357	857	89663
13	M	27	450	759	110019	437	811	105882	411	872	91721
14	M	33	240	651	60737	398	973	28911	408	957	26888
15	M	19	601	1644	297874	583	1534	311092	651	1452	300922
16	M	22	539	712	110183	525	1023	87999	455	1066	79639
17	M	25	270	435	28031	336	672	9021	429	639	7058
18	M	27	246	733	22660	259	755	27898	211	781	22844
20	M	29	321	560	11144	333	829	11453	308	800	18522
21	F	21	370	391	173178	392	722	121022	502	967	59633
22	M	28	345	992	14519	333	947	17900	329	788	25983
23	M	30	171	510	209796	199	615	207678	251	611	298667
24	M	26	680	1219	17131	612	1121	16752	482	899	59633
25	M	22	163	258	737148	172	427	547288	144	389	685214
26	M	19	298	505	98813	323	504	102007	389	585	73695
27	M	18	566	1447	27960	535	1399	33872	524	1189	49685
28	F	39	227	489	398022	254	500	358966	233	496	211589
29	M	42	188	412	678876	189	397	402587	161	385	54833

Patient 2, 11 and 19 discontinued.

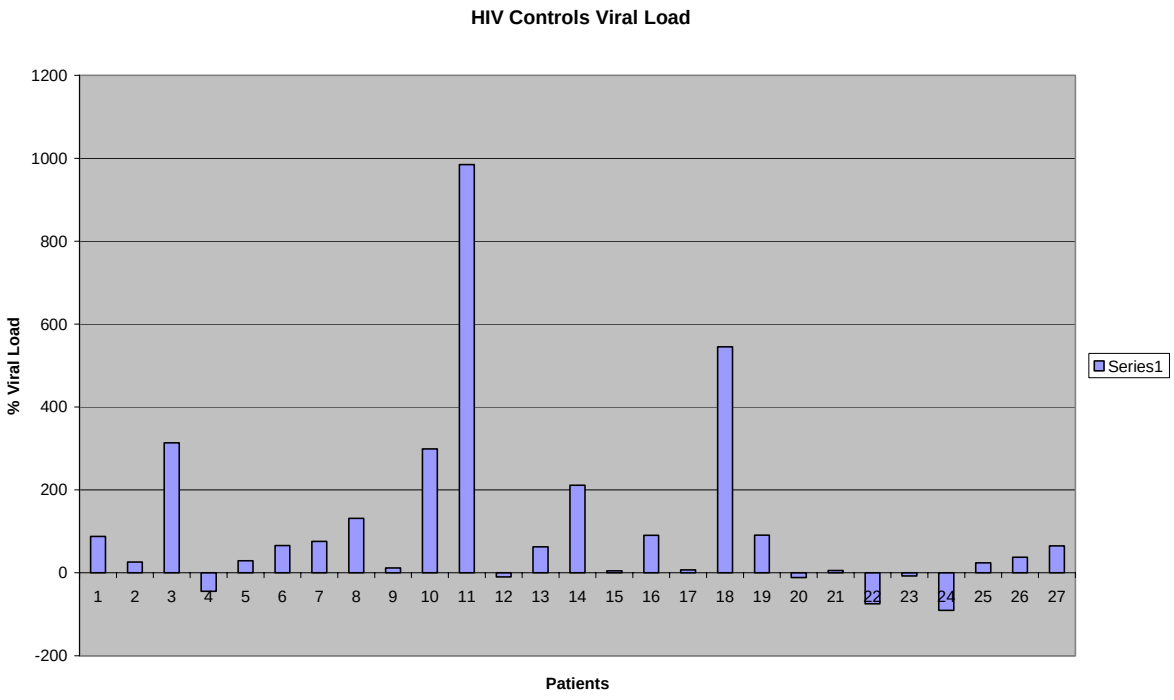
Table 2 Untreated patients:

