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Effects of HIV and Drugs of Abuse on the Blood-Brain Barrier

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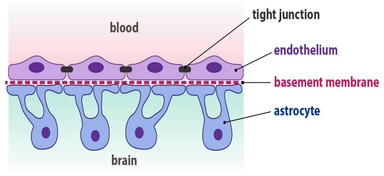
INTRODUCTION

Despite effective systemic therapy, HIV-1 infection within the brain results in neuronal degradation and neurocognitive dysfunction. This neurocognitive dysfunction is worsened in the setting of opiate abuse. The central nervous system (CNS) is protected by the blood-brain barrier (BBB), a selective barrier regulating the passage of substances from peripheral circulation into the CNS. The BBB is composed of microvascular endothelial cells encased by basal lamina, pericytes, and perivascular astrocyte endfeet. Intracellular junctional complexes comprising of adherens and tight junctions are located between the endothelial cells and form tight barrier, preventing traffic of compounds between cells (paracellular flux). Clinical and *in vitro* data suggest that BBB integrity is compromised in HIV infection, which leads to a leaky barrier. Brain microvascular endothelial cells also express efflux transporters that are responsible for the extrusion of substances from the brain back into the blood. P-glycoprotein is a drug efflux transporter involved in the efflux of many antiretroviral drugs and overexpression of P-glycoprotein can limit therapeutic concentrations of substrate drugs within the brain. Additionally, P-glycoprotein expression and/or function may be altered in the setting of HIV infection and in the setting of drug abuse.



Lavríková P, Fontana J. *Functions of Cells and Human Body,* Chapter 12.11, 2014.

OBJECTIVES

The **purpose of this study** was to analyze the impact of HIV-1 Tat and morphine on P-glycoprotein (Pgp) as a means to study the impact of substance abuse drugs on drug-efflux proteins in the BBB. This will be done by:

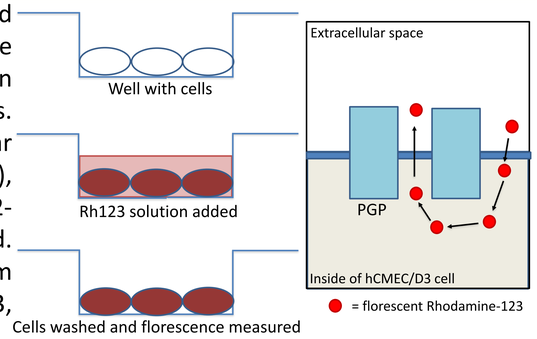
Analyzing Pgp function through Rhodamine-123 cellular accumulation studies

Analyzing Pgp expression through Western Blots

METHODS

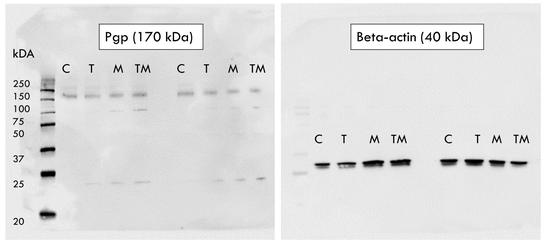
Experiment #1 — Rhodamine-123 Accumulation

In order to study the impact of morphine, a commonly used opiate drug of abuse, on drug-efflux proteins at the BBB, the effects of morphine and the HIV-1 protein Tat on P-glycoprotein function were studied via intracellular accumulation studies. hCMEC/D3 cells, a human derived brain microvascular endothelial cell line, were pre-treated for 24h with Tat (100nM), morphine (500nM), or Tat (100nM) + morphine (500nM). 12-well plates with 3 replicates of each treatment group were used. Accumulation was determined by measuring florescence from the prototypical P-glycoprotein substrate, rhodamine-123, following cell lysis.



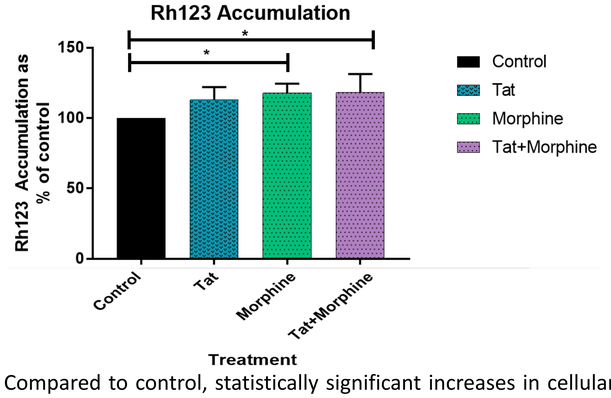
Experiment #2 — P-glycoprotein Western Blots

The effects of Tat and morphine on Pgp protein expression were measured by immunoblot. Gels were run in an electrophoresis box and protein was transferred to the membrane using a wet transfer. Blots were then blocked with milk and probed for both P-glycoprotein and beta-actin (to provide controls for each sample). C219 (1:200 dilution) was used for Pgp primary antibody, and anti-beta actin (1:4000 dilution) was used for beta-actin primary. Both samples used anti-mouse HRP-linked substrate (1:20000 dilution) for secondary antibodies.



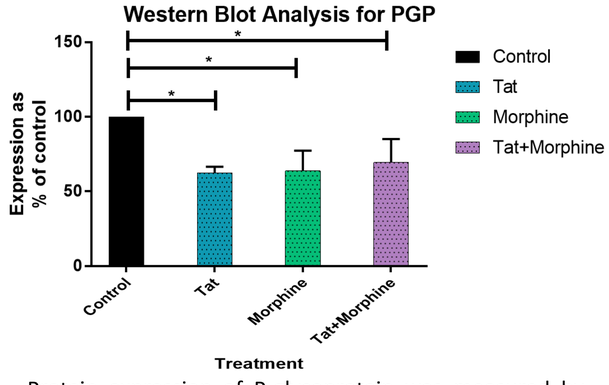
RESULTS

Finding #1 — Rhodamine-123 Accumulation



Compared to control, statistically significant increases in cellular accumulation of rhodamine-123 were observed in both the morphine (mean±SEM; 118 ± 6.5%, p<0.05) and Tat+morphine (118 ± 13.1%, p<0.05) groups. Increased Rh-123 accumulation indicates decreased P-glycoprotein function due to less substrate being effluxed from the interior of the cell.

Finding #2 — P-glycoprotein Western Blots



Protein expression of P-glycoprotein was measured by immunoblot analysis. Pgp expression was significantly decreased in all treatment groups as compared to control; Tat (63±4.2%, p< 0.05), morphine (64±13.5%, p<0.05) and Tat+morphine (69 ± 15.6%, p<0.05).

CONCLUSIONS

Increase in accumulation of Rhodamine-123 within cells treated with Tat, morphine, and Tat+morphine treatment groups indicate decreased ability of P-glycoprotein to efflux substrates out of the cell. This is complemented with decreased expression of the P-glycoprotein protein in endothelial cell membranes under each of the treatment groups. Both experiments indicate increased leakiness of the BBB when exposed to HIV-1 protein and opioids. This has implications in both determining substances that lead to increased BBB breakdown and factors that lead to increased permeability of cells to antiretroviral therapy drugs.

FUTURE STUDIES

Areas for further study include:

- Analysis of Tat and morphine on other drug efflux proteins, such as breast cancer resistance protein (BCRP) and the multidrug resistance proteins (MRPx).
- Intracellular accumulation of drugs used in the treatment of HIV (such as atazanavir or lopinavir) would allow for analysis of the impact of P-glycoprotein in efflux of antiretroviral therapy drugs.

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ACKNOWLEDGMENTS

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