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HIV: reactive oxygen species, enveloped viruses and hyperbaric oxygen

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Summary This paper demonstrates that there are many examples in the literature of contradictory data concerning reactive oxygen intermediates (ROIs), responsible for producing cellular oxidative stress (OS), and their enhancement or diminution of viral replication. Nevertheless, ROIs repeatedly have been shown to be virucidal against enveloped-viruses, like the human immunodeficiency virus (HIV). Hyperbaric oxygen therapy (HBOT) increases the production of ROIs throughout the body, leaving no safe harbor for the virus to hide outside the genome. This technique already has been tried on acquired immune deficiency syndrome (AIDS) patients, with exciting results. Historically, the biggest setback to demonstrating HBO's antiviral effects has been the investigator's folly of studying non-enveloped viruses or failing to initiate ROI production. ROIs specifically attack areas of unsaturation occurring in the polyunsaturated fatty acids of cell membranes and viral envelopes. Moreover, it consistently has been shown that a peroxidized viral envelope breaches, and a breached viral envelope causes viral disintegration. © 2000 Harcourt Publishers Ltd

INTRODUCTION

There is a very simple property which many viruses possess that medical science has yet to exploit: the viral envelope. Virologists in fact do not even classify viruses by this property. Many viruses known to cause human disease possess a lipid envelope. Table 1 is a partial listing.

The literature is amply supplied with examples showing that breaching the lipid bilayer of lipid-enveloped viruses leads to virus disintegration (1–7). Therefore it seems logical that research toward eradicative drug therapy should be directed at the lipid bilayer.

Indeed, Moore (1) and associated stated in 1992 that 'some viruses, and especially retroviruses such as HIV-1, may be extremely sensitive to agents that perturb the structure of their membranes and/or envelope glycoproteins'. They further suggested that 'antiviral drugs targeted at this stage of the virus life cycle should be evaluated routinely for their effect on virion integrity'.

Although in vitro agents do not usually lend themselves to the clinical picture, it has been shown that chlorine and iodine destroy HIV (8, *9*). But who would suggest that free halogens should be used as antiviral drugs? As far-fetched as that idea might sound, oxygen has many of the same properties as the halogens, and it is a normal cellular nutrient and oxidant.

Table 1 Selected enveloped viruses

1.	Animal immunodeficiency viruses: human (HIV), simian (SIV), feline (FIV), bovine (BIV), and murine (MIV);
2.	Herpes simplex virus, type 1 (HSV-1);
3.	Cytomegalovirus (CMV);
4.	Hepatitis B virus (HBV);
5.	Epstein–Barr virus (EBV);
6.	Influenza virus (IV);
7.	Pox viruses (e.g. Vaccinia, Variola, Molluscum contagiosum)

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In the early 1990s, experiments both in vitro and in vivo were conducted in Baltimore using oxygen under pressure. Oxygen under pressure is a *drug*. Hyperbaric oxygen, as it is termed, was found to be virucidal to HIV in vitro (10) and HIV viremia was found to drop to undetectable levels in vivo in a number of patients treated by 1999 (11, 12). Even a physician with AIDS has tried the technique on himself and found great relief from his chronic fatigue (13).

CELLULAR VS VIRAL OXIDATIVE STRESS: SCIENTIFIC PING-PONG

Most published papers to date seem to ignore the utility of attacking the lipid-bilayer of lipid-enveloped viruses with oxygen radicals, or otherwise, while focusing completely on the ravages of oxidative stress (OS) caused by HIV and other viral infections.

If one reads only papers dealing with the OS imposed on host cells during viral infections, the whole notion of using oxygen radicals as a defense against viruses sounds absurd. Still, point of view is the all-important criterion here. Most of the viruses purported to cause OS in the host are, coincidentally, lipid-enveloped viruses. This fact is most curious.

