Novel adjunctive therapies for cerebral malaria that target metabolism

Tuan M. Tran,1,* Emile B. Gordon,1 Geoffrey T. Hart,1 Michael Waisberg,2 Munir Akkaya,1 Ann Kim,1 Sara E. Hamilton,2 Minna Penäl,1 Takeye Yazew,1 Chen-Feng Qi,1 Louis H. Miller,2 Jonathan D. Powell3 and Susan K. Pierce1
*These authors contributed equally to this work

Laboratory of Immunogenetics and 1Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, 2Sidney Kimmel Comprehensive Cancer Research Center, Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD, and 3Department of Laboratory Medicine and Pathology, Center for Immunology, University of Minnesota Medical School, Minneapolis, MN, 4Division of Infectious Diseases, Indiana University School of Medicine, Indianapolis, IN

Abstract (Presentation #1606)

Background: Cerebral malaria (CM) caused by Plasmodium falciparum has a case fatality rate of 30–50%. Infants die within 24–48 hours of presentation in 600,000 malaria deaths annually despite effective antimalarial chemotherapy. Currently, there are no adjunctive treatments for CM, emphasizing the need to identify novel targets for therapy. Using the mouse model of CM, experimental CM (ECM), we have demonstrated that targeting specific metabolic pathways important to the immune response protects against the progression of ECM and improves survival. We recently showed that treatment with sirolimus, which inhibits the kinase mammalian target of rapamycin (mTOR), within 4 days of infection increased survival and decreased the accumulation of CD4+ and CD8+ T cells as well as parasitized erythrocytes in the infected brain despite an increase in systemic inflammatory responses. Transcriptomic analysis of infected brains revealed inhibition of cellular trafficking and cellular proliferation during sirolimus treatment. In the current study, we targeted glutamine metabolism, which plays a critical role in the activation and proliferation of T cells.

Methods: We infected C57BL/6 mice with P. berghei ANKA, which causes ECM, and treated mice with an active site inhibitor of glutaminase or saline during late-stage ECM, at a time when animals exhibit profound neurological signs of infection. We determined differences between treatment and control groups in terms of survival, clinical scores, immunopathology in the brain, and metabolomics within the brain, liver, and serum.

Results: Treatment of infected mice with the glutaminase inhibitor, but not saline, rescued mice from ECM, restored blood brain barrier integrity, decreased brain swelling, reduced the function of activated effector CD8+ T cells, and reversed infection-induced metabolic changes specifically in the brain.

Conclusions: These results provide evidence that targeting metabolic pathways critical to the host immune response may be a useful strategy for the development of highly selective adjunctive therapies for CM.

Significance

CM is a deadly complication of P. falciparum infection in African children despite effective antimalarial treatment. Once signs of neurological disease have commenced, there is no adjunctive treatment for CM, and overall mortality remains high. Thus, a treatment that arrests disease and promotes healing in the late stages is urgently needed. Here we report a novel model of CM, that the glutamine (Gln) analog 6-diazo-5-oxo-l-norleucine (DON) is an effective therapy even when treatment is initiated after infected animals show neurological signs of disease. Within hours of DON treatment, blood-brain barrier integrity was restored, and brain swelling was reduced. These results suggest DON as a strong candidate for an effective adjunctive therapy for CM in African children.

Study Design

FIG. 1. DON treatment schedule. DON treatment (1.3 mg/kg) was initiated on day 5 p.i. at 7:00 AM (D0x Rx d5a), or day 6 p.i. at 7:00 AM (D0x Rx d6a) and was continued every day or every other day as shown.

Results

FIG. 2. DON treatment reduced the mortality associated with ECM. C57BL/6 mice were infected with PBA on day 1 p.i. and were injected ip. with saline (NaCl) (n = 49) or with DON (1.3 mg/kg) beginning on day 5 p.i. at 7:00 AM (DON Rx d5a) (n = 28), on day 6 p.i. at 7:00 AM (DON Rx d6a) (n = 28). DON treatment was continued every day or every other day as shown in FIG. 1. (A) Kaplan-Meier survival plots. (B) Clinical scores from 0 (no symptoms) to 10 (morbund) of mice in A. (C) Peripheral blood parasitemia in mice in A. (D) Data for DON Rx d5a were combined from three independent experiments, data for DON Rx d6a were combined from two independent experiments, and data for DON Rx d5a were combined from two independent experiments. Data in A (and B) are shown as mean ± SEM. (E) Fold change in PBA 18S RNA in brains of DON-treated and untreated PBA-infected mice on day 6 p.i. Data were analyzed with PBA 18S RNA in brains of PBA-infected mice on day 6 p.i. Each dot represents a mouse with the mean and SD given. The results shown are combined from three independent experiments, each having three or four mice per group. A Mann-Whitney test showed no significant difference.

FIG. 3. DON treatment promoted BBB function and reduced brain swelling but did not accurately resolve brain hemorrhages in PBA-infected mice. All mice were infected with PBA and treated with saline or DON (1.3 mg/kg) on day 5 p.i., and the brains were removed and analyzed on the days and times indicated. (A–C) Representative images of the brains of mice injected with EB. (B) EB levels in the brains were quantified and expressed relative to the EB levels in the brains of PBA-infected, untreated mice on d13a p.i. Each symbol represents one mouse. The data are combined from three independent experiments and are shown as mean ± SD. (D) Brain water content expressed as the weight of each brain after desiccation divided by the weight before desiccation X 100 is given. Each symbol represents one mouse. Data are combined from two independent experiments and are shown as mean and SD. (E) Representative images of brain sections showing hemorrhages in the late stage were stained with anti-glyceraldehyde-3-phosphate dehydrogenase antibodies and were visualized by white arrows. (F) Quantification of brain hemorrhages. Each symbol represents one mouse. Data are combined from three independent experiments. Mann-Whitney tests were used for comparison of groups (**P<0.005, ***P<0.0005).

Discussion

1) The anti-parasitic activity of DON may contribute to its ability to arrest ECM and promote healing, but we do not believe it plays a critical role.
- DON had little effect on the parasite load in the brains of PBA-infected mice during the critical period when the BBB was restored and brain swelling decreased in DON-treated mice.
- DON treatment of PBA-infected mice did not prevent or decrease the accumulation of CD8+ T cells in the brain, but the number of CD8+ T cells that depleted was decreased following DON treatment.
- This suggests that DON’s protective effect is mediated by blocking CD8+ T-cell effector function rather than by preventing the proliferation and generation of CD8+ effector cells.
2) More than 10 metabolic changes in the brains of infected mice compared with uninfected mice that were either reversed or blocked by DON.
- The complexity of the fluctuations in Gln and Glu levels in the brain during infection and upon treatment most likely reflects DON’s inhibition not only of glutamine activity but also of Gln transport and other Gln-using enzymes.

Summary

1. The Gln analog DON is an effective therapy for ECM even when treatment is first initiated after infected animals show neurological signs of disease.
2. This clinical response was accompanied by the ability of DON to inhibit pathology as measured by decreases in BBB dysfunction, brain swelling, and degradation of parasite-specific CD8+ T cells that accumulated in the brain.
3. DON is able to reverse or prevent metabolic changes associated with the disease state.
4. The ability of DON to promote survival at such a late stage of the disease when animals are suffering from BBB dysfunction and brain swelling distinguishes our findings from all other attempts to treat and reverse ECM.

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Suggested References

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