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Morphine Exposure During HIV Encephalitis in SCID Mice

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Abstract

HIV encephalitis (HIVE) is often complicated by opiate abuse. Based on human pathological, animal and in vitro studies, opiates are thought to exacerbate HIVE. To test this hypothesis we exposed 10 week old SCID mice with HIVE to morphine and examined histopathological parameters. Mice inoculated intracerebrally with either HIV-infected or uninfected (control mice) human macrophages were immediately implanted subcutaneously with pellets containing saline, morphine or morphine plus naltrexone. They were sacrificed after 10 days. Immunostaining for astrocytes (GFAP), mouse mononuclear phagocytes (CD45) and neuronal dendrites (MAP2) was analyzed by densitometry. HIVE mice exposed to either saline, morphine or morphine plus naltrexone also had brain sections counted for HIV+ human macrophages. Typical HIVE pathology was present, consistent with previously published studies. Surprisingly, there were no effects on astrogliosis, microgliosis and MAP2 decreases in the HIVE, morphine treated group. There was also no effect of morphine exposure on numbers of p24+ human macrophages. These results emphasize the complexities of modeling opiate effects in HIVE and the potential significance of opiate abuse on HIVE in humans.

Introduction

The use of drugs such as alcohol, methamphetamine, cocaine and opiates are associated with a higher risk for contracting HIV infection (1). HIV associated neurocognitive disorders (HAND) remain an important complication of HIV infection, affecting as many as 50% of HIV-infected individuals, despite combined antiretroviral therapy (ART) (2). It is generally accepted that drug abuse predisposes to HAND and associated pathology (ie, HIV encephalitis or HIVE) (3), however, this is not without controversy (4). Drug abusers typically are polydrug users, thus making it practically impossible to determine the effects of a single agent. They are also often non-compliant with ART putting them at greater risk for HAND and opportunistic infections of the central nervous system (CNS). Whether drug abuse overall predisposes HIV-positive patients to HAND remains unclear. A recent large study suggested no increased risk (5).

Bell et al. originally reported in an Edinburgh population of purportedly exclusive heroin users that HIVE was more common at autopsy in HIV-positive heroin users than in a cohort of HIV-positive non-users (6). However, other studies have either failed to demonstrate the association of increased brain pathology in HIV-positive intravenous drug users through neuropsychological testing (7) or showed only a weak association of intravenous drug abuse with the development of neurocognitive dysfunction in HIV-positive individuals (8).

Nevertheless, there are significant effects of opiates on astrocytes and microglia in association with HIV (9). Studies have shown HIV tat and morphine interactions resulting in increased *in vitro* expression of chemokines and cytokines, substances which have been hypothesized to exacerbate HAND and HIVE (10–12). However, curiously these types of

synergistic morphine and tat effects on astrocytes recently were not reproduced, thus adding to confusion concerning synergistic neuropathological effects of opiates and HIV (13). Nonetheless, others have also suggested that morphine and tat have combined effects on inflammatory mediator production by cultured microglia (14) and when neurons are exposed *in vitro* to gp120 and tat in the presence of morphine, enhanced toxicity occurs (15, 16). Finally, studies in transgenic rodent models suggest that HIV and opiates have synergistic effects on brain pathology and behavior (17–20).

Given the discrepancies outlined above as to whether opiates clearly have effects on HAND, we sought to determine whether morphine exposure alters neuropathology in a model of HIV encephalitis in SCID mice (21–23). Consistent with previous studies (22, 24, 25), HIVE mice had significant differences from control mice in increased microgliosis and astrogliosis and decreased neuronal integrity (ie, MAP2 expression). Morphine exposure had no effect on these parameters or viral load.

Methods

Preparation of HIV-infected human monocytes, mouse injections and treatments

Primary human macrophages were cultured for 1 week and infected with HIV-1ADA for 10 days. Approximately 1×10^7 monocytes/culture were collected after viral infection for inoculation into B6.CB17-Prkdcscid/SzJ male mice or NOD.Cg-Prkdcscid Il2rg/SzJ male mice (10 weeks old, Jackson Laboratory, Cold Harbor, ME). Mice were inoculated intracranially (IC) with 10^5 HIV-infected or uninfected (control) human monocytes in 30 μ l PBS. Immediately following i.c. inoculation of cells, mice were subcutaneously implanted with pellets containing either morphine (25 mg total; 0.208 mg/hr) or saline or morphine plus naltraxone (60 mg total; 0.37 mg/hr). The morphine and naltrexone pellets were obtained from NIDA (National Institute on Drug Abuse) and are standard dosages used in mice (11, 17). These pellets release their contents over a period of 5 days; therefore, they were replaced at day 5. Importantly, mice who received morphine pellets exhibited lethargy over the first 24 to 48 hours after pellet insertion. This was not observed in mice inserted with both morphine and naltrexone pellets. Groups therefore included: 1. Control mice inserted with saline pellets (n=11), 2. Control mice treated inserted with morphine pellets (n=14), HIV mice inserted with saline pellets (n=16), HIV mice inserted with morphine pellets (n=18) and HIV mice inserted with both morphine and naltrexone (60 mg total; 0.37 mg/hr) pellets (n=6). Mice were sacrificed at day 10 post i.c. inoculation and brains were extracted, snap frozen and stored at -70°C until they were cryo-sectioned.