A little over 5 years ago, Greenspan (14) asserted that several prominent researchers were investigating the role of reactive oxygen and free radicals in the activation of latent HIV in infected individuals. He went on to say that there was a premise for the existence of OS as an important element in HIV progression. Nor was Greenspan alone in this opinion.

In 1995, Pace and Leaf (15) published a paper stating that evidence had accumulated suggesting that HIV-infected patients were under chronic OS as a results of perturbations in their antioxidant defense system. They further stated that OS has been observed in asymptomatic HIV-infected patients early in the course of the disease. Other authors also have suggested, along with Pace and Leaf, that OS contributes to increased viral replication or to increased viral transcription or to reactivation of latent infection (16–18).

This author maintains that viral infection does not cause OS; rather, the host's defense against the viral infection (or any infection) is the reason for the perceived viral-induced OS. There is just as compelling rationale for inducing OS as therapy against the infective virus itself.

For example, Polonis and Anderson (19) published a paper in 1991 that completely refutes OS. They found that *anoxia* induces HIV expression. Thus, it appears we are in the middle of a scientific ping-pong tournament.

Combating cellular oxidative stress

Much of the hoopla about OS causing an increase in viral infection has occurred because reactive oxygen intermediates (ROIs) activate nuclear factor κ B (NF κ B). Even low levels of ROIs appear to have this mitogenic effect on cells (20). Weiss (21) and associates demonstrated that N-acetyl-L-cysteine (NAC) not only removes ROIs but also inhibits hepatitis B virus (HBV) replication by a mechanism independent of the intracellular level of ROI.

Treatment of HBV-producing cell lines in vitro with NAC resulted in an at least 50-fold reduction of viral DNA in the tissue culture supernatant within 48 hours. Moreover, it was discovered that the decrease in viral DNA/virion production was caused by a disturbance in the virus assembly and not by a reduction of viral transcripts.

The results of another study, employing another lipid-enveloped virus, HIV, by Staal (22) and associates demonstrated that antioxidants regulate NF-κ B activation and signal transduction pathways leading to HIV expression. They also showed that NAC inhibits NF-κ B activation and HIV expression under conditions in which glutathione (GSH) is depleted and NAC cannot be converted into GSH, such as during OS. In addition to NAC, a variety of chemically unrelated antioxidants were also shown to inhibit NF-κ B activation and/or transcription directed by HIV long terminal repeats.

The above results, however, only demonstrate conditions for reactivation of infection by essentially 'opening up' the genome and allowing HIV to replicate. It is essential to remember that the whole point of this essay is show that OS kills *vegetative* virions.

Aillet (23) and associates advocated prudence in the design of antioxidant-based therapies aimed at suppressing HIV replication. They found in vitro that antioxidants like BHA (butylated hydroxyanisole) and NAC inhibited tumor necrosis factor (TNF)-induced NF-κ B activity in U1 cells, as well as the sustained NF-κ B activity permanently induced by the virus itself in chronically HIV-infected U937 cells. However, this resulted only in partial inhibition of TNF-induced HIV replication in U1 cells, and had no detectable effect on HIV replication in chronically infected U937 cells.

Another limitation Aillet and associates found was that antioxidant concentrations high enough to block NF-κ B activation were shown to have a suppressive effect on immune functions in vitro because they blocked interleukin (IL) 2-induced peripheral blood mononuclear cell (PBMC) proliferation.

Destroying enveloped viruses in vivo

Ironically, many authors seem to focus on singlet oxygen and oxygen radical damage to cell molecules as if the infective viruses are completely immune to them. Perhaps one reason is because it has been demonstrated that sublethal doses of singlet oxygen ($^1O_2$) are enough to

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reactivate HIV-1 latently infected promonocytes or lymphocytes in vitro (17).

The best way to stop an infection is to *kill* the infective agent, not just suppress it. Granulocytes protect their host by employing an arsenal of oxidative, enzymatic, and polypeptide (defensin) weapons (24, 25). Curiously, in the latter case, defensins have been shown to inactivate only lipid-enveloped viruses (26).