			Month 0			Month 1			Month 2		
ID	Sex	Age	CD4	CD8	Viral Load	CD4	CD8	Viral Load	CD4	CD8	Viral Load
1	M	26	379	795	87177	311	660	89155	308	489	163588
3	M	19	561	1338	29227	544	1125	39565	452	1159	36852
4	M	42	371	1393	38237	312	915	71589	215	689	158009
5	M	38	194	365	349193	204	411	334568	261	497	193578
6	M	30	635	1484	57671	678	1358	65487	587	1258	74269
7	M	46	261	671	204635	258	615	219284	199	422	339658
8	M	40	565	1246	32381	572	968	29567	518	702	56892
9	M	19	319	1041	102341	288	777	198557	333	693	236952
10	M	19	525	1452	34802	535	1544	20568	439	1423	38978
12	M	37	549	1531	38977	515	1422	37552	482	1112	155369
13	M	27	602	1317	23262	438	891	43255	339	672	252361
14	M	29	594	1156	33517	598	1211	29658	502	1005	30251
15	M	29	466	1122	116590	483	1534	135985	389	1025	189752
16	M	20	212	635	30907	241	702	28564	149	583	96352
17	M	23	219	637	154793	215	672	155888	226	545	162358
18	M	19	519	1285	74609	485	1158	95685	389	901	142365
20	M	40	193	497	183090	202	544	175882	159	452	196358
21	F	22	451	965	29391	435	789	37551	242	498	189635
22	M	23	307	694	33068	333	752	31588	242	593	63258
23	M	22	171	438	172352	170	411	218678	193	452	152981
24	M	38	503	1101	17579	544	1004	16884	539	996	18596
25	M	37	508	1040	62644	333	775	221479	406	779	15639
26	M	45	242	640	200383	244	581	200875	269	621	185269
27	M	22	158	335	275711	148	347	338547	158	385	25689
28	F	25	145	440	394090	140	451	401585	129	421	489366
29	M	25	535	1114	26904	517	1220	27568	506	1107	36952
30	M	27	412	811	12459	388	795	16587	376	763	20562

Graph of HIV Viral Load Changes Over 2 Months for Individual Patients in the Treated Group



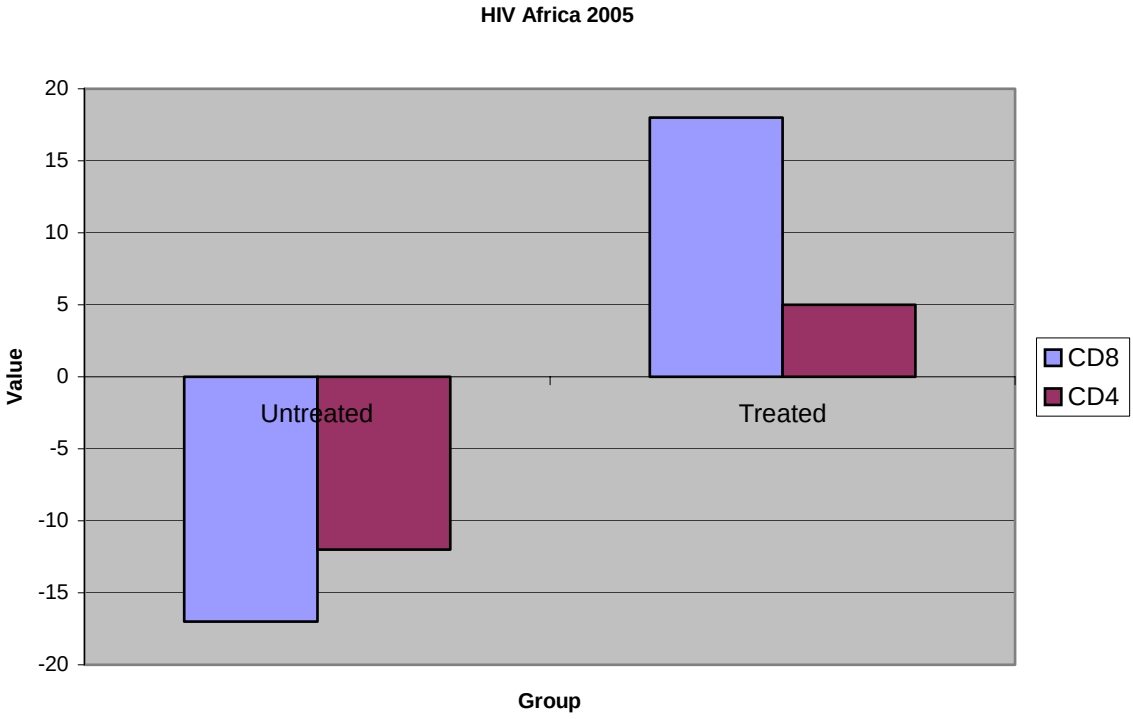
Graph of Change in HIV Viral Load Over 2 Months for Individual Patients in the Untreated Group



