[1]HMRS in a monkey model of AIDS and opiates

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Introduction: Opiate abuse is a major co-morbidity and risk factor for human immunodeficiency virus (HIV1). However, very little is known about possible interactions between HIV1 and opiates in the brain. To control for variables in human studies (e.g. other drugs of abuse, time of infection), several animal models have been developed to study HIV1-like infections. However, the simian (SIV) model is best suited for the study of HIV1 and drug use interactions since primates can be administered drugs in a similar way to humans, and SIV is virologically closest to HIV1 and produces similar neuropathology as HIV encephalitis.[1] Both human epidemiologic and in vivo monkey studies suggest that opiates can have both beneficial and harmful influences on AIDS progression.[2]

The Aim of this study is to investigate the possible interactive effects of SIV and opiates (morphine) on the brain using ex vivo [1]HMRS.

Methods: Four-year old male Rhesus macaques were randomly assigned to 1 of 2 treatment groups: SIV positive, saline controls (n=14); SIV positive, morphine dependant (n=16). Two healthy animals of similar ages that were not given injections and were not SIV infected were also included. During the first week of the study, the animals received 4 morphine (or saline)-injections daily, with a staggered ramping-up of dose from 1mg (2 days), 2 mg (2 days) to 3mg. After 2 weeks of morphine exposure, animals were infected with the sooty mangabey strain of SIVsmm9. They were maintained on morphine for variable times until sacrifice for, up to, 4 years. To avoid undue suffering from SIV related complications, animals were carefully monitored and euthanized by a barbiturate overdose after which they were exsanguinated followed by saline perfusion. Their brains were quickly removed, dissected, and stored at –80ºC. Sections of frontal gray matter, frontal white matter, putamen, and caudate were homogenized in 5 volumes 0.04 M HClO4 (based on wet weight) and centrifuged, twice. Supernatants were combined and 3-(Trimethylsilyl)- Propionic acid-D4, sodium salt (TSP) was added (final concentration 2.5 mM). A 0.425 ml sample was analyzed on a Bruker ADVANCE 400 spectrometer (9T) with a 5mm QNP probe at room temperature. The acquisition parameters were: 30º pulse, 6µs, 4100Hz spectral width, 0.125Hz/point, 128 averages, and acquisition time 4s. Signal areas were determined using integrals of the peak area referenced to TSP, following FT, phasing, and baseline correction. N-acetyl aspartate (NA), glutamate, gamma-aminobutyric acid (GABA), choline, creatine (CR), and myo-inositol (MI) concentrations were measured. Samples were run in duplicates and their values averaged. Metabolite concentrations were analyzed by repeated measure ANOVA for region (within subject) and drug exposure (between subjects). Post hoc analyses were performed to further define the significant effects.

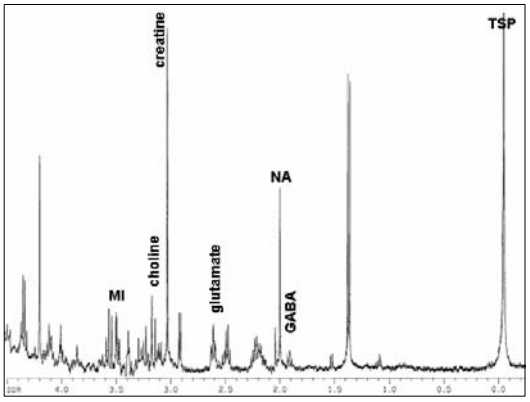

Figure 1: Typical [1]HMRS spectrum

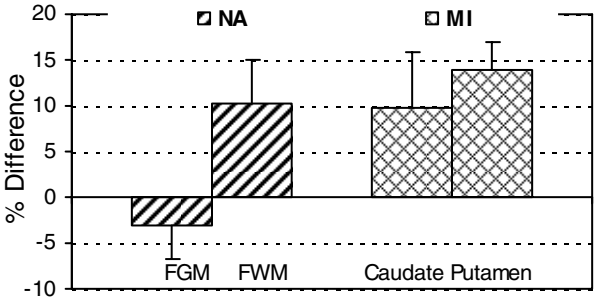

Figure 2: Percent change in metabolite concentrations in morphine-dependant SIV positive animals as compared to SIV positive saline-control animals. NA: N-acetyl aspartate, MI: myo-inositol, FGM: frontal gray matter, FWM: frontal white matter

Results: There were no significant differences in disease severity between SIV groups as measured by CD4 count, CD4/CD8 ratios, or survival time from infection. The average age at time of death was 9.7±0.3 years, with no significant difference between groups. Consistent with prior studies, there were significant regional variations in all measured metabolites. ANOVA also showed region by treatment interactions for NA (p=0.0003) and MI (p=0.04). Post-hoc analysis showed the morphine-dependant animals had significantly higher (10%, p=0.01) frontal white matter NA (saline control levels were non-significantly lower than the 2 healthy animals), as well as strong trends for higher caudate (10% p=0.06) and putamen MI (14% p=0.09). Morphine-dependant animals had slightly lower NA in the frontal gray matter than saline controls (3% p=0.09).

Discussion: Similar to studies of HIV1[3] and other SIV models,[4] the decreased frontal gray matter NA and increased striatal MI in morphine-dependant compared to saline control animals suggest neuronal injury and glial activation in these regions. However, the white matter appears to be spared in the morphine-dependant animals. These findings are consistent with combined HIV and other drugs of abuse studies.[2] Our findings are also in agreement with other SIV and opiate studies that reported opiates may have differing influences on disease progression based on SIV strain, opiate dosing, dependence, and or withdrawal state, leading to either exacerbation of disease severity or to protective effects.[2] These findings demonstrate that our model is useful for understanding the combined or interactive effects of viral-induced immunosuppression and opiates.

References

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