



Review

Substance Abuse and HIV-gp120: Are Opiates Protective?

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Abstract. There has long been a popular conceptual linkage between human immunodeficiency virus (HIV) acquisition and substance abuse involving “needles”. Indeed, *in vitro* studies demonstrate that these substances promote the replication of HIV. Included in these *in vitro* studies is a linkage or association of tissue damage and viral load with the actions HIV envelope protein gp120 with substances of abuse. However, detailed epidemiological studies have not supported this association of substance abuse and HIV acquisition, viral load and exacerbated tissue damage. It is with this understanding that we undertake a reevaluation of the *in vitro* studies within the context of the microvascular immune environment. In this regard, a counter-intuitive hypothesis emerges, namely, that specific substances of abuse may afford a degree of protection from HIV infection. This new hypothesis involves the neural, immune, and vascular signaling molecule nitric oxide.

Key words: gp120; morphine; monocytes; HIV; immunovascular regulation; nitric oxide.

Immunocyte-Endothelium Interactions

The interaction of leukocytes with the vascular endothelium is a critical component in immune activation^{3, 52, 61, 80, 81}. The interplay of the components and their interaction is crucial to the health of the underlying tissues. Communication among the different cells in the vascular microenvironment (those which form the vasculature and those of the immune system) constitutes a local regulatory process, referred to as autoimmuno-vascular regulation (AIVR)⁸¹. Normally, the interaction among these cells occur in stages involving a progressive change in the synthesis and release of adherence molecules^{3, 52, 61, 80, 81}. For example, immunocyte rolling involves the selectin family of adhesion molecules whereas transendothelial migration involves PECAM-1⁸¹.

Morphine, Anandamide and gp120 Signaling in the Microvascular Environment

Given the significance of AIVR and the role of opioid and opiate signaling molecules in immune function⁹¹, the actions of the same signaling molecules was investigated in vascular tissues. In examining this microvascular environment for opioid and opiate involvement several novel actions were found. For one, the response of the cells of the vascular microenvironment depends on the length of exposure: chronic exposure to opiates such as morphine enhances monocyte adherence to endothelium and the cells exhibit chemokinesis whereas acute exposure results in a lack of monocyte adherence^{5, 86, 88}. In this regard, acute exposure of human saphenous vein or internal thoracic artery endothelium to either morphine or anandamide, an

Abbreviations used: HIV – human immunodeficiency virus, No – nitric oxide, NOS – nitric oxide synthase, AIVR – autoimmuno-vascular regulation, LPS – lipopolysaccharide, IFN- γ – interferon γ .

endogenous cannabinoid, results in nitric oxide (NO) release whereas exposure to the human immunodeficiency virus envelop protein gp120 does not⁸⁶. Following the acute exposure of the vessel endothelium monocyte adherence is diminished with morphine and anandamide treatment (jointly by – 80%) whereas it is enhanced with that of gp120 (approximately 40%), indicating that gp120 enhances the ability of the endothelium to adhere monocytes⁸⁶. Chronic or continuous exposure of the endothelia to all agents results in a significant enhancement of monocyte adherence ($p < 0.05$) which is further increased when exposed to either morphine and anandamide plus gp120. This appears to be due to a desensitization of the endothelium to further NO release after the initial exposure to either anandamide or morphine⁸⁶. The results, at first glance, indicate that in individuals abusing opiates and/or cannabinoids a tissue, i. e., CNS, viral load may be higher, and AIDS may progress more rapidly since monocyte adherence and mobility is significantly increased, suggesting a higher level of transmembrane migration.