Summary of Average CD4 and CD8 Changes in Untreated and Treated Groups – Percentage Change

	Untreated	Treated
CD8	-17%	+18%
CD4	-12%	+5%

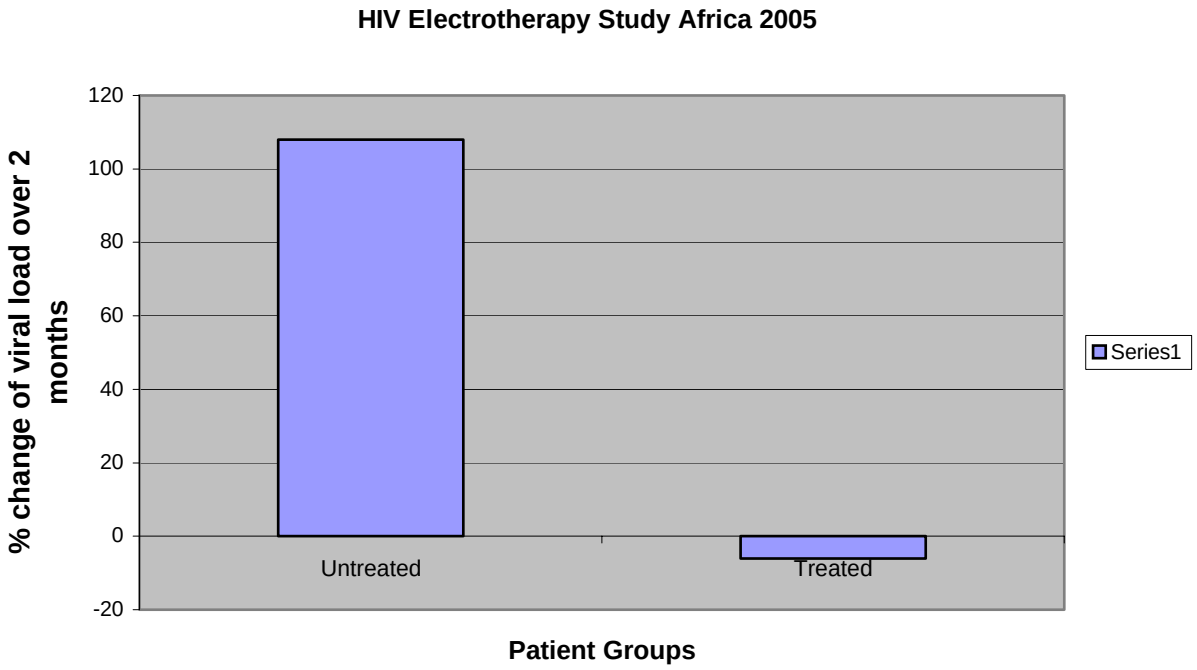
CD4 + CD8 Graph – Summary Results - % Change over 2 months



Summary of Average Changes in HIV Viral Load for the Untreated and Treated Groups – Percentage Change

	Viral Load Change
Untreated Group	+108%
Treated Group	-6%

Viral Load Changes Graph – Summary Results



Discussion

There seems to be an early obvious positive effect of treatment with The Bio-Lyfe electrotherapy machine on CD8 cell count followed by a positive effect on CD4 count and viral load in many of the HIV-infected patients. Large individual variations in response were also observed. We have previously observed positive effects in CD8 cell counts in various cancer patients and in three HIV patients (unpublished data).

The multi wave device provides an electrical signal to two hand held electrodes that boost CD8 immune system cell numbers.

By boosting these CD8 cells the viral load is reduced, and the treated patients experience an increase in CD4 cells and also often an improvement in quality of life.

Research at Duke University has shown that high levels of CD8 cells have a positive effect on prognosis in AIDS-infected patients. The proposed mechanism of action is that in asymptomatic patients, CD8 cells can identify infected CD4 cells, latch onto them, and release compounds that cause the infected cell to burst, killing it.

CD8 cells also possess a non-cytolytic weapon as well, and this stops HIV replication.

Specifically Tomaras and Greenberg's experiments (2000) at Duke University show that CD8 cells affect the virus after it has already entered the CD4 cell, which is different to the way beta-chemokines work. The CD8 cells stopped HIV from hijacking the CD4 cell's genetic machinery to reproduce itself.

