Morphine Enhances the Permeability Across Vascular Endothelial Cell Barriers

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Morphine Enhances the Permeability across Vascular Endothelial Cell Barriers

by

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Submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology from the Department of Biology of Seton Hall University

May, 2001
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Abstract

Using three types of in vitro endothelial cell barrier models, we investigated the direct effects and mechanisms of morphine on vascular permeability. Our current studies illustrate LPS induces permeability across these VEC barriers, and is significantly enhanced when co-treated with morphine, displayed using the $[^{14}\text{C}]$-inulin paracellular marker. Incubation with morphine alone induces permeability in a concentration-dependent manner, and is not blocked by the addition of naloxone. Morphine enhances the detrimental effects of LPS on cell viability and alone also decreases endothelial cell viability, concentration-dependently, which is also not affected by naloxone, as demonstrated by the trypan blue exclusion assay. Using 4',6-diamidino-2-phenylindole (DAPI) nuclear stain, we reveal LPS- and morphine-induced cell death is through an apoptotic mechanism. Morphine-induced apoptosis is concentration-dependent and not blocked by naloxone once again, and cotreatment with LPS synergizes to induce enhanced apoptosis. Morphine has been shown to induce Fas-mediated apoptosis in immune cells (Yin et al., 1999); however, our VEC models demonstrate a Fas-independent apoptotic mechanism. Lastly, we confirm that pretreatment of the monolayer with morphine enhances viral penetration, using The Amplicor® Viral RNA Detection Assay. These results suggest that morphine enhances permeability and viral penetration across the vascular endothelium by promoting apoptosis, via a Fas-independent pathway. This study reveals insight as to how morphine exposure may enhance HIV-1 penetration and further implicate the effects of endotoxins on these physiological barriers.
**Introduction**

The vessels of the vascular system are lined by a continuous monolayer of endothelial cells containing intercellular adherent and tight junctions. These specialized junctions function as a barrier against uncontrolled paracellular permeability (Kevil et al., 1998; Rubin and Staddon, 1999; Li and Mrsny, 2000). Thus, the vascular endothelial cell (VEC) barrier provides a crucial interface between the circulating blood and the underlying tissues (Hoek, 1992), and serves as a boundary to protect vital organ systems from invasion by micropathogens such as the HIV-1 virus (Baba et al., 1988; Zietz et al., 1996; Notter, 1999). Mechanical stress, inflammation, or exogenous factors such as drugs of abuse, including the opiate, morphine, may disrupt the VEC layer, leading to edema and significant damage to the underlying tissues.

Previous studies have shown that chronic morphine exposure increases the penetration of microorganisms across the epithelial lining of the GI tract (Hilberger et al., 1997; MacFarlane et al., 2000; Bannerman and Golblum, 1997). These studies suggest that morphine may modulate the permeability of the GI tract epithelium, which contains intercellular junctions similar to VEC. However, the specific cellular and molecular mechanisms underlying morphine's effects on the VEC barrier still need to be elucidated.

One mechanism by which morphine may alter VEC permeability is through the promotion of programmed cell death or apoptosis. Apoptosis, which is a normal cellular process, may be induced prematurely by morphine's activation of certain intracellular signals, such as the pro-apoptotic Fas-dependent pathway. A recent study reported that morphine induces the expression of the death receptor, Fas, in immune cells (Yin et al., 1999). The Fas-dependent pathway is initiated by the binding of the Fas-Ligand (FasL)
to the Fas receptor, thus, activating the Fas-Associated Death Domain (FADD) of the receptor. In turn, a cascade of caspases is triggered, mediating apoptotic cell death. In addition, a soluble form of the receptor, sFas, can exist in circulation, and has been reported to block Fas-dependent apoptosis (Cheng et al., 1994).