In another report we demonstrate that immediate exposure to gp120 (5 min; 0.1 $\mu\text{g/ml}$) results in a significant shift of the macrophage population to an amoeboid and motile category and prior exposure with anti-gp120 antagonizes this shift⁸⁸. Acute exposure of the macrophages to morphine (10^{-6} M) or anandamide (10^{-6} M) resulted in the cells rounding up and becoming non-motile. The action is blocked by prior treatment with the specific antagonists naloxone and SR 141716A, respectively. Chronic exposure (6 h) of the cells to all three agents resulted in a random migration pattern⁸⁸. Further, all agents blocked chemotaxis induced by DAMA and IL-1. Observation of the cells behavior during chronic exposure revealed a sporadic activity pattern with gp120 whereas morphine and anandamide first induced a period of inactivity which is followed by a period of activity (chemokinesis)⁸⁸. Recent work from our laboratory has demonstrated that both morphine and anandamide acutely stimulate constitutive macrophage NO release^{56, 85}, which then induces macrophage rounding and inactivity. It was therefore of interest to examine their behavior by exposing macrophages to the NO-donor SNAP (S-nitroso-N-acetyl-DL-penicillamine). In a concentration dependent manner SNAP exhibited the same behavioral actions as both substances of abuse⁸⁸. Given this, we next determined if macrophages exposed to gp120 would release NO⁸⁸. We demonstrated that NO was released only when exposed to morphine and anandamide not gp120. Thus, this study also suggests that because of the chemokinetic inducing activities of these

agents a higher viral load in various organ systems should be found since immunocyte adherence and random migration are stimulated.

Nitric Oxide and the Microvascular Environment

Clearly, the recent work just referenced implicates NO in substance abuse actions. NO downregulates immunocyte adherence and the ability of the endothelia to attract leukocytes^{81, 91}. NO is involved in the regulation of immunocyte adherence in several different tissues^{2, 42–46, 53, 73, 78, 93} and appears to inhibit adhesion molecule expression^{18, 19, 44, 66}. Experiments have also indicated that NO represses VCAM-1 gene transcription by inhibiting NF- κ B¹⁸.

Regarding a link between opiates and cannabinoids and NO recent reports indicate that morphine analgesia, tolerance and dependence appear to involve NO production^{4, 16, 24, 26, 41, 57, 96, 97, 99} as does suppression of splenic lymphocyte proliferation²⁵, gastrointestinal regulation³⁴ and food intake¹². In the vascular system, opiates and NO are coupled via a novel μ 3 opiate receptor subtype, first isolated in our laboratory⁸³. This receptor is opiate alkaloid selective and opioid peptide insensitive. It is found on human monocytes, granulocytes, endothelial cells and various other tissues from different animals^{21, 58, 83, 91}. The receptor is coupled to NO release in monocytes and granulocytes and in saphenous vein and internal thoracic artery endothelial preparations^{5, 50, 55, 85}. In human endothelia, NO release leads to vasodilation and the inhibition of monocyte adherence^{5, 84}. We have also demonstrated that the morphine-stimulated release of NO is antagonized by naloxone and nitric oxide synthase (NOS) inhibition^{5, 84, 91} and is specific for μ 3 agonists since opioid peptides do not induce NO production in these tissues. The effects of cannabinoids, such as anandamide, occur via separate receptors and exerts the same actions⁸⁵.

Having established the linkage between substances of abuse and NO we now must differentiate the origin of NO. In another recent study we demonstrated that constitutive NO synthase (cNOS) can regulate inducible (i)NOS⁸⁷. This is quite important since cNOS appears to be very important regulator of the microvascular environment in both endothelial cells and immunocytes. Additionally, constitutive NO release is associated with positive biomedical phenomena, whereas iNOS associated NO release with detrimental consequences in regard to endothelial inflammatory activities⁸⁷. As yet, an important link demonstrating why one is activated over the other is not available. Activation of human endothelial cells, obtained from the sa-