Discussion Of Other Relevant Studies

Immunologic characteristics have previously been studied in 103 patients with multiple myeloma, acute leukemia, chronic lymphocytic leukemia and non-Hodgkin's disease following in vitro exposure of blood to a low-intensity static field (SF) and alternating field (AF) or pulsating magnetic field (PF) (Bessmel'tsev 2001). In this study of multiple myeloma, a 30 minute exposure had a positive effect on expression of tumor cells and T-cell markers and stimulated the regulatory function of T-lymphocytes. With SF-AF and PF application alternating, the expression of both +CD3 and +CD4 and the +CD3/+CD4 ratio increased suggesting the lowering of immunological deficiency. In acute leukemia, a combined application of the magnetic fields had an effect on the helper activity of the T-lymphocyte sub-population. The phagocytic activity of leukocytes increased significantly while their digestive ability rose to a moderate degree.

The effect of leukocyte subsets, total leukocyte isolates or full blood samples subjected to medium-strength square-wave electric impulses (100 V/cm field force, 5 ms duration) has also been reported (Filipic 2000). On the surface of the leukocytes, the expressions of several markers (CD3, CD4, CD8, CD11a, CD11b and ICAM-1) were determined in order to study the influence of pulsed ionic currents on different aspects of the cellular immune response. Large individual differences were also observed in this study among randomly chosen healthy donors, both in the initial expression rate and in the response patterns of different antigens. As a general conclusion, it can be stated that electric impulses with the above parameters activate the state of immune response alertness of human leukocytes. Elevation in the activities of several enzymes, such as lactate dehydrogenase and superoxide dismutase in the serum in response to electric impulses suggested an antiviral and immune activated condition. It was concluded that ex vivo blood treatment with medium-strength electric impulses seems to be a promising adjuvant course for the establishment of acute immune potentiation and an antiviral state in patients undergoing dialysis treatment.

The effects of uncontrollable and controllable electric shocks on the immune system in rats (the proportion of CD4+, CD8+ or CD25+ T lymphocytes to total lymphocytes was measured in the peripheral blood, spleen, and thymus) have also been observed (Nakata 1996). The rats were given either controllable shocks, identical uncontrollable shocks, or no shocks, and then small shocks 24 h later (reinstating shocks). The proportion of CD4+ T lymphocytes relative to total lymphocytes in both the peripheral blood and spleen of uncontrollable rats (URs) was significantly smaller than was found in no-shock rats (NRs). Similarly, the proportion of CD4+ T lymphocytes in the thymus of controllable rats (CRs) was significantly smaller than in NRs. In contrast, the proportion of CD8+ T lymphocytes in the thymus of URs was significantly larger than in NRs and CRs. The CD4+ to CD8+ T lymphocyte ratios (CD4+/CD8+ ratios) in the peripheral blood, spleen and thymus of URs were significantly smaller than in NRs; also, the ratios in the peripheral blood and spleen of URs were significantly smaller than in CRs. The white blood cell (WBC) count of URs was significantly smaller than those of NRs and CRs, and the WBC count of CRs was significantly smaller than those of NRs and CRs, and the WBC count of CRs was significantly smaller than that of NRs. These results suggest that

decreases in CD4+ T lymphocytes (and/or an increase of CD8+ T lymphocytes) in the peripheral blood, spleen, and thymus are caused by uncontrollable stress followed by a reinstating stress condition, leading to the decrease of WBC in the peripheral blood and decreases in the CD4+/CD8+ ratios in these tissues.

Electrical stimulation can also affect neuropathies in nerves and circulating neurotransmitter levels (Herzberg 1995). This indirectly can affect the immune system (Lechin 2002).
To study the possible mechanism by which peripheral nerves mediate immune responses in target tissues, electrical stimulation of the sciatic nerve was combined with subcutaneous microdialysis of the hind paw (Herzberg 1995). Following unilateral stimulation of the sciatic nerve, an ipsilateral rise in substance P and a bilateral rise in VIP levels were observed in dialysate samples from experimental vs control animals. Electrical stimulation of the sciatic nerve induced a marked hyperemia and swelling of the ipsilateral paw. Quantitative immunocytochemical analysis of paraffin-embedded sections of the hind foot pads demonstrated T lymphocyte migration ipsilateral to the stimulated nerve. These findings suggest that peripheral nerves can directly modulate local immune and inflammatory responses.