Bacterial LPS is a complex glycolipid isolated from the cell walls of Gram-negative bacteria. LPS acts as a potent endotoxin, leading to an acute inflammatory response that can ultimately result in endotoxic shock (Roy et al., 1999). LPS has been shown to induce production of an array of immune mediators, including the pro-apoptotic proteins, FasL and sFas, in sinusoidal endothelial cells (Muschen et al., 1998).

Synergistic effects of morphine and LPS have been observed for various parameters of immune responses (Roy et al., 1999). For example, chronic exposure to morphine has been shown to enhance LPS induced production of pro-inflammatory cytokines that can increase leukocyte adhesion to endothelial cells (Chang, 2001). These cytokines have been shown to enhance the permeability of the VEC barrier (Fiala et al., 1997).

In this study, we investigated whether morphine's actions on endothelial cells could augment the effects of LPS and enhance the permeability of the VEC barrier, thus, allowing viruses, such as HIV-1, to penetrate these physiological barriers. We also examined the mechanisms of in vitro endothelial cell death produced by LPS and morphine, including the involvement of the Fas/FasL system.
Methods

Chemicals and reagents. Morphine sulfate-pentahydrate, naloxone-hydrochloride, LPS from Escherichia coli, [14C]-carboxylated inulin, Dulbecco's phosphate buffered saline solution (DPBSS), and 4',6-diamidino-2-phenylindole (DAPI) nuclear stain were all purchased from Sigma Chemical Co. (St. Louis, MO). Trypan blue solution (0.2%) was purchased from GibcoLife Technologies, Inc (Rockville, MD). Recombinant human Fas/FC Chimera (soluble Fas protein) was purchased from R & D Systems, Inc. (Minneapolis, MN). Fas-Ligand recombinant protein was purchased from Oncogene Research Products (Cambridge, MA). PRG-2 trypsin/EDTA solution for the rodent endothelial cell line was purchased from Cell Systems, Inc. (Kirkland, WA).

Cell cultures. Low passage (3rd or 4th) human coronary artery (hCAEC) and human brain microvascular (hBMVEC) endothelial cells were purchased from Biowhittaker/Clonetics (Walkersville, MD). Cells were cultured in Clonetics EGM-2MV Bullet Kit media supplemented with EGM-2 MV, and maintained at 37°C in a humidified 5% CO2 incubator. A Reagent Pack (Biowhittaker) containing Hepes buffered saline solution, trypsin/EDTA solution, and trypsin neutralizing solution was also used for culturing the human endothelial cell lines. Immortalized rat brain microvascular endothelial cells (rBMVEC), generously provided by Dr. D.B. Stanimirovic and Dr. A. Muruganandam (National Research Council of Canada), were maintained as previously described (Morley et al., 1998).
**In vitro VEC barrier model.** An in vitro VEC barrier model was constructed as described (Fiala et al., 1997) using hBMVEC, rBMVEC, and hCAEC cells, passage numbers 5-8. Briefly, the endothelial cells were seeded at a density of 20,000 cells/0.3 mL into a cell culture insert (8.0 µm pore size) housed in a 24-well tissue culture plate (Fisher Scientific, Springfield, NJ), containing 1 mL/well of culture medium.

For the LPS experiments, cells at 100% confluency, the medium in the top insert chamber was replaced with either 0.3 mL of fresh medium (for controls), 0.3 mL of fresh medium containing LPS (1, 10, or 100 µg/mL), or 0.3 mL of fresh medium containing 10 µM morphine plus 10 µg/mL LPS. For the morphine experiments, the medium in the upper chamber was replaced with morphine-supplemented medium (1, 10, or 100 µM). The medium in all the lower chambers was replaced with 1.0 mL of fresh medium, and the plates were incubated for 24 h at 37°C. Naloxone sensitivity was determined by pre-treating the cells in the top chamber with 0.3 mL of fresh medium containing 100 µM naloxone for 20 min. The naloxone-supplemented medium was then replaced with 0.3 mL of morphine (10 µM)-supplemented medium, and the plates were incubated for 24 h at 37°C.