phenous vein, with morphine or anandamide stimulated NO release (35 nM and 28 nM, respectively) that peaked within 5 min and returned to basal levels within 10 min of agonist stimulation, consistent with cNOS activation. Significant release of NO from endothelial cells stimulated with lipopolysaccharide (LPS) and interferon γ (IFN- γ) occurred after 2.6 h post-exposure and remained significantly elevated over basal levels for 24–48 h (28 nM), consistent with iNOS activation. Preincubation of endothelial cells with morphine or anandamide prior to, but not after, the addition of LPS+IFN, blocked iNOS activity. Exposure of endothelial cells to the NO donor, SNAP, prior to the addition of LPS+IFN, blocked iNOS induction, whereas preincubation of endothelial cells with inhibitors of NOS, prior to morphine or anandamide exposure, restored LPS+IFN induction of iNOS, suggesting a direct impact of NO on the regulation of iNOS activity. Morphine and anandamide stimulation of endothelial cells did not stimulate cAMP accumulation, whereas a marked increase in cAMP was observed in endothelial cells treated with LPS+IFN (8.2 to 33 pmol/mg protein). Treatment of endothelial cells with LPS+IFN did not induce cAMP accumulation in endothelial cells treated with morphine, anandamide or SNAP prior to LPS+IFN exposure. These data suggest that cAMP is required for the induction of iNOS in endothelial cells and that NO may directly impair adenylate cyclase activity, preventing iNOS activation⁸⁷. One can also surmise that iNOS activation would be sufficient to halt the spread of HIV in the body, given its strong and lasting actions.

Substance Abuse and HIV

Opiate abuse, which is often accompanied by the sharing of needles, has also been linked to susceptibility to HIV. At first glance, this association appears to be almost a given since opiates have been shown to limit or diminish immune capabilities⁹¹, theoretically making the host more vulnerable to HIV infection. *In vitro* studies have shown that opiates enhance viral replication, thus supporting this concept^{15, 68, 69, 75}. Despite these studies and others noted earlier, however, epidemiology research does not support such a linkage²². This problem is made more perplexing given the data generated from our own laboratory. We surmise that once HIV is present in an individual abusing opiates, for example, the infection may progress more rapidly due to the combined immunoinhibitory effects of opiates and HIV, especially in the presence of its envelope protein gp120.

In examining the HIV-gp120 reports we find much evidence for tissue damage by these substances. Associated with AIDS are various neurological and neuropsychiatric deficits including progressive dementia^{39, 74}. HIV may gain entrance into the brain via infected macrophages^{39, 74} and then negatively affect neurons via binding of gp120 (CD4)^{39, 74}. Thus, the interaction of monocytes with the vascular endothelium is important since these cells must interact before the cells can have access to the brain. Briefly, once HIV is in the CNS, gp120 can damage neurons without cellular penetration by the virus⁸. Two different gp120 isolates and a recombinant gp120 have been shown to produce neuronal death⁷. Glycoprotein 120 can act *in vitro*, that is in the absence of CD4; in rat retinal ganglion cells in culture the effects of gp120 can be abrogated by antibodies to gp120 but not those directed against CD4³⁸. HAROUSE et al.³⁵ found that in two of these CD4-negative cell lines antibodies against galactosyl-ceramide (Galc) were able to inhibit HIV infection, suggesting that Galc may also play a part in viral entry. Thus, evidence exists that demonstrates that we do not know all the gp120 “target” sites.

It is important to note that AIDS-associated neurological disorders^{37, 40, 98} include pain, headaches, fever, sensory and motor dysfunction as well as cognitive changes³¹. The involvement of pain suggests dysfunction of an opioid mechanism that must be explored. Furthermore, HIV entry into the brain is accompanied by a rise in cytokine-levels^{28, 29, 33, 39, 47, 62, 63, 74}. Recent studies suggest that HIV/gp120 primes macrophages^{32, 92} via the production of potential immunostimulatory toxins from the hosts immunocytes (i. e., cytokines, eicosanoids, quinolate and NO)³². Further, gp120 inhibits macrophage chemotaxis⁷¹ and promotes chemokinesis as does morphine⁹². Glycoprotein 120 up-regulates intercellular adhesion molecule-1 (ICAM-1) expression in glial cells⁷⁷ and neuroadhesiveness of monocytoid lines⁶. This inhibition of chemotaxis can help contribute to the incidence of opportunistic infections in AIDS (i. e., cryptococcal meningitis)^{13, 14, 64}.