For every article condemning oxidative stress, there is probably at least one article extolling the antiviral effects of oxidants like singlet oxygen, and superoxide and hydroxyl free radicals, on lipid-enveloped viruses; with singlet oxygen getting the most publicity (27–31).

Singlet oxygen ($^1O_2$) has a completely empty electron orbital and preferentially attacks areas of high electron density. Double bonds are areas of high electron density (24), hence, the propensity of singlet oxygen to attack the polyunsaturated fatty acids contained in cell membranes and viral envelopes.

At 37°C, the efficiency of $^1O_2$ in inactivating enveloped viruses has been shown to reach 8 $\log_{10}$ virions/ml ($TCID_{50}$)(27). Specifically, $^1O_2$ has been shown to inactivate enveloped viruses of particular current medical interest, such as HIV-1, herpes simplex virus type 1 (HSV-1), and cytomegalovirus (CMV)(29).

Oxygen radicals also have been shown to oxidize proteins, DNA, and RNA under hyperbaric conditions (32, 33). Pressure is not a requirement. It would, however, increase the number of oxidative species over that expected at sea level or 1 atmosphere, absolute (1 ATA). With regard to RNA viruses, Gendimenico and Haugaard (33) have shown that hyperbaric oxygen had adverse effects on both uridine incorporation and uridine kinase activity in rat neuroblastoma cells. Therefore, one could extrapolate that such effects would adversely affect the replication of any RNA viruses present in cells under such adverse conditions.

It almost seems that virologists are afraid to study hyperbaric oxygen enhancement of the effects of reactive oxygen and nitrogen species on the enveloped viruses. This is said in light of the fact that the western literature is almost devoid of research in this area. Moreover, when there is a paper published, the virus selected for study invariably is not enveloped and HBO is shown to have no effect – a predictable outcome based on current information. On theoretical grounds alone, research into the effects of HBO on lipid-enveloped viruses seems to scream for attention.

In 1997, Howell (34) and associates determined (again in vitro) that HIV-infected monocytes and macrophages produced less superoxide free radical than uninfected cells from the same donor after artificial stimulation of the cells to undergo respiratory burst. Their guarded conclusion was to suggest that in some cases impairment of ROI production might contribute to the pathogenesis of HIV infection, as was also shown by Polonis and Anderson (19) with anoxia in HIV-infected T-cells. This conclusion is in direct opposition to the proponents of oxidative stress, who constantly publish papers showing that OS increases HIV replication.

The influenza virus paradox and hepatitis B virus

Influenza virus (IV), another lipid-enveloped virus, appears not to have been the subject of any hyperbaric oxygen experiments. Furthermore, it has been shown to flourish in an oxidative environment, at least initially. Peterhans (20) has described how IVs interact with the phagocyte cell membrane to activate respiratory burst. Theoretically, respiratory burst should be unhealthy for *all* lipid-enveloped viruses. What is happening with IV? Perhaps the answer lies in the type of lipids in the virion envelope; that is, cholesterol-squalene, for example, versus cholesterol-polyunsaturated phospholipids (?).

With all the enveloped viruses to choose among, it is an incredible fact that there apparently are very few studies of the effects of HBO on lipid-enveloped viruses published in the USA, Canada, or the UK.

Unlike in the English-speaking world, the use of hyperbaric chambers is more or less standard practice in eastern countries like Russia. Additionally, hyperbaric medicine is a highly developed medical specialty in Cuba (35). A literature search did reveal a number of Russian citations, which inconveniently lacked an abstract. However, the work of Gabrilovich (36) and associates did have an English abstract.