At the end of each treatment period, the medium in the upper chamber was replaced with 0.3 mL of fresh medium containing [¹⁴C]-carboxylated inulin, and the medium in the bottom chamber was replaced with 1 mL of fresh medium. The cells were incubated at 37°C for an additional 2 h to allow for diffusion. Fifty microliters of the medium from each top and bottom chamber were then collected, mixed with liquid scintillation cocktail, and counted in a liquid scintillation counter. The permeability coefficient
(cm/min) for [14C]-inulin (MW 5000) was calculated using the formula previously described by Fiala et al. (1997):

\[ \frac{\{V / (A \cdot D)\} \cdot (pR / pT)}{V = \text{volume of the receptor chamber (1.0 cm}^3) \), A = \text{area of the cell monolayer on the membrane (0.35 cm}^2), D = \text{initial amount of the marker molecule in the donor chamber, } pR / pT = \text{amount of the marker molecule in the receiver solution (} pT = 1 \text{ hr. or 4 hr.}) \]

After each experiment, the upper chambers were stained with crystal violet and observed with an Olympus Model IMT-2 inverted bright-phase compound microscope to ensure minimal damage to the confluent monolayers.

**Viability studies.** The primary endothelial cells were seeded at a density of 50,000 cells/well (passages 5-9) into 6-well plates containing 4 mL/well of fresh medium, and grown until ~70% confluent. Fresh medium (for controls), or medium containing morphine (1, 10, or 100 µM), naloxone (100 µM), morphine (10 µM) plus naloxone, LPS (10 µg/mL), or morphine (10 µM) plus LPS (10 µg/mL) was added, and the cells were incubated for an additional 24 h at 37 °C. The monolayers were examined with a phase contrast microscope to ensure that they remained intact. The supernatant was discarded, and each well was washed twice with Hepes buffered saline solution (Biowhittaker/Clonetics, Walkersville, MD). The human endothelial cells were detached with trypsin/EDTA, and the trypsin was inactivated with trypsin neutralizing solution. PRG-2 (Passage Reagent Group) trypsin/EDTA solution was used to detach the rodent endothelial cell monolayers, which was then inactivated by addition of twice the amount
of cell culture medium. The cells were centrifuged at 700 rpm at room temperature for five minutes, and resuspended in fresh culture medium. Cell viability was determined on a hemacytometer by the trypan blue exclusion assay.

*Nuclear staining assay.* All three endothelial cell types (passages 6-9) were grown to 70% confluence on round glass coverslips (Fisher Scientific) in 24 well-plates. One milliliter of fresh medium containing the appropriate treatment was added in quadruplicate, and the cells were incubated for an additional 24 h at 37°C. The final concentrations of the treatments were: 10 µM morphine ± 100 µM naloxone, 100 µM naloxone, or 10 µg/mL LPS ± 10 µM morphine. At the end of the 24 h incubation, the cells were stained with 4',6-diamidino-2-phenylindole (DAPI), which intercalates DNA, and, under UV light, the condensed, irregularly nuclei of apoptotic cells were differentiated from the large, round nuclei of normal cells. Briefly, DAPI staining was performed by fixing the cells onto coverslips with 4% paraformaldehyde for 5-8 min, rinsing the cells 3 times with PBS, then permeabilizing the cells with 95% ethanol/5% acetic acid for 5-8 min. The cells were then rinsed 3 times with PBS, and incubated in the dark at room temperature with DAPI (final concentration = 300 nM in PBS) for 1-3 min. After 3 PBS rinses, the coverslips were mounted with Permount onto microscope slides. The cells were examined with an Olympus BX40 system microscope with an Olympus BX-FLA reflected light fluorescence attachment at an excitation wavelength of 358 nm and an emission wavelength of 461 nm, and photographed with an Olympus camera (Model number C-35AD-4) and 400 speed Kodak film with an exposure time of 1 minute.
Assessment of the Fas/Fas-Ligand pathway. The hBMVEC cell monolayers (passage 8-10) were grown on round glass coverslips in 24-well plates, as described above, and maintained at 37°C in a humidified environment. When the cells reached 70% confluence, the medium was replaced with 900 µL of fresh medium and 100 µL of medium containing the appropriate treatment (4 wells/treatment). The final concentrations of the treatments were: 200 ng/mL FasL alone, 200 ng/mL FasL ± 50 ng/mL sFas, 10 µM morphine ± 50 ng/mL sFas, 10 µg/mL LPS ± 50 ng/mL sFas, or 50 ng/mL sFas alone. The cells were incubated for 24 h at 37°C, then analyzed for apoptotic nuclei by fluorescent microscopy using the DAPI nuclear staining assay.