It has been shown in previous studies that cell poration (i.e. reversible permeabilization of cell membrane) and cell fusion can be induced by applying a pulse (or pulses) of high-intensity DC (direct current) electric field (Chang 1989). The same group also suggested that such electro-poration or electro-fusion can also be accomplished by using an oscillating electric field. The DC field relies solely on the dielectric breakdown of the cell membrane to induce cell fusion. The oscillating field, on the other hand, can produce not only a dielectric breakdown, but also a sonicating motion in the membrane that could result in a structural fatigue. Thus, a combination of a DC field and an oscillating field is expected to enhance the efficiency of cell poration and cell fusion. Pulses of high-intensity, DC-shifted RF (radio frequency) electric field were used to induce a highly efficient cell poration and cell fusion on human red blood cells and on a fibroblast cell line.

Another study demonstrated a safe and effective way to introduce exogenous genes into cells, a new method of electroporation which uses a radio-frequency (RF) electric field to permeabilize the cell membrane has been uncovered (Chang 1991). This RF method has several advantages over the conventional electroporation method which uses a direct current (DC) field. It was shown that the RF electroporation method can be used to introduce marker genes into a wide variety of cell lines and was able to increase substantially the efficiency of gene transfection. Interestingly, the transfection efficiency was shown to be affected by a number of factors, including cell type, field strength, pulse protocol and medium buffer. Because of its wide range of applications, high transfection efficiency and lack of harmful side-effect, the RF electroporation method would be particularly useful for introducing genes into human cells for gene therapy.

Morphological changes of HIV infected cells following application of low electrical potential were induced due to a decrease in the plasma membrane fluidity and

deformation of cytoskeletal structure including F-actin (Aizawa et al. 1999; Yaoita et al. 1989). It has also been suggested (Kumagia 2004) that the swelling and breakdown of cell membrane due to application of electrical potential might have occurred due to an increase in the adhesion area between the positively charged ITO electrode and the negatively charged cells after electrical stimulation. Several other factors possibly contribute to the higher cellular damage due to electrical stimulation in P6 HeLa/HIV-1_{LAI} cells than in P6 HeLa cells. It was considered that the most important factor to be the large surface area of chronically HIV-1 infected cells due to the budding of HIV-1 particles at the plasma membrane, which is not observed in uninfected cells (Barré-Sinoussi et al. 1983; Chrystie and Almeida 1988). In other words, budding of HIV-1 would increase the negative charge of infected cells, and hence lead to increased reception of the effect of electrical stimulation. Another factor would be that some oxidized species might have developed due to the electrochemical reaction at the ITO electrode surface in the supernatant of P6 HeLa/HIV-1_{LAI} cells. Collectively, it has been shown (Kumagai 2004) that the difference in sensitivity to electrical stimulation between uninfected cells and cells chronically infected with HIV-1 could be useful not only for the detailed elucidation of HIV control mechanisms but also for the development of new therapies.

Summary - In Vivo Pulsed High Potential Electrotherapy

Pulsed high potential electrotherapy technology appears to offer a novel approach to treating diseases related to immunosuppression, such as infection with HIV.

The technique involves the use of brief high potential spikes which disrupt the skin's dielectric barrier and allow specific electrical potentials to penetrate into the body's tissues. These potentials carry specific waveforms and frequencies which can have positive effects on the healthy function of a range of tissues in the body. In addition these high electrical potentials can disrupt the weakened membranes of infected cells, causing cell death. These potential also appear to have a specific anti-microbial effect. It is thought that electromechanical and electrostatic stresses are induced by these high voltage pulses at multiple audio and radio frequencies.

The electromechanical stresses, cell electroporation, and ion exchange processes induced by high voltage DC pulses are well documented in vitro. These processes may act to both destroy infected cells and to stimulate the immune system. (Bryant 1987, Chang 1989, Dao-Sheng 1990, Dimitrov 1990)

Inactivation and destruction of bacteria by electric treatment methods is well established in vitro. In addition pulsed high voltage electrotherapy at multiple frequencies appears to inactivate pathogenic micro-organisms in vivo. (Allen 1966, Hamilton 1967, Gillibrand 1967)

In addition high potential electrotherapy is showing promise in treating inflammatory conditions (Johnson 2004), and in treating cancer – both as a direct cytotoxic agent (Fedorowski 2004, Hernandez-Bule 2004, Kirson 2004, Pinero 1997) and also by means of enhancing the efficacy of chemotherapy and radiotherapy through electroporation effects on the membranes of cancer cells (Ito 2001, Engstrom 2001, Gray 2000, Li 2003, Rabussay 2002, Rols 2000, Sun 2003).