They found that 8–10 sessions of HBO at 1.5 ATA and 45 minutes' duration produced favorable effects in patients suffering from HBV infection if used during the early stages of the infection (first week of hospitalization). Yet, when HBO was used later in the course of the infection (4–5 weeks) no well-defined clinical effect was observed. The stated effect was a significant reduction in the rate of exacerbations and residual phenomena, plus a reduction in the number of T- and B-lymphocytes by the- 10th treatment. They stated additionally that leukocyte activity rose during the use of HBO.

The authors considered HBO treatment in HBV infection to be most indicated in prophylaxis and during the acute infection. Very likely the reduction in viremia (not discussed in the abstract) leads to a reduction in the production of antibody against HBV, and thus to favorable outcomes concerning 'residual phenomena'.

Cuban physicians (35) also are using HBO in conjunction with cases of HBV infection.

HISTORIC VIROLOGIC FOLLY

In 1877, a French physician and physiologist named Paul Bert published the results of his extensive study of the effect of a hyperbaric hyperoxic environment on the

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viability of various animals, bacteria and one virus (37). As his study subject, Bert picked the pox virus, variola.

Bert subjected the virus-containing fluid from the pustules of an infected child to 23 atmospheres (23 ATM) of pure oxygen for 7 days and then vaccinated newly born babies. Pustules subsequently formed on the children, indicating that hyperbaric oxygen had no effect.

Variola (smallpox) and vaccinia (from Jenner's original cowpox isolate?) are members of the Poxviridae family, which also are characterized as enveloped viruses. Moss (38) describes vaccinia's envelope as being composed of 5% lipid composed of cholesterol and phospholipid. Five percent lipid seems quite low; perhaps low enough to prevent breaching of the viral envelope (?). Contrast this 5% lipid to influenza virus at 20% lipid derived from the host cell membrane (39) and to fowlpox at about 33% lipid composed of squalene and cholesterol (35).*

Because of the very high partial pressure of oxygen used in Bert's experiment (24 ATA), and the extreme time at pressure (7 days), it is compelling to conjecture that a low lipid content in the virion envelope, or possibly a lack of any transition metal ions in the incubation medium, could account for the long-term survival of the pox virus. But who knows? The experiment apparently has not been repeated in modern times.

Orsi (40) and associates found enhancement of coxsackievirus infection in mice subjected to hyperbaric oxygen. Mice were subjected to hyperbaric oxygen either before or after infection and manifested increased mortality because of the treatment. The authors indicated that they chose coxsackievirus because it has ubiquitous human distribution and predictable decreasing sensitivity in mice with increasing age. In retrospect, coxsackievirus was a very poor choice for a potentially great discovery, since this is a nonenveloped virus.

Gottlieb (41) (circa 1971) confessed that no systematic studies have been performed on the direct in vitro and in vivo effects of HBO on viruses. This is still true in 1999. In his paper, Gottlieb discussed the results of numerous studies, but only on species viewable by the light microscope.

In his brief review of viruses and HBO, Gottlieb does mention one exciting bit of data. White mice had been afforded protection against lymphocytic choriomeningitis and acute human disseminated encephalomyelitis by HBO. Five to 10 hours at 2 ATA was maximal time of exposure to protect mice against both neurotropic viral infections. According to *Field's Virology*, the former virus has a lipid envelope. The author could not identify the second neurotropic virus by the given name. However, it may be measles, an enveloped virus of the Paramyxoviridae family.

COMBATING AND CAUSING OXIDATIVE STRESS

Host cell antioxidants vs a viral antioxidant

In normal animal physiology, the deleterious (oxidative) properties of molecular oxygen are controlled by such antioxidant biochemicals as catalase, peroxidases (e.g. glutathione peroxidases), zinc-copper and manganese superoxide dismutases, ascorbic acid, glutathione, cysteine, vitamin E, vitamin A, beta-carotene, and uric acid (24). Without these defenses, cells are severely damaged, even killed by oxygen and nitrogen radicals (42–46).