HIV-I penetration assays. These experiments were performed by our collaborators, Drs. Gujuluva and Fiala, of the department of Neurology at UCLA School of Medicine, Los Angeles, California. Briefly, the VEC barrier model was constructed using the hCAEC cell line, as described previously in the in vitro VEC barrier models used for the [14C]-inulin permeability assays (Fiala et al., 1997). When the cells reached 70% confluence, the macrophage-tropic virus strain, HIV-1JR.rfl (150,000,000 RNA copies), was added to the upper chamber with control vehicle, and incubated at 37°C for either 2 h or 24 h. To determine the effects of morphine on viral penetration across the VEC barrier, either morphine (10 µM or 100 µM), or naloxone (100 µM) and morphine (10 µM) was added to the upper chamber and incubated at 37°C for 24 h. Approximately 150,000,000 RNA copies of the HIV-1 virus was then added to the upper chamber, and the plate was again incubated at 37°C for 24 h. After incubation, 50 µL samples were collected from both
the upper and lower chambers, and the number of HIV RNA copies was determined according to Fiala et al. (1997) using the Amplicor® HIV-1 monitor test developed by Roche Corp. Diagnostic Laboratories (Nutley, NJ). The amount of virus in the morphine-treated samples was determined, and compared to the control vehicle standard. This experiment was repeated at least twice with reproducible results.

Statistical analysis. For all studies, statistical significance of the results were determined using ANOVA or the Student's t-test using the GraphPAD InStat software (GraphPad Software, San Diego, CA). Post-hoc statistics were computed using adjusted t-tests with p-values corrected by the Bonferroni method. Significance was evaluated using a standard 95% confidence interval (p<0.05). Individual samples were tested in triplicate or quadruplicate for each experiment, and each experiment was repeated at least twice with similar results.
Results

**Morphine's effects on LPS-induced permeability of the VEC barrier.** The effects of the bacterial endotoxin, LPS, on the hCAEC VEC barrier model were examined by determining the permeability of the hCAEC monolayer to $[^{14}\text{C}]$-inulin. Pre-treatment of the hCAEC with 1, 10, and 100 µg/mL LPS for 24 h significantly increased $[^{14}\text{C}]$-inulin permeability in a concentration-dependent manner (Figure 1A). Co-treatment of the model with 10 µM morphine plus 10 µg/mL LPS enhanced $[^{14}\text{C}]$-inulin permeability compared to LPS alone (Figure 1B).

Morphine's effects on three different VEC barrier models were determined by pre-treating the hCAEC (A), hBMVEC (B), and rBMVEC (C) monolayers for 24 h with either morphine alone (1, 10, or 100 µM), 100 µM naloxone alone, or 10 µM morphine plus naloxone, then examining $[^{14}\text{C}]$-inulin permeability (Figure 2). Treatment with either morphine or naloxone alone increased $[^{14}\text{C}]$-inulin permeability (Figure 2), and co-treatment with morphine plus naloxone resulted in an additive effect on $[^{14}\text{C}]$-inulin permeability in all three of the endothelial cell lines tested (Figure 2).