The relationship of opiate substance abuse to HIV progression is more circumstantial, in part, due to the general lack of studies designed to examine this question. Morphine is clearly immunoinhibitory⁹¹. We have demonstrated that the effects of morphine and anandamide, a cannabinoid ligand, on the vascular system are coupled to NO release^{5, 82, 89, 91} which could explain many of their immune inhibitory actions^{10, 11, 20, 30, 51, 60, 79, 85, 94, 100}.

Others have reported that gp120, like morphine, will induce the release of NO and through this mechanism

directly cause tissue damage^{17, 48, 49, 65, 70, 92}. In these particular studies, the NO measurements were performed 15 h to several days following gp120 exposure. However, we have demonstrated that gp120, unlike morphine, does not directly stimulate NO release^{86, 88}. We surmise that gp120 actually induces a cytokine by its stimulatory signaling cascade, i. e., IL-10 (STEFANO, unpublished), which in turn releases NO⁸². Recently we demonstrated that gp120 enhances the adherence of monocytes to human saphenous vein endothelium⁵⁹. Since NO inhibits adhesion molecule expression gp120 cannot stimulate its release as surmised by others. Furthermore, we demonstrated⁵⁹ that chronic endothelial exposure to opiates also enhances monocyte adhesion, a phenomenon that is further increased if these agents are co-administered with gp120. Thus, these are the first studies examining the joint actions of opiates and gp120.

Clearly, these studies, and those by others, strongly suggest that in individuals abusing opiate alkaloids, HIV infection may progress more rapidly resulting in higher gp120 levels; the increased gp120 could initiate more tissue damage. Thus, gp120 combined with an opiate presence, should profoundly influence the behavior of macrophages to promote AIDS associated pathologies. However, this conclusion is not supported by strong epidemiological studies that also examined various immune parameters^{22, 23}.

Nitric Oxide and Viruses

Regarding NO and viruses in general, mice deficient in iNOS were susceptible to herpes simplex virus-1 infection and they also exhibited a delayed clearance of the virus from dorsal root ganglia⁵⁴. Furthermore, macrophage NO is implicated in resistance to a number of viruses, including ectromelia virus and vaccinia virus⁷². Interferon- γ -induced production of NO also inhibited growth of murine hepatitis virus type 3 in a murine macrophage cell line (RAW 264.7) an action also found with the NO donor SNAP⁷². NO has also been implicated in an antihepatitis C infection⁷⁶. In support of these observations the antiretroviral agent (R)-9-(2-phosphonomethoxypropyl) adenine stimulates cytokine and NO production¹⁰¹. In HIV-1-associated dementia iNOS levels were elevated and coincided with increased expression of the HIV-1 coat protein gp41 that has been shown induce iNOS in primary cultures of mixed rat neuronal and¹. BUKRINSKY et al.⁹ found NO expressed in HIV-1-infected monocyte cultures. In another report HERMANN et al.³⁶ found that infection of human monocyte-derived macrophages with HIV-1 did

not seem to induce detectable NO release or iNOS mRNA accumulation.

This conflict may be better understood within the framework of experimental design. For example, superoxide (O_2^-) and NO metabolites may also cause harm to the host besides exerting their antimicrobial and antiviral actions. As also noted above low levels of these agents and their metabolites can also facilitate viral replication because of their mitogenic effects on cells⁶⁷. Additionally, most viruses stimulate their host cells since they grow better in proliferating cells^{67, 95}. Indeed, we have demonstrated that gp120 may exert such a stimulatory influence in diverse cells, suggesting that its immune and vascular cell activation is intentional and comprises an important step in the infection process^{86, 88, 92}. Furthermore, universal mechanisms in this regard probably are not found and variation does exist. Additionally, these studies did not measure or take cNOS stimulated NO in consideration. However, there is general agreement on the point that NO may exert antiviral actions on particular viruses.

