181

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Enhancement of HIV-1 Infection by Buprenorphine

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Abstract

Background: Medication-assisted treatment (MAT) with buprenorphine is widely prescribed to treat addiction to heroin and other illicit opioids. There is evidence that illicit opioids enhance HIV-1 replication and accelerate AIDS pathogenesis, but the effect of buprenorphine is unknown.

Methods: We obtained peripheral blood mononuclear cells (PBMCs) from healthy volunteers and activated them with phytohemagglutinin and interleukin 2 for 72 hours in the presence of morphine or buprenorphine. We determined the level of expression of CCR5 both by qPCR and flow cytometry. We infected the cells with a replication-competent CCR5-tropic HIV-1 reporter virus encoding a secreted nanoluciferase gene, and measured infection by luciferase activity in the supernatants over time. We also surveyed opioid receptor expression by qPCR in PBMCs, vaginal leukocytes, and Langerhans cells derived from CD34+ hematopoietic stem cells.

Results: Buprenorphine increased HIV-1 infectivity at the three MOIs tested (0.1, 0.2 and 0.5) and over all timepoints (24, 48 and 72 hours; n=5 experiments, n=4 donors). For example, at 72 hours, HIV infection was three to six times higher in the presence of 2 nM buprenorphine ($p<0.0001$). Morphine also enhanced HIV-1 infection, but to a much lesser extent, and only at the higher dosage; this effect was variable across donors. At 100 µM, morphine caused a 1.4-fold increase in infection after 72 hours ($p<0.0001$), whereas at 1 µM, infectivity remained unchanged across time points. Neither CCR5 mRNA levels nor CCR5 protein expression was affected by buprenorphine treatment. We did not find expression of the classical opioid receptors (mu, kappa or delta) in any of the leukocyte types tested, but the nociception/orphanin FQ receptor (NOP also known as OPRL1) was present in blood and vaginal lymphocytes.

Conclusions: Our results suggest that buprenorphine, much more than morphine, may increase the susceptibility of leukocytes to HIV-1 infection *in vitro*, and the mechanism may occur at the post-translational level. Given that leukocytes do not express the classical opioid receptors, this increase in HIV susceptibility must occur through either NOP (OPRL1) or an unknown pathway. These findings are a first step toward understanding how opioids, including those used for MAT, affect HIV infection.

Overarching Hypothesis

Opioids or MAT drugs are not inert with regard to HIV infection, replication, reactivation and/or latent cell proliferation.

Significance

Knowledge of the specific effects of MAT drugs may help with the choice of MAT in the setting of HIV risk, infection and curative approaches.

Methods and Results

Secreted NanoLuc™ Reporter Virus

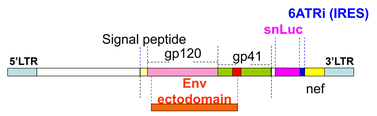

Figure 1. Simplified schema of the replication-competent CCR5-tropic HIV-1 reporter virus vNL-sNLuc.6ATRi-B-Bal.ecto. This vector was based on the replication competent molecular clones of HIV expressing *renilla* luciferase reporter (C. Ochsenbauer *et al.* AIDS Res Hum Retroviruses. 2015 Dec 1; 31(12): 1278–1296). The snLuc.IRES cassette was inserted between the NL.Bal.ecto *env* and *nef* genes. 6ATRi is a fragment derived from the encephalomyocarditis virus (EMCV) IRES.

Experimental Approach

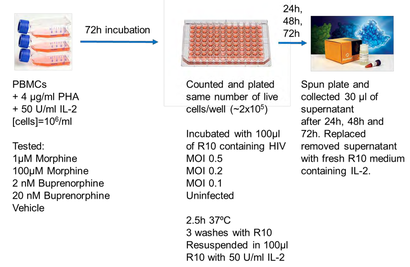

Figure 2. Experimental approach to assess early events during HIV-1 infection. In these initial experiments, cells were incubated with opioids for 72h but these drugs were withdrawn during and after HIV-1 infection. "R10" is the abbreviation for RPMI medium containing 10% heat inactivated serum.

HIV mRNA and HIV *tat* Copies in Buprenorphine Treated Cells

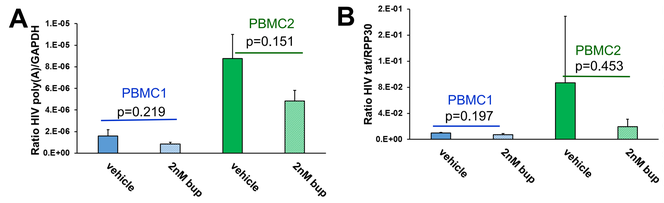

Figure 7. A. ddPCR analysis of HIV-LTR-driven poly(A) transcripts after 24h of HIV infection. Y-axis indicates HIV poly(A) copies relative to GAPDH (average + 1SD). The increase of luciferase was 90% and 30% for donor 1 and 2, respectively. **B.** ddPCR analysis of HIV *tat* copies after 24h of HIV infection. Y-axis shows HIV tat copies relative to the endogenous control RPP30, used as normalizer for input DNA.