**Morphine's effects on cell viability of endothelial cells.** To determine if the change in the VEC permeability produced by LPS and morphine occurred as a result of endothelial cell death, the viability of the hCAEC (A), hBMVEC (B), and rBMVEC (C) cells was examined. Treatment with either morphine (10 µM and 100 µM) or naloxone (100 µM) significantly decreased the viability of all three endothelial cell lines compared to control ($p<0.005$, Figure 3). Co-treatment with morphine (10 µM) plus naloxone lowered cell viability in an additive manner compared to morphine alone (Figure 3).
LPS (10 µg/mL) treatment decreased the viability of the hCAEC, hBMVEC, and rBMVEC cells (Figure 4A, B, and C, respectively) compared to untreated control cultures. All three endothelial cell types co-treated with morphine (10 µM) plus LPS had a significantly lower percentage of viable cells compared to LPS treatment alone (Figure 4).

Apoplolic effects of morphine on endothelial cells. Decreased cell viability can result from either necrosis (physical or chemical damage to cells) or apoptosis (programmed cell death). Necrosis due to physical damage was ruled out because microscopic examination of the cell monolayers showed that the cells remained morphologically intact following morphine treatment (data not shown). Apoptosis can be identified by characteristic morphological changes that occur in the nucleus of cells. Using DAPI nuclear staining, the nuclear morphology of the hBMVEC treated with 10 µM morphine with and without 10 µg/mL LPS was examined. The condensed, irregular nuclei of apoptotic cells were then differentiated from the large, round nuclei of normal cells. Untreated control hBMVEC cells contained morphologically normal nuclei (Figure 5A); whereas apoptotic nuclei were observed in the morphine- and LPS-treated cells (Figure 5B and C, respectively). Co-treatment with morphine plus LPS resulted in both cells with apoptotic nuclei and cells with normal nuclei (Figure 5D).

The percentage of apoptotic cells was calculated for the hCAEC (A), hBMVEC (B), and rBMVEC (C) following treatment with 10 µM morphine, 100 µM naloxone, morphine plus naloxone, 10 µg/mL LPS, or morphine plus LPS. Morphine treatment induced apoptosis in all three endothelial cell types; this effect was not blocked by
naloxone (Figure 6). In fact, the number of apoptotic cells was additive following treatment with morphine plus naloxone compared to morphine treatment alone (Figure 6), which is consistent with the decreased cell viability observed with morphine plus naloxone treatment.

Treatment with LPS also resulted in apoptosis of the endothelial cells, which was significantly enhanced by co-treatment with morphine (Figure 6).

**Morphine's effects on Fas-mediated endothelial cell apoptosis.** Binding of FasL to the Fas receptor has been shown to promote apoptosis. The soluble form of Fas, sFas, is thought to inhibit the Fas/FasL apoptotic signaling pathway by blocking the binding of FasL to the Fas receptor (Cheng et al., 1994). We, therefore, investigated if the Fas pathway could be involved in morphine's apoptotic effects on the VEC barrier.

hBMVEC cells treated with either 200 ng/mL FasL or 50 ng/mL sFas underwent significant apoptosis when compared to untreated control monolayers (p<0.005, Figure 7A). However, co-treatment of the monolayer with FasL plus sFas inhibited apoptosis (Figure 7A). Consistent with our previous results, treatment with 10 µM morphine alone induced apoptosis (Figure 7A); whereas co-treatment with morphine plus sFas induced apoptosis in an additive manner (Figure 7A).