Immunohistochemistry and densitometry

Briefly, mouse brains were snap frozen in tissue-freezing medium, and approximately 6 sets of 5 μ m coronal sections were taken starting at the frontal lobe through the temporal-parietal lobe. Intervening sections (not immunostained) were saved for mRNA extraction. Immunoperoxidase staining, which has been previously described in detail (ref), included human macrophages (1:50 EBM11; DAKO, Denmark), HIV (1:20 p24; Dako), astrocytes (1:750 glial fibrillary acidic protein; Chemicon, Temecula, CA), microglia (1:75 CD45; AbD Serotec, UK), and neuronal dendrites (1:200 microtubule associated protein-2 [MAP2]), Chemicon. The CD45 antibody is specific for mouse CD45. Slides were then reviewed using light microscopy (Olympus microscope: Melville, NY) and two (MAP2 and CD45) or three (GFAP) slides were used for each antibody analyzed, corresponding to the area closest to the injection track of the human macrophages (approximately 50–60 μ m in each direction). This pathology was quantified using densitometry analysis for microgliosis, astrogliosis, and dendritic arborization using NIH ImageJ software. For p24 cell counts all sections (usually a total of 6/mouse) that contained p24+ cells were counted for each mouse.

These were then analyzed according to HIV mouse groups as described above and in Figure 1.

RNA extraction and cDNA synthesis

Intervening sections not used for pathological analysis were collected and RNA was extracted according to the RNeasy Mini Kit (Qiagen) protocol. Approximately 1 µg of RNA was used for cDNA synthesis. First-strand cDNA synthesis was performed using the iScript cDNA Synthesis Kit (Biorad).

Real-time PCR

Levels of HIV (gag) were analyzed using real-time PCR with Biorad C1000 Thermocycler. Glyceraldehyde-3-phosphatedehydrogenase (GAPDH), a housekeeping gene, was used as the endogenous control for all experiments. HIV and GAPDH primers were designed using Assays-by-Design (Applied Biosystems). cDNA samples were added to Sybr Green Supermix (Biorad) and the primer from DNA Technology or Applied Biosystems. Relative quantification of target gene was done using GAPDH levels of each sample. The relative changes in gene expression were analyzed using $2^{-\Delta C_t}$ method by Livak and Schmittgen.

Statistics

Analysis of densitometry readings was completed using an unpaired t test using SPSS software. Significance was set at $p < 0.05$ for all analyses.

Results

HIV in brain

Samples from SCID mouse brains injected IC with HIV-infected human monocytes and given placebo pellets (n=4), SCID mouse brains injected IC with HIV-infected human monocytes and given morphine pellets (n=4), SCID mouse brains injected IC with HIV-infected human monocytes and given morphine and naltrexone pellets and control mice injected with uninfected human monocytes (n=3) were subjected to RT-qPCR for HIV gag. As expected, control samples were negative. Gag RNA levels in all mice injected with HIV-infected human monocytes revealed low copy numbers and there was no apparent effect of morphine treatment. This data was corroborated by p24 + cell counts in brain sections (Figure 1). There was no significant effect of morphine treatment on the number of p24+ cells counted in mouse brains from the three groups of mice injected with HIV-infected human monocytes.

Densitometry for mouse mononuclear phagocytes and astrocytes

Consistent with previously reported data (22), mice injected with HIV-infected human monocytes had a significantly greater amount of CD45 staining (ie, mononuclear phagocytes including macrophages and microglia) than controls (Figure 2). There was no effect of morphine treatment. Also consistent with previously reported data, mice injected with HIV-infected human monocytes had a significantly greater amount of GFAP staining (ie, astrocytes) than controls (Figure 3). Interestingly, mice treated with morphine plus naltrexone had significantly more astrogliosis than mice treated with morphine alone, suggesting that there is an effect of morphine on reducing astrogliosis.

Densitometry for dendritic staining

These results were also consistent with previously reported data in that mice injected with HIV-infected human monocytes had a significantly lesser amount of dendritic staining for

MAP2 than control mice (Figure 4). As with the presence of HIV and mononuclear phagocyte staining, there was no effect of morphine treatment.

Discussion

Morphine was given subcutaneously via slow release pellets to SCID mice inoculated IC with either HIV-infected or uninfected (controls) human monocytes over a 10 day period. This dose of morphine resulted in a visible physiological effect (ie, lethargy) that was abolished with naltrexone pellet insertion. In addition, the mice exhibited habituation to this dose typically after 2 days. Therefore, it was a relevant dose of morphine. Nevertheless and contrary to our hypothesis, this dose of morphine had no effect on HIVE in these mice. p24 cells counts (Fig 1) were unchanged, as were microgliosis (Fig 2), astrogliosis (Fig 3), and MAP2 immunostaining between morphine HIV and placebo HIV groups (ie, dendritic arborization, Fig 4).