Opioid Receptor Expression by qPCR

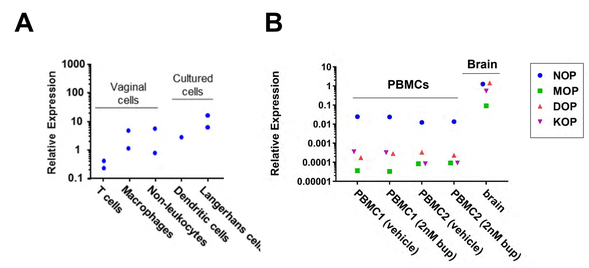

Figure 8. A: Expression of opioid receptors in leukocytes. RNA isolated from the indicated cell types was assessed for expression of opiate receptors by qPCR. T cells, macrophages, and non-leukocytes were sorted from vaginal tissues, dendritic cells and Langerhans cells were cultured from precursors, and brain RNA was purchased for use as a positive control. Expression is plotted on the y-axis relative to expression of a housekeeper gene (PP1A). MOP, DOP and KOP only amplified in brain. **B. Opioid receptor analysis in PBMCs** isolated from two healthy donors. Samples were treated with either vehicle or 2nM buprenorphine for 72h.

Effect of Opioids on Viability and Proliferation

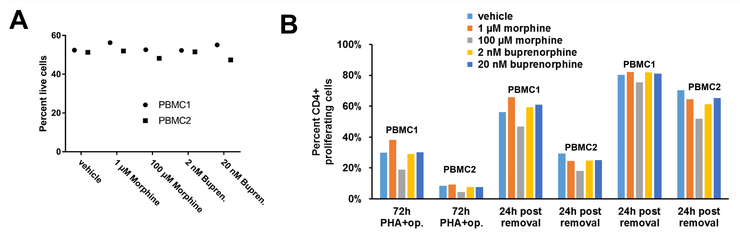

Figure 3. A. Viability analysis of 72h PHA, IL-2, opioid-treated PBMCs by Guava ViaCount assay (Millipore). **B. Proliferation analysis** after 72h of PHA, IL-2 and opioid treatment and at 24h and 48h upon opioid removal. Y-axis indicates percent of CD4+ proliferating cells (% Celltrace Violet^{low} cells).

Representative Nanoluciferase Experiment

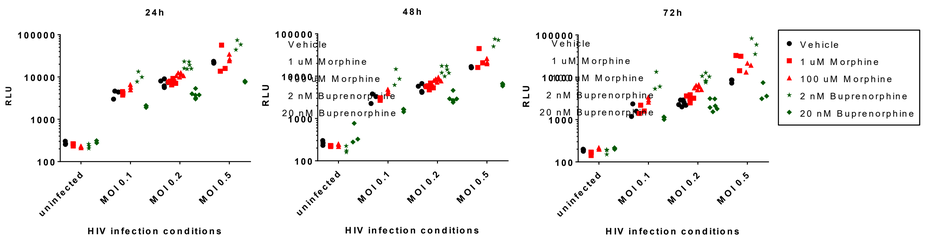

Figure 4. Representative Nanoluc Experiment. PBMCs were cultured for 72h in the presence of PHA and IL-2 and morphine (1 µM or 100 µM), buprenorphine (2 nM or 20 nM) or vehicle control. Supernatants were taken at 24h, 48h and 72h after HIV-1 infection. The luminescence signal (*Y*-axis, Relative Luciferase Units) was determined using the Promega Nano-Glo© kit. For MOI 0.2, we measured the luminometric signals of n=6 replicates; for the rest of the conditions, n=3 replicates.

Summary of Infection Experiments

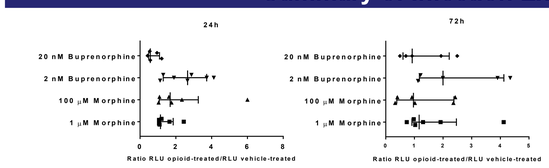

Figure 5. Summary plots showing the effect of opioids on nanoluciferase activity measured in supernatants at 24h (left) and 72h (right) after HIV-1 infection. Y-axis indicates different treatments and concentrations. X-axis shows the ratio of the luciferase activity measured in vehicle-treated cultures/ luciferase measured in opioid-treated cultures. Long vertical bars indicate the medians for each treatment and shorter bars show the interquartile range.

