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CSF1R Inhibition Targets CNS Macrophages in an SIV/Macaque Model of HIV CNS Disease

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Abstract

CNS macrophages, including microglia, serve as a latent HIV cellular reservoir.[1,2] Colony stimulating factor 1 receptor (CSF1R) is expressed exclusively on cells of myeloid lineage and is essential for microglial survival.[3] We have previously shown that CSF1R protein levels increase with SIV infection and remain elevated in the CNS despite suppressive ART.[4] Other groups have described the same pattern of CSF1R protein expression with HIV infection.[5] Consequently, CSF1R is an ideal drug target to reduce the CNS latent reservoir. PLX3397 (Pexidartinib) is an FDA-approved, small-molecule inhibitor of CSF1R[6] and has been shown to effectively eliminate microglia in rodent models.[7] Here, we cultured primary brain and spinal cord microglia and treated cells with 10μM PLX3397 for three days. Microglial viability was significantly reduced as observed by calcein (live) and ethidium (dead) staining, as well as interferon-beta qPCR ($P < 0.0001$, Two-Way ANOVA). *In vivo* studies were conducted using daily oral treatment of 165mg/kg PLX3397 in ART-suppressed SIV-infected pigtailed macaques (N = 2). Two weeks after the start of PLX3397 treatment, ART was stopped while PLX3397 treatment continued for 16 days. Drug treatment was well tolerated; animals did not show side-effects or develop monocytopenia. PLX3397 treatment did not significantly affect plasma viral rebound kinetics. However, treatment did prevent CSF rebound in one animal. In addition, neither SIV RNA nor DNA was detected in cultured primary microglia from this animal. In the second animal, viral RNA was isolated from CNS macrophages cultured from both brain and spinal cord. We conclude that PLX3397 reduced CNS macrophage viability *in vitro*, demonstrating that targeting CSF1R may reduce CNS macrophages, including those harboring HIV. Treatment was associated with a lack of SIV rebound from the CNS and a decrease in IBA1+ CNS macrophages in one of two animals after stopping ART. These studies demonstrate the potential of targeting CSF1R to reduce the HIV latent reservoir in the CNS.

Methods

Animals: All samples were collected at necropsy from male juvenile pigtailed macaques (*Macaca nemestrina*). Groups included:

1. Uninfected controls (mock inoculated)
2. SIV IV inoculated with SIV 17E-Fr (neurovirulent molecular clone) and B670 (immunosuppressive swarm) + ART (subcutaneous injection 2.5mg/kg Dolutegravir, 20mg/kg PMPA, 40mg/kg FTC; 20mg oral Maraviroc). Fully suppressed for >150 days and then released from ART. Euthanized at peak plasma viral loads (indicated by plateau). (Release)
3. SIV IV inoculated + ART + Release; + 165mg/kg daily oral PLX3397 2 weeks prior to ART release. PLX3397 treatment continued until euthanasia (approx. 2 weeks post release). (PLX1; PLX2)

All animal use was approved by the Johns Hopkins Animal Care and Use Committee.

Microglia in vitro: Microglia from uninfected pigtailed macaques were isolated as previously described.[8] Microglia were treated with 10uM PLX3397 or vehicle alone for 72 hours. Brightfield immunofluorescence images were taken at each timepoint. Immunofluorescence for live (calcein; cat. C1430, ThermoFisher) and dead (ethidium; cat. E1169, ThermoFisher) was performed as previously described.[9] Cell lysates were collected at each timepoint. IFN-beta gene counts were used to determine cell count as previously described.[1] Microglia from PLX3397-treated animals were conducted in the same manner. SIV RNA was collected every two days and quantified by qRT-PCR.

Viral load quantification: SIV RNA was quantified in plasma and CSF by qRT-PCR and ddPCR as previously described.[1] Plasma and CSF were collected every four days post ART release.

IBA1 IHC: IHC was performed on STF-fixed frontal cortex sections of Release and PLX3397-treated animals using anti-IBA1 (cat. 019-19741, Wako) at a dilution of 1:500 and counterstained with hematoxylin. Positive staining was visualized using the Bond Polymer Refine Red kit (Leica Biosystems). Images were taken using Nikon Elements.

Results

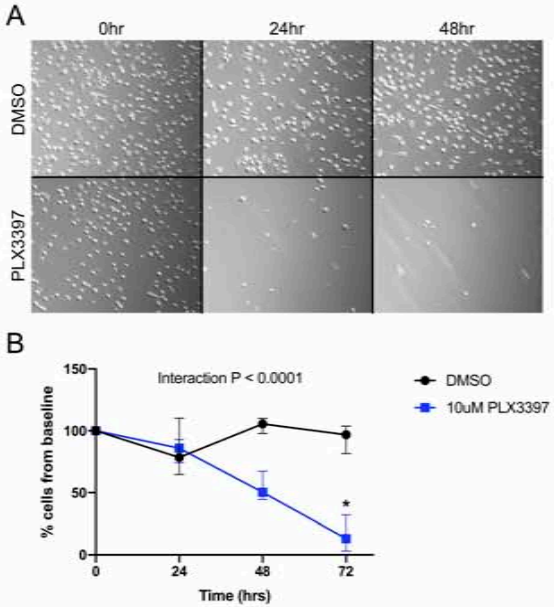

Figure 1. 72 hours of PLX3397 treatment depletes microglia *in vitro*. Uninfected pigtailed macaque microglia were treated daily with 10μM PLX3397 in DMSO or with vehicle (DMSO) alone. (A) Phase microscopy showed a visible decrease in adherent cells over 48 hours of PLX3397 treatment compared to vehicle control. (B) Cell counts were determined with interferon-beta qPCR. After 72 hours of PLX3397 treatment, microglia cell number decreased approximately 84% from baseline. This decrease was significant ($P = 0.03$, Sidak's multiple comparisons test) and was due to PLX3397 treatment over time (Two-way ANOVA $P < 0.0001$).