It seems logical to assume that lipid-enveloped viruses obtain any antioxidant protection that they may possess from the host's cell membrane (fat-soluble antioxidants) and from trapped cytoplasm (water-soluble antioxidants) during budding from the host cell. Otherwise, it has been assumed that viruses do not produce their own antioxidants.

Unfortunately, from the standpoint of human antiviral strategies, some viruses do produce an antioxidant. McFadden (47) recently reviewed virus counter strategies to host cell anti-virus mechanisms. Of the various viral mechanisms of counter strategy known, the discovery that the molluscum contagiosum virus (MCV) synthesizes an antioxidant selenoprotein is shocking news. This selenoprotein, dubbed MCO66L, is the first discovery of such a protein being expressed by a virus. Moreover, MC066L is 74% identical to glutathione peroxidase (GP_x).

Hyperbaric oxidative therapy

By far the greatest utility in using HBO is to set up a situation of oxidative stress throughout the entire body by producing a host of different oxidative species independent of the activation of leukocytes or production of cytokines by lymphocytes. That the number of hyperbaric chambers in the USA, for example, is vastly limited in comparison to the potential patient population is the subject of another article, and would certainly entertain some novel engineering feats.

From the beginning of the AIDS pandemic through today's current state-of-the-art chemotherapy, we continue to aim for a balance between protecting the host and killing the pathogen or cancerous cell. This same philosophy was suggested in the previous decade by Yarchoan (48) and associates, who stated: 'One must … always consider the balance between harm to the pathogen and harm to the host … For a life-threatening illness such as AIDS, one may have to accept drugs with a lower therapeutic index, at least in the beginning'.

* Vaccinia virus is infectious either with or without its envelope and thus the Poxviridae may possess an envelope artifactually. At any rate, the envelope is not integral in its survival.

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Even today, we are still dealing with drugs with a low therapeutic index in the fight to stop the spread of HIV from infected cells to uninfected cells. The best way to accomplish this is simply (in theory, anyway) to kill/deactivate the virus, a feat already accomplished in vitro in blood banks.

The literature already contains some data showing that hyperbaric oxygen therapy (HBOT) has had nullifying effects on AIDS-related complications and virucidal effects upon HIV, both in vitro and in vivo (10–13, 49, 50). This author feels that the data contained in the literature overwhelmingly support a continuation of research in this area. Moreover, the theoretical rationale for why we should pursue the use of HBO to treat lipid-enveloped viral infections is staggeringly explicit.

We need to determine at what oxygen partial pressure and time at pressure viral lipid membrane peroxidation becomes lethal to the virus. Reaching deep into body compartments with slow rates of perfusion and/or diffusion will take a much longer time than that required for a thin layer of cells on a petri dish, hence the need for clinical data in this area.

Oxidative host defense biochemistry

Table 2 gives the specific chemical reactions involved in generating hydrogen peroxide, hydroperoxides, superoxide and hydroxyl free radicals, singlet oxygen, and the peroxynitrite free radical. All reactions in Table 2 can be produced within activated granulocytic leukocytes (24, 51). Moreover, granulocytes are not unique in this capacity. Sperm cells, oocytes, fibroblasts and many other nonphagocytic cells produce ROI species when activated to do so (52).

Note that in Reaction 4 (Table 2) both singlet oxygen ($^1O_2$) and hydroxyl free radical (HO•) are formed. HBOT is expected to increase the number of all these oxidative species, but Reaction 4 is perhaps the most pivotal of all. Not only does Reaction 4 produce two highly reactive oxygen derivatives; it requires the presence of a transition metal (Fenton chemistry), such as iron or copper, to do so.

Reillo (11, 12) inadvertently used Ondrox™ tablets as a pretreatment for her patients undergoing HBOT to 'protect' their cells from oxidative damage caused by the treatment. The significance of this is that Ondrox tablets are a timed-release formulation of vitamins and minerals. Specifically, Ondrox time-releases iron, copper, and a small amount of vitamin C. Iron and copper – in the presence of vitamin C – increase the formation of oxygen species at just 1 ATA (53). At pressure, the slow steady absorption of these formula ingredients was no doubt beneficial to the success of Reillo's protocol by producing more oxidative species in the blood and tissues with which to attack HIV and possibly to deactivate tumor necrosis factor (TNF) and other catabolic species involved in cachexia.