Potential Counter-Intuitive Protective Action of Morphine

Clearly, from the above noted in vitro reports as well as those coming from our laboratory it would appear that HIV infection and viral load with associated pathologies in substances abusers should be great. However, this is not the case^{22, 23}. Therefore, the answer to this interesting riddle may lie in the general biological actions of these substances, i. e., morphine. In this regard, we must realize that morphine is a naturally occurring signal substance⁹¹. Additionally, it selectively can increase NO levels in monocytes⁵⁶ and endothelial cells⁸⁴ by stimulating cNOS, a process requiring intracellular calcium²⁷. Taken together, cNOS stimulation may represent an antiviral event. This may be ascertained from examining immune and endothelial cells following morphine or NO-donor exposure. In either case, the cells become round and non-mobile as well as difficult to stimulate^{56, 83, 84, 91}, a condition not favoring viral replication. However, after prolonged opiate exposure these same cells become hyper-activated, resulting from a simple rebound effect following their inhibition^{86, 88}. Thus, in this scenario the initial opiate exposure would down regulate the cells while simultaneously producing antiviral NO. Thus, morphine or cannabinoid substance abuse may exert a protective action against HIV infection initially upon specific cellular exposure.

Indeed, if this is the case, opiate addicts would have

a lower incidence of HIV as well as a lower viral load with associated pathologies. This too is not the case^{22, 23}. Here, what may be now operating is the actions of chronic opiate exposure. This will include enhanced immunocyte activation, adherence and enhanced endothelial activation as well. These have all been documented to occur upon chronic opiate exposure to these tissues^{81, 86, 88}. According to our reports, this effect would result from a temporary inhibition of iNOS activity as noted elsewhere and under the regulation of cNOS stimulation⁸⁷. Thus, in examining the immune and endothelial actions of acute and chronic opiate exposure we appear to have actions that support viral infection and those that would inhibit it. This balance between the two processes may ultimately yield data that would contradict the superficial view that substance abusers, i. e., opiate, may have higher viral loads. Additionally, it must be noted that many of the studies examining the actions of substances of abuse on HIV replication were examined during chronic opiate exposure. Very few studies, probably, none, have examined this process for acute actions. Thus, our observations regarding opiate actions appear to be novel, however, they just represent early events. Furthermore, these early events appear to be responsible, as would be expected, for opiate and cannabinoid chronic actions.

In the scenario just examined there also exists a process that would keep this "cycle" of events going, that is, the alternating periods of HIV resistance and susceptibility associated with substance abuse and HIV-coupling. This process is tolerance. In the past tolerance has been defined by our group as a naturally occurring process that deactivates naturally occurring morphine presence in a microenvironment, since its half life can be in hours⁹⁰. Here, the cycle of HIV resistance and susceptibility associated with substance abuse would continue since addicts must take more opiate to get the desired action over time. As a consequence of this phenomenon, the results of acute (immediate bolus) and chronic opiate exposure would continue. We can also surmise from gp120 actions, in stimulating vascular endothelial cells and various immunocytes^{86, 88, 92}, that it resembles chronic opiate and anandamide exposure. This may serve to indicate that without the immediate bolus of new drug introduced into ones body, both chronic opiate exposure and immediate gp120 actions may be enough to activate specific cells so that viral replication may occur unimpeded. However, once the injected material enters the body and because of tolerance, the new material initiates the antiviral actions of cNOS stimulation⁸⁷.

In conclusion, it is important to note that this argument at the present time is in part speculation that is based on existing data. Other scenarios are probably at work as well and in this regard other interpretations of the data may also be valid. However, for the moment it does offer a novel explanation of the discrepancy between *in vitro* reports and those examining broader *in situ* findings. This new hypothesis takes advantage of the new acute opiate exposure experiments verses the old chronic exposure experiments, often occurring over hours. As such, it may provide the conceptual framework for future studies into AIDS and substance abuse.

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