Conclusion

There are multiple studies in the literature suggesting that electrical stimulation can induce positive immunological changes both in vivo and in vitro, and negatively effect HIV-infected cells with no effect on non-infected cells.
The Bio-Lyfe electrotherapy apparatus seems to have an obvious positive effect on HIV-infected individuals in this pilot study.
However it's mechanism of action for inducing these changes is currently unclear.
Further more detailed and larger trials on the efficacy and safety of The Bio-Lyfe machine are warranted, as are studies into the possible mechanism of action of The Bio-Lyfe machine.
It is of note that The Bio-Lyfe machine appears to be a cheap, safe and easy to use treatment system. It may even be appropriate for home use.
The Bio-Lyfe machine looks to be a real possibility to add to the very limited arsenal of weapons to fight the current global HIV epidemic.
Further studies are therefore warranted.

References

Aizawa M, Koyama S, Kimura K, Haruyama T, Yanagida Y, Kobatake E (1999) Electrically stimulated modulation of cellular function in proliferation, differentiation, and gene expression. Electrochemistry 67:118–125

Allen M, Soike K. Sterilization by Electrohydraulic Treatment. Oct. 1966; Science; pp. 155-157.

Barr-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, Dauguet C, Axler-Blin C, V_zinet-Brun F, Rouzioux C, Rozenbaum W, Montagnier L (1983) Isolation of a T lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 220:868–871

Berg H, Augsten K, Bauer E, F_rster W, Jacob H-E, M_hlig P, Weber H (1984) Possibilities of cell fusion and transformation by electrostimulation. Bioelectrochem Bioenerg 12:119–133

Bessmel'tsev SS, Abdulkadyrov KM, Gonchar VA, Lavrushina TS. The in-vitro effect of constant and pulsating magnetic field on immunocompetent blood cells of hematologic patients. Vopr Onkol. 2001;47(1):59-65. Russian.

Bryant G, Wolfe J. Electromechanical Stresses Produced in the Plasma Membranes of Suspended Cells by Applied Electric Fields. (1987); J. Membrane Biol.; vol. 96; pp. 129-139.

Cameron DW, Heath-Chiozzi M, Danner S, Cohen C, Kravcik S, Maurath C, Sun E, Henry D, Rode R, Potthoff A, Leonard J (1998) Randomised placebo-controlled trial of ritonavir in advanced HIV-1 disease. Lancet 351:543–549

Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ, Copper DA (1998) A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. AIDS 12:F51–F58

Chang DC, Gao PQ, Maxwell BL. High efficiency gene transfection by electroporation using a radio-frequency electric field. Biochim Biophys Acta. 1991 Apr 17;1092(2):153-60.

Chang DC. Cell poration and cell fusion using an oscillating electric field. Oct. 1989; Biophys. J.; vol. 56; pp. 641-652.

Chang DC. Cell poration and cell fusion using an oscillating electric field. Biophys J. 1989 Oct;56(4):641-52.

Chiron Diagnostics. Paediatric protocol of branched DNA. Quantiplex HIV RNA 2.0 Assay (bDNA) 50 mL Format. Manufacturer's information. Beijing: Chiron Corporation; 1997.

Chrystie IL, Almeida JD (1988) Further studies of HIV morphology by negative staining. AIDS 2:459–464

Colgrove RC, Pitt J, Chung PH, Welles SL, Japour AJ (1998) Selective vertical transmission of HIV-1 antiretroviral resistance mutations. AIDS 12:2281–2288

Dao-Sheng L, Astumian R, Tsong T. Activation of Na and K Pumping Modes (Na, K)--ATPase by an Oscillating Electric Field. 1990; J. Biol. Chem. vol.265; pp. 7260-7267.

Dimitrov D, Sowers A. Membrane Electroporation - Fast Molecular Exchange by Electroosmosis. 1990; Biophys. Act; vol. 1022; pp. 381-392).