Table 2 Normal oxidative reactions increased by hyperbaria

1.	Formation of hydrogen peroxide, a byproduct of all oxidase reactions: O_2 + partial (1/2) reduction → HOOH (H_2O_2)
2.	Symmetrical or asymmetrical chemical decomposition of H_2O_2: HOOH → HO• + •OH or HOO•
3.	Formation of superoxide free radical, also a byproduct of oxidase reactions: O_2 + partial (1/4) reduction → $•O_2$
4.	The Haber–Weiss reaction, typically with either iron or copper: $•O_2 + H_2O_2$ + transition metal → $^1O_2 + {}^{-}{:}OH + HO•$
5.	The myeloperoxidase (MPO) reaction: $ClO^- + H_2O_2 + MPO \rightarrow {}^1O_2 + Cl^- + H_2O$
6.	Formation of nitric oxide: O_2 + arginine → 2 NO•
7.	Formation of peroxynitrite: NO• + $•O_2$ → ONOO•

On the other hand, Jordan (50) believes that HBO acts to slow the uncontrolled activation of monocytes that leads to the overproduction of cytokines. In discussing his results, Jordan cites 5 pro and 3 con reasons for using/not using HBOT. The first reason he gives against HBO is that it is too expensive and not cost-effective. The second reason Jordan gives against using HBO it that it may potentiate underlying Kaposi's sarcoma. This is a valid concern because HBOT could feed the sarcoma. Additionally, HBO will hyperoxygenate the first couple of millimeters of skin in a one-man (monoplace) chamber. This author's counter argument is that if HIV is killed, perhaps Kaposi's sarcoma will undergo spontaneous remission due to lack of the virus that is stimulating the cancer in the first place. Reillo (12) (p. 44) states that Kaposi's sarcoma 'responds to HBOT and alpha-interferon'.

DISCUSSION AND CONCLUSION

The idea of using HBO on AIDS patients started among a small group of scientists and clinicians. Jordan (50) credits Bocci (54) as being first to suggest the use of HBO on AIDS patients in 1987. However, this author is quite sure that others were contemplating its use around that same time. For example, Baugh first thought of using oxygen radicals against HIV when he read in a review by Badwey and Karnovsky (55) that ROI are also antiviral. This information was used subsequently during a lecture he delivered to the American Chemical Society in 1987 (56), and mentioned again in two subsequent published articles on oxygen toxicity (24, 57).

Reillo was not far behind Bocci and Baugh, and she appears to be among the first clinicians involved in the

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use of HBO on human HIV-infected subjects in 1990. She also was among the first to demonstrate the effect of HBO on HIV in vitro.

Reillo's book, *AIDS Under Pressure* (12), was reviewed recently by the Undersea and Hyperbaric Medical Society in *Pressure* (58). It is agreed with the reviewer that this book suffers from irreconcilable flaws. Nonetheless, historically, kernels of truth have been found within some of the most outlandish theories or publications. Even the absurdity of alchemy has, in modern times, been shown to be possible in an atomic accelerator.

This paper has presented substantial references to show that properly controlled and designed experiments to show the effects of HBO on enveloped viruses, particularly HIV, are well worth our time, effort, and budgetary considerations.

This review concludes with a recent proclamation. Although it was said more like a whisper than a bellow, Sandstorm (59) and associates, in discussing how antioxidant defenses influence HIV-1 replication and associated cytopathic effects, stated that '*these results indicate that exposing HIV-1-infected cells to moderate oxidative stress may provide a novel therapeutic approach in the treatment of AIDS*'.

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