Treatment with 10 µg/mL LPS also induced significant apoptosis (Figure 7B). However, this apoptotic effect was blocked by co-treatment with LPS plus sFas (Figure 7B).
Morphine's effects on HIV-1 penetration of an in vitro VEC barrier. Since exposure to morphine resulted in a significant increase in VEC permeability, we then investigated if morphine could affect the penetration of viral particles, such as HIV-1, through the VEC barrier. Using the ultrasensitive Amplicor® viral RNA detection assay, we determined the average number of viral RNA copies that penetrated into the lower chamber of the hCAEC barrier model at 2 h and 24 h after addition of HIV-1 to the upper chamber (Figure 8). A significant number of viral RNA copies penetrated into the lower chamber at 24 h compared to the number at 2 h (p<0.005). Treatment with morphine (10 µM or 100 µM) significantly increased the average number of HIV-1 RNA copies penetrating into the lower chamber in a dose-dependent manner (Figure 8).
Figure 1
Figure 2.
Figure 3.
Figure 4.
= 20 μm
Figure 6.
Figure 7.
Figure 8.

Average # of Viral RNA Copies

HIV alone (2 h)  HIV alone (24 h)  10 μM Mor. (24 h) + HIV  100 μM Mor. (24 h) + HIV

*  #
Discussion

The vascular endothelial cell (VEC) barrier is important in providing an impermeable interface between the circulating blood and the underlying tissue. Structurally, this barrier is critical for protecting the host against pathogenic microorganisms such as the HIV-1 virus. However, the VEC can be compromised by infection and chronic opioid abuse. In this study, we explored the mechanisms by which morphine enhances LPS-induced permeability of VEC barriers and promotes HIV-1 penetration through those barriers.

LPS is a bacterial endotoxin that affects the integrity of the vascular endothelial lining (Bannerman and Goldblum, 1997). Under phase contrast light microscopy, there are no obvious differences in the morphology of these cells; all three cell types, rBMVEC, hBMVEC, and hCAEC grow into a confluent monolayer and present contact inhibition of cell growth. In our studies, both LPS and morphine decreased VEC viability and increased the [\(^{14}\)C]-inulin permeability in these three endothelial cell types tested. Furthermore, morphine co-treatment significantly enhanced LPS-induced permeability of the VEC, suggesting that the detrimental effects of LPS on the VEC barrier could be significantly greater in the presence of morphine.

We then investigated the mechanism by which VEC viability and permeability could be affected by morphine and LPS. Cell death can occur as a result of either necrosis or apoptosis (Farber, 1994; Trump et al., 1997). Necrosis is characterized by cell membrane swelling and blebbing, and the simultaneous death of large numbers of cells. Apoptosis is programmed cell death that results from intracellular signal transduction pathways, and usually involves only a single or a few isolated cells at any given time. Nuclear staining
with DAPI and morphological examination confirmed that the decrease in cell viability following treatment with either morphine or LPS alone, and in combination was due to apoptosis, and not necrosis. Such morphine-induced programmed cell death could certainly contribute to enhanced VEC permeability and increase the possibility of penetration by HIV-1 through the VEC barriers.