Engstrom PE, Persson BR, Brun A, Salford LG. A new antitumour treatment combining radiation and electric pulses. Anticancer Res. 2001 May-Jun;21(3B):1809-15.

Fedorowski A, Steciwko A, Rabczynski J. Low-frequency electromagnetic stimulation may lead to regression of Morris hepatoma in buffalo rats. J Altern Complement Med. 2004 Apr;10(2):251-60.

Filipic B, Kovacs K, Somogyvari F, Ihan A, Ocsovszky I, Koren S, Toth S. The effects of medium-strength electric impulses on human blood. Bioelectrochemistry. 2000 Sep;52(1):29-36.

Gallo RC, Salahuddin SZ, Popovic M, Shearer GM, Kaplan M, Haynes BF, Palker TJ, Redfield R, Oleske J, Safai B, White G, Foster P, Markham PD (1984) Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and risk for AIDS. Science 224:500–503

Gillibrand S, Speck M. Inactivation of Microorganisms by Electrohydraulic Shock. 1967a; Appl. Microbiol.; vol. 15(5); pp. 1038-1044.

Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA, Saxon A (1981) Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men. Evidence of a new acquired cellular immunodeficiency. N Engl J Med 305:1425–1431

Gray JR, Frith CH, Parker JD. In vivo enhancement of chemotherapy with static electric or magnetic fields. Bioelectromagnetics. 2000 Dec;21(8):575-83.

Hamilton W, Sale A. Effects of High Electric Fields on Microorganisms II. Mechanism and Action of the Lethal Effect. 1967; Biochem. Biophys. Acta. vol. 148; pp. 789-800.

Hernandez-Bule ML, Trillo MA et al. Nonthermal levels of electric currents applied in capacitive electric transfer therapy provokes partial cytotoxic effects in human neuroblastoma cultures. Neurocirugia (Astur). 2004 Aug;15(4):366-71; discussion 371.

Herzberg U, Murtaugh MP, Mullet MA, Beitz AJ. Neuroreport. Electrical stimulation of the sciatic nerve alters neuropeptide content and lymphocyte migration in the subcutaneous tissue of the rat hind paw. 1995 Sep 11;6(13):1773-7.

Hogg SR, O'Shaughnessy MV, Gataric N, Yip B, Craib K, Schechter MT, Montaner JSG (1997) Decline in deaths from AIDS due to new antiretroviral. Lancet 349:1294

Ito K, Wong L, Ando H et al. Pharmacodynamics induced by direct electric current for the treatment of 5-fluorouracil resistant tumor: an animal experiment. Int J Colorectal Dis. 2001 Sep;16(5):326-30.

Johnson MT, Waite LR, Nindl G. Noninvasive treatment of inflammation using electromagnetic fields: current and emerging therapeutic potential. Biomed Sci Instrum. 2004;40:469-74.

Kirson ED, Gurvich Z, Schneiderman R et al. Disruption of cancer cell replication by alternating electric fields. Cancer Res. 2004 May 1;64(9):3288-95.

Kojima J, Shinohara H, Ikariyama Y, Aizawa M, Nagaike K, Morioka S (1992) Electrically promoted protein production by mammalian cells cultured on the electrode surface. Biotechnol Bioeng 39:27–32

Kumagai E, Tominaga M and Harada S, Sensitivity of chronically HIV-1 infected HeLa cells to electrical stimulation. Appl Microbiol Biotechnol. 2004 Feb;63(6):754-8. Epub 2003 Aug 8.

Lechin F, Van der Dijs B, Lechin M. Neurocircuitry And Neuroautonomic Disorders – Reviews And Therapeutic Strategies. Karger. Switzerland. 2002. ISBN 3-8005-7413-4

Li H, Wang Z, Yue B et al. Study of high-intensity electric pulse inhibited sarcoma for improving antitumor drug effect. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi. 2003 Dec;20(4):612-4.

Mie M, Ohgushi H, Haruyama T, Kobatake E, Aizawa M (1996) Electrically enhanced osteogenic differentiation of rat bone marrow stromal stem cells. Cell Eng 1:153–158

Morizono K, Harada S (1998) Human immunodeficiency virus type 1 (HIV-1) infection and transcytosis activity of a HIV-1 susceptible clone from HeLa cells. Microbiol Immunol 42:313–320

Nakata A, Araki S, Tanigawa T, Sakurai S, Yokoyama M. Effects of uncontrollable and controllable electric shocks on T lymphocyte subpopulations in the peripheral blood, spleen, and thymus of rats. Neuroimmunomodulation. 1996 Nov-Dec;3(6):336-41.