CCR5 mRNA and *CCR5* Cell Surface Levels Following Opioid Treatment

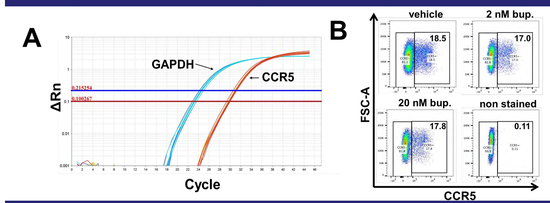

Figure 6. CCR5 mRNA and CCR5 cell surface analyses by qPCR (A) and by FACS (B). A. Amplification plots for GAPDH and CCR5 for samples that were either vehicle, (1µM and 100 µM) morphine or (2 nM and 20 nM) buprenorphine treated for 72h. All curves overlap. **B. Flow results for CCR5 cell surface staining** in one representative donor. PBMCs were treated for 72h with 2nM, 20nM buprenorphine or vehicle. Numbers in the upper right corner indicate %CCR5+ cells.

Results Summary

- Buprenorphine increased nanoluciferase activity at the three MOI tested (0.1, 0.2 and 0.5) and the effect was sustained (at least up to 72h).
- Morphine effects were milder (only seen at 100µM and in 2/4 individuals).
- Inter-subject variation: 0.35% to up to 600% increase after buprenorphine treatment; the enhancement was observed in 4/4 individuals tested.
- Neither morphine nor buprenorphine upregulated *CCR5* mRNA levels at 72h post treatment.
- Buprenorpine, when added at 2nM, did not enhance CCR5 cell surface levels in any of the two donors tested after 72h post treatment.
- We did not detect any significant difference in the relative levels of HIV transcripts or HIV *tat* genomic copies when we compared vehicle vs. 2nM buprenorphine-treated cells after 24h of HIV infection.
- In PBMCs as well as in leukocytes purified from vaginal explants and in CD34+-derived dendritic cells, we found higher expression of the OPRL1 receptor than the classical opioid receptors.
- Buprenorphine alone did not reactivate HIV-1 from latency at nM doses and did not enhance the reactivating effect mediated by TNF-α.
- Buprenorphine impeded in one donor the intracellular release of cytokines following superantigen (SEB) challenge.

Buprenorphine and Immunosuppression

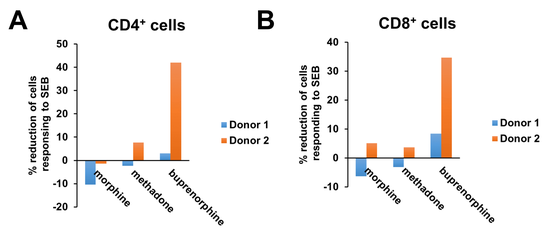

Fig. 9. Effect of opioid pre-treatment on CD4⁺ and CD8⁺ T cell responses to the superantigen staphylococcus enterotoxin B (SEB). PBMCs isolated from two healthy donors were pretreated with the indicated opioids, then exposed to the superantigen SEB for 6 hours and the production of cytokines was assessed by intracellular cytokine staining for IFN-γ, IL-2, and TNF-α. Y-axis indicates the % reduction of cells responding to any cytokine.

Buprenorphine did not Reactivate HIV-1 at nM Doses

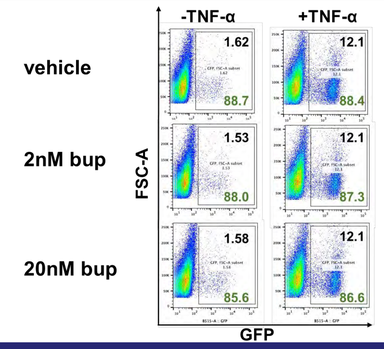

Figure 10. Effect of buprenorphine on HIV-1 reactivation. HIV-1 latently infected CD4⁺ T-cells [J-lat 11.1, developed by Dr. Verdin, Jordan, A., Bisgrove, D., & Verdin, E. (2003). EMBO J, 22(8), 1868-1877] were treated for 4 days with either vehicle, 2 nM or 20 nM buprenorphine and later activated for 24h with 1 ng/ml TNF-α. The data shows the flow analysis for GFP expression, which is a surrogate marker for HIV-1 reactivation in this model. Numbers in the upper right and lower right corners denote % GFP⁺ and % live cells, respectively.

Follow up Experiments

- Verify the enhancement of HIV infection after buprenorphine in more donors
- Analyze the effect of buprenorphine in over longer culture periods and in the presence of buprenorphine
- Compare buprenorphine to methadone
- Determine the impact of naloxone on buprenorphine
- Extend the studies to mucosal infection
- Study the effects of heroin and MAT *in vivo* in our clinical cohort (HEEX-001 study)
  - Infectivity
  - Immunological effects

Acknowledgements

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