Preliminary studies showed that FasL, the natural ligand for Fas, induced apoptosis in our VEC in a concentration-dependent manner, and that morphine pre-treatment enhanced FasL-induced apoptosis, confirming the expression of a functional Fas receptor in our cell types (data not shown). Morphine has been shown to induce expression of Fas in a T-cell hybridoma (A1.1), in mouse splenocytes, and freshly isolated human peripheral blood lymphocytes (Yin et al., 1997). Taken together, these results suggested that morphine may prime the VEC to undergo FAS-mediated apoptosis. The soluble form of Fas, sFas, reportedly blocks Fas-dependent apoptosis (Cheng et al., 1994). In this study, we, therefore, used sFas to examine if morphine’s apoptotic effects were Fas-dependent. As expected, sFas did inhibit FasL-induced apoptosis. Furthermore, when the VEC monolayer was co-treated with LPS and sFas, apoptosis was inhibited, suggesting that LPS-induced apoptosis is mediated via a Fas-dependent pathway. Unexpectedly, sFas alone induced apoptosis of the endothelial cells, and co-treatment with morphine resulted in increased rather than decreased apoptotic effects. These data suggest that the molecular mechanisms underlying morphine-induced apoptosis in the VEC monolayer are mediated through Fas-independent pathways although the VEC has demonstrated functional Fas/Fas-L mediated apoptotic pathways. Our studies are consistent with previous studies showing variable data regarding the susceptibility of
vascular endothelial cells to Fas-mediated cell death and the expression of Fas-regulatory components (Walsh and Sala, 1999). Another possible mechanism by which morphine may affect endothelial cell permeability is through the mu opioid receptor pathway. The presence of opioid receptors on endothelial cells has been shown by our laboratory (Vidal et al., 1998) and others (Stefano et al., 1995; He et al., 2000). While expression of mu opioid receptor mRNA has been demonstrated in endothelial cells by both semi-quantitative and competitive RT-PCR (Vidal et al., 1998; Stefano et al., 1995), mu-opioid binding sites have not been identified. Furthermore, in our study, morphine’s actions were not reversed by naloxone, a mu opioid receptor antagonist. Although we do not completely exclude the possibility that morphine’s effects on [14C]-inulin permeability, cell viability, and apoptosis are mediated via opioid receptors, the data suggest that morphine may act on these endothelial cells via a distinct, separate pathway, especially considering a study by Melzig et al. (1998) showing binding of [3H]-morphine within the nuclei of endothelial cells. These experiments demonstrate a mechanism of action for morphine that is not mediated via opioid receptors, but rather binding to a separate membrane or nuclear fraction of the endothelial cells. Therefore, the underlying mechanism of morphine, as reported here, still remains to be clarified.

The VEC barrier is one of the defense mechanisms through which vital organs are protected from attack by pathogenic viruses such as HIV-1. While HIV-1 penetration through our hCAEC barrier model was limited when exposure duration was short, the penetration of HIV-1 through the VEC barrier was significantly increased following prolonged exposure to virus. These results are consistent with a previous report, which examined an in vitro blood-brain barrier model constructed from endothelial cells.
isolated from microvascular vessels (1998). In addition, pre-treatment of the hCAEC barrier model with morphine enhanced HIV-1 penetration. Taken together, these findings suggest that the VEC barrier breaks down with prolonged exposure to HIV-1, and that the integrity of the VEC barrier could be compromised further by the presence of morphine. Consequently, in opioid addicts or in patients chronically treated with morphine for pain relief, the VEC barrier may be compromised, allowing enhanced penetration of pathogenic microorganisms, such as the HIV-1 virus.

HIV-1-associated encephalitis and cardiomyopathy are characterized by the pronounced entry of the virus into the organ tissue environment (Fiala et al., 1997; Persidsky et al., 1997). The increased penetration of cell-free or cell-associated pathogens that results from endothelial damage may contribute to the pathogenesis associated with HIV-1 infection (Berger et al., 1999). In the presence of morphine, these cell-free viruses may penetrate the physiological VEC barriers more effectively. Our results provide some insight into the mechanisms underlying how morphine exposure may enhance HIV-1 penetration through VEC barriers, and the detrimental effects that endotoxins, such as LPS, may have on these physiological barriers.
Summary

The VEC barrier is one of the defense mechanisms by which vital organs are protected from attack by pathogenic viruses such as HIV-1. Our findings suggest that the VEC barrier breaks down with prolonged exposure to HIV-1, and that the integrity of the VEC barrier is compromised further in the presence of morphine. Consequently, in opioid addicts or in patients chronically treated with morphine for pain relief, the VEC barrier may be breached, allowing this enhanced penetration of pathogenic microorganisms, such as the HIV-1 virus. Our results provide some insight into the mechanisms underlying how morphine exposure may enhance HIV-1 penetration through VEC barriers, and the detrimental effects that endotoxins, such as LPS, may have on these physiological barriers.
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