There are inconsistencies in the literature (see Introduction) as to whether opiates truly have adverse effects on the development and course of HAND. One possible explanation for discrepancies in our model and in the literature could be the CNS viral load of HIV at the time of assessment of outcomes. A previous preliminary study in our lab reported as an abstract suggested that morphine exposure during HIVE in SCID mice reduces astrogliosis and microgliosis (26). Under those experimental conditions there was some suggestion that less HIV was present than in the current study. These results might be consistent with what is seen clinically and with *in vitro* and *in vivo* basic investigations

The study by Bell et al. was an autopsy study of patients with HIVE who abused heroin (6). An association was seen between heroin use and the development of HIVE. These individuals were almost certainly assessed when viral load in the brain would be at its highest. This is opposed to studies examining neuropsychological test results, early in HIVE, when presumably viral load is relatively low. As stated in the Introduction, these cognitive studies in humans showed no real effect of IV drug use on test scores (7, 8). We believe the SCID mouse model of HIVE represents a relatively mild expression of HIVE, with relatively low viral loads (27, 28). Therefore morphine effects might not be seen in this model because of relatively low HIV CNS viral load.

Relatively low [or high] CNS viral loads may also be explanations for discrepancies between *in vitro* studies, which demonstrate opiate effects on glia and neurons (9–16, 29), this report and clinical studies, which show little to no effect of IV drug abuse on the development of HAND (7, 8). The dosages of tat used during *in vitro* investigations could be construed as relatively high doses, which may be more likely seen clinically in cases of frank dementia (ie, HIV associated dementia or HAD) or at autopsy, which represent clinical scenarios where HIV CNS viral load is likely to be higher. This may also be true for studies utilizing transgenic models of tat (17, 18) where again, the local CNS dose of tat may be more representative of more progressive stages of HAND (ie, HAD). In the SCID mouse model of HIVE used in this study, CNS viral loads are presumed to be relatively low because HIV is more or less confined to the human macrophages that are inoculated into the right frontal lobe. Under these conditions, presumably the amount of tat present is also relatively low. The SCID model therefore differs from severe, end stage human HIVE (6) and tat transgenic models where HIV and tat are more pervasive. That is why we believe the SCID mouse HIVE model may be more representative of early stages of HIV infection of the brain that are reflected in the human studies described above that tested cognitive function and where opiates had little or no effects (7, 8).

Alternatively or in addition, morphine effects in HIVE may be dependent on the presence of multiple putative neurotoxins postulated to be involved in the pathogenesis of HAND and HIVE (30, 31). Severe forms of HAND (ie, HAD) may only result from the combination of multiple neurotoxins, both viral (eg, tat) and immune mediated (eg, interferon-alpha) (28). It may be that only in this milieu, when the combined onslaught of many neurotoxins is in play that morphine effects are most pertinent.

Finally, it may be that current animal models that employ whole retrovirus genomes are either relatively insensitive to the effects of morphine or that [relatively subtle] morphine effects are more likely to be evident through behavioral assessments of animals (20). Future studies using the SCID HIVE model will focus on behavioral parameters (eg, the object recognition test). Alternatively, animal models that represent more severe pathology and incorporate the effects of multiple neurotoxins need to be developed to examine opiate influences. Simian immunodeficiency virus encephalitis may represent just such an opportunity (32–34).

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Figure 1.

Number of p24 positive human macrophages counted in brain sections for HIV mice inserted with saline pellets (n=16; "Placebo HIV"), HIV mice inserted with morphine pellets (n=18; "Morphine HIV") and HIV mice inserted with both morphine and naltrexone pellets (n=6).

Figure 2.

Densitometry of CD45 (mouse mononuclear phagocyte) staining in brain sections for: 1. Control mice inserted with saline pellets (n=11; "Placebo Ctrl"), 2. Control mice treated inserted with morphine pellets (n=14; "Morphine Ctrl"), HIV mice inserted with saline pellets (n=16; "Placebo HIV"), HIV mice inserted with morphine pellets (n=18; "Morphine HIV") and HIV mice inserted with both morphine and naltrexone (n=6; "Morph+Naltrexone HIV").

Figure 3.

Densitometry of GFAP (astrocytes) staining in brain sections for: 1. Control mice inserted with saline pellets (n=11; "Placebo Ctrl"), 2. Control mice treated inserted with morphine pellets (n=14; "Morphine Ctrl"), HIV mice inserted with saline pellets (n=16; "Placebo HIV"), HIV mice inserted with morphine pellets (n=18; "Morphine HIV") and HIV mice inserted with both morphine and naltrexone (n=6; "Morph+Naltrexone HIV").

Figure 4.

Densitometry of MAP2 (neuronal dendrites) staining in brain sections for: 1. Control mice inserted with saline pellets (n=11; "Placebo Ctrl"), 2. Control mice treated inserted with morphine pellets (n=14; "Morphine Ctrl"), HIV mice inserted with saline pellets (n=16; "Placebo HIV"), HIV mice inserted with morphine pellets (n=18; "Morphine HIV") and HIV mice inserted with both morphine and naltrexone (n=6; "Morph+Naltrexone HIV").