Palella FJ, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD, HIV outpatient study investigators (1998) Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection.N Engl J Med 338:853–860

Pinero J, Lopez-Baena M, Ortiz T, Cortes F. Apoptotic and necrotic cell death are both induced by electroporation in HL60 human promyeloid leukaemia cells. Apoptosis. 1997;2(3):330-6.

Powell KT, Derrick EG, Weaver JC (1986) A quantitative theory of reversible electrical breakdown in bilayer membranes. Bioelectrochem Bioenerg 15:243–255

Rabussay DP, Nanda GS, Goldfarb PM. Enhancing the effectiveness of drug-based cancer therapy by electroporation (electropermeabilization). Technol Cancer Res Treat. 2002 Feb;1(1):71-82.

Rols MP, Bachaud JM, Giraud P et al. Electrochemotherapy of cutaneous metastases in malignant melanoma. Melanoma Res. 2000 Oct;10(5):468-74.

Sarmati L, Nicastri E, Parisi SG, d'Ettorre G, Mancino G, Narciso P, Vullo V, Andreoni M (2002) Discordance between genotypic and phenotypic drug resistance profiles in human immunodeficiency virus type 1 strains isolated from peripheral blood mononuclear cells. J Clin Microbiol 40:335–340

Sun CJ, Xie L. Antitumor effects of electrothermal and electrochemical therapy on mice with sarcoma180. Zhonghua Kou Qiang Yi Xue Za Zhi. 2003 Sep;38(5):351-4.

Tomaras GD, Lacey SF, McDanal CB, Ferrari G, Weinhold KJ, Greenberg ML. CD8+ T cell-mediated suppressive activity inhibits HIV-1 after virus entry with kinetics indicating effects on virus gene expression. Proc Natl Acad Sci USA. 2000 Mar 28;97(7):3503-8.

Tominaga M, Kumagai E, Harada S (2003) Effect of electrical stimulation on HIV-1-infected HeLa cells cultured on an electrode surface. Appl Microbiol Biotechnol 61:447–450

Tominaga M, Kumagai T, Takita S, Taniguchi I (1993) Effect of surface hydrophilicity of an indium oxide electrode on direct electron transfer of myoglobins. Chem Lett 1771–1774

UNAIDS. Report on the global HIV/AIDS epidemic, July 2002. Geneva: Joint United Nations Programme on HIV/AIDS, 2002: 226P.

USAID. HIV/AIDS, President's Emergency Plan for HIV/AIDS relief, fact sheets. http://www.usaid.gov/our_work/global_health/aids/pepfarfact.html (accessed June 15, 2004).

Vergne L, Bourgeois A, Mpoudi-Ngole E *et al.*, Biological and genetic characteristics of HIV infections in Cameroon reveals dual group M and O infections and a correlation between SI-inducing phenotype of the predominant CRF02_AG variant and disease stage. *Virology* **310** (2003), pp. 254–266.

WHO, UNAIDS. Treating 3 million by 2005: making it happen—the WHO strategy. Geneva: World Health Organization, 2003. Available at: http://www.who.int/3by5/publications/documents/en/3by5StrategyMakingItHappen.pdf (accessed June 7, 2004).

WHO. Prequalification project: procurement, quality and sourcing project—access to HIV/AIDS drugs and diagnostics of acceptable quality. http://mednet3.who.int/prequal/ (accessed June 7, 2004).

WHO. Scaling up antiretroviral therapy in resource-limited settings: treatment guidelines for a public health approach—2003 revision. Geneva: World Health Organization, 2003. Available at: http://www.who.int/hiv/pub/prev_care/en/arvrevision2003en.pdf (accessed June 7, 2004).

Yaoita M, Aizawa M, Ikariyama Y (1989) Electrically regulated cellular morphological and cytoskeletal change on an optically transparent electrode. Exp Cell Biol 57:43–51

Zimmerman U (1982) Electric field-mediated fusion and related electrical phenomena. Biochim Biophys Acta 694:227–277

Zimmerman U, Pilwat G, Riemann F (1974) Dielectric breakdown of cell membranes. Biophys J 14:881–899