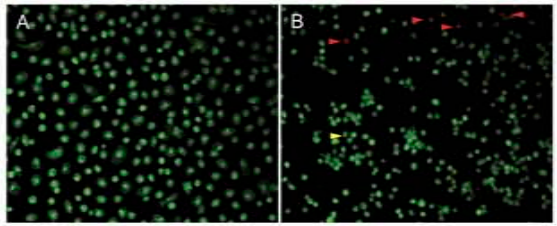

Figure 2. PLX3397 kills primary pigtailed macaque microglia *in vitro*. Primary microglia isolated from an uninfected pigtailed macaque were treated for 48 hours with vehicle (DMSO) (A) or 10μM PLX3397 (B). Live microglia were identified with the uptake of calcein (green), and dead cells were identified with the uptake of ethidium homodimer (red). PLX3397 treatment (B) killed cells, and the remaining live cells were smaller and arranged in clusters.

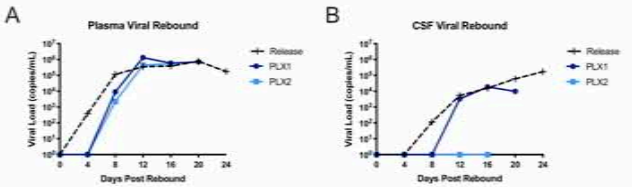

Figure 3. The effect of PLX3397 on plasma and CSF viral rebound kinetics in SIV-infected macaques released from ART. (A) The kinetics of viral rebound in the plasma of PLX3397 treated animals was no different compared to procedural control animals (Release). (B) While the viral rebound kinetics in the CSF was not affected in PLX1, CSF viral rebound did not occur in PLX2 for 16 days post-release from ART.

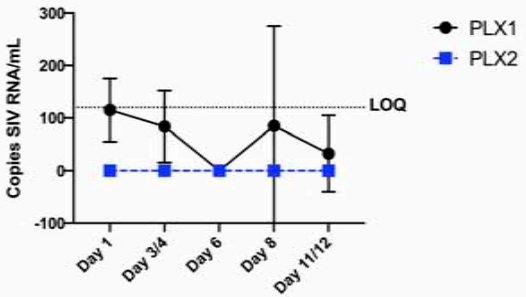

Figure 4. Viral outgrowth of microglia isolated from SIV-infected macaques treated with PLX3397 and released from ART. Virus RNA was detectable in the supernatants of microglia isolated from the brain of PLX1 (black). However, virus RNA was undetectable in the supernatants of microglia isolated from the brain of PLX2 (blue). Limit of quantitation (LOQ) was 120 copies viral RNA/mL. Limit of detection was 1 copy viral RNA/mL.

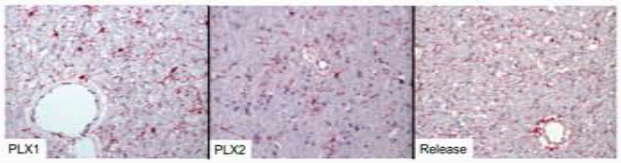

Figure 5. The effect of PLX3397 treatment on microglial viability *in vivo*. IBA1+ immunostaining (red) in frontal cortex white matter of PLX1 showed altered microglial morphology, including enlarged nuclei and longer projections as compared to procedural controls (Release). In PLX2, there was a reduction in IBA1+ staining compared to procedural controls. Additionally, the remaining microglia had similar morphologic changes as seen in PLX1.

Discussion

Addressing all sites of the HIV latent reservoir is essential to curing HIV. CNS macrophages, including microglia, serve as a latent reservoir for HIV.[1,2] We propose that PLX3397 treatment, which blocks CSF1R signaling, is an effective way to deplete this latent reservoir. While PLX3397 treatment did not totally deplete microglia, it did cause a decrease in the microglial number. This may be due to a lack of maximal drug penetrance. It is not clear whether maximal drug concentrations in plasma and CSF were reached in macaques in this study. Further pharmacokinetic and pharmacodynamic studies will need to be conducted to determine the proper drug dosing strategy.

There was no evidence of viral rebound in the CSF of PLX2. We hypothesize this is due to a reduction in the viral reservoir in the CNS. It is possible that if this study were taken out longer, PLX2 would eventually rebound in the CSF. However, this delay is important to note, and is most likely due to a reduction in the latent reservoir. PLX3397 treatment had no effect on plasma viral rebound in either animal, showing that this drug targets the CNS reservoir rather than all tissue viral reservoirs. Treating more animals with PLX3397 will elucidate whether this drug is consistently effective at reducing the SIV CNS latent reservoir. PLX3397 may be an effective treatment to reduce the viral reservoir in the CNS.

Conclusions

- PLX3397 effectively killed pigtailed macaque microglia *in vitro*
- PLX3397 treatment was well tolerated in pigtailed macaques with no observable side-effects.
- CSF viral rebound did not occur in one of two animals treated with PLX3397 after release from ART.
- Microglia isolated from this animal did not produce replication-competent virus.
- PLX3397 treatment in two SIV-infected pigtailed macaques reduced the number of microglia *in vivo*. Remaining microglia had an altered morphology compared to procedural controls.

Acknowledgments

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