

Doxycycline alters inflammatory immune reactions to *Plasmodium Berghei* ANKA and inhibits experimental cerebral malaria in infected C57BL/6 mice

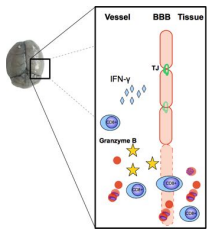
Janina M. Küpper¹, Kim E. Schmidt¹, Beatrix Schumak¹, Judith Alferink^{2,3}, Sabine Specht¹ & Achim Hörauf¹

1 Institute of Medical Microbiology, Immunology and Parasitology (IMMIP), University of Bonn

2 Institute of Molecular Psychiatry, University of Bonn

3 Department of Psychiatry, University of Münster

INTRODUCTION



Cerebral Malaria (CM) in humans is life-threatening complication due to infection with *Plasmodium falciparum*. CM Symptoms include convulsion and coma, which are caused by sequestration of parasites in the brain and local inflammation. CM treatment is based on anti-parasitic approaches but is not specific for cerebral symptoms.

Doxycycline (DOX) is an antibiotic, which has in addition well-known anti-parasitic effects. The tetracycline derivate elicits also anti-inflammatory as well as anti-apoptotic properties and inhibits matrix metalloproteinases, relevant for blood-brain-barrier stability.

Aim: Further investigation of DOX-mediated effects on inflammation after experimental *Plasmodium* infection, with regards to the previous finding that DOX prevents experimental CM in a mouse model by inhibiting parasite growth and cerebral inflammation¹.

METHODS

Experimental model / Infection

C57 Bl/6 mice were infected with *Plasmodium berghei* ANKA (PbA) → Well known model for experimental cerebral malaria (ECM)

For antigen specific assays, a transgenic strain containing ovalbumin was used (PbTG)

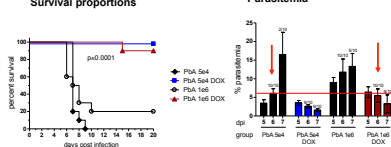


Analysis

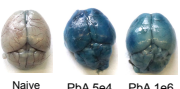
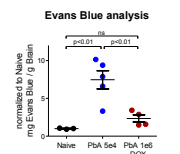
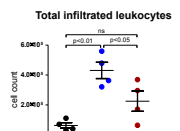
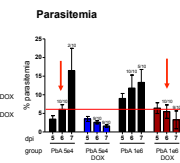
- Survival & Parasitemia
 - Monitoring of mice from dpi 4 till coma or severe anemia
- Ex vivo analysis on dpi6
 - Blood brain barrier integrity (Evans Blue assay)
 - Cytokine production of lymphocytes from brain and spleen (ELISA and FACS)
 - Cell composition and activity of brain and spleen (FACS)
 - In vivo cytotoxicity (in vivo kill) to analyse OVA-derived MHC class I peptide SIINFEKL specific CD8 lytic activity

RESULTS

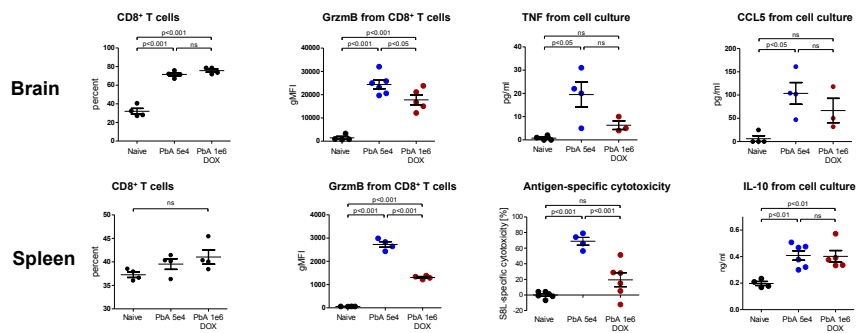
A.) DOX-treatment prevented ECM in PbA infected mice even if these received an elevated parasite dose (1e6 iRBCs vs 5e4 iRBC). Parasitemia of DOX-treated mice infected with a high dose of iRBCs was comparable to control-infected PbA mice (infected with 5e4 iRBC) without DOX treatment.



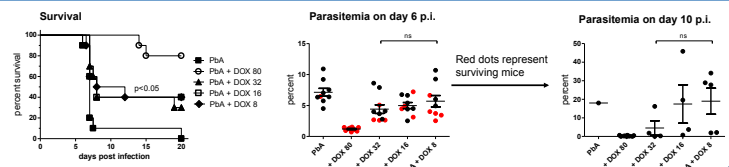
The blood brain barrier remained stable in DOX treated 1e6 PbA mice.



B.) DOX treatment did not change the composition of immune cells in brain or spleen but attenuated their (antigen-specific) activity, in parallel to reduced expression of activation markers and cytokine production. Release of immunoregulatory interleukin 10 was not affected by DOX treatment.



C.) Sub-anti-microbial doses of DOX prevented ECM independently of parasite reduction



SUMMARY & DISCUSSION

- We could previously show that DOX-treatment of PbA-infected mice from day 4 p.i. resulted in a 100% protection from experimental CM and a reduced inflammatory response. However, DOX also reduced the parasitemia from dpi 6 on (two days after start of treatment)¹ due to well-known antiparasitic effects.
- To exclude that the protective effects were solely caused by decreased parasite burden, we infected mice with a 20fold elevated parasite dose (1e6 iRBCs) to reach comparable parasitemia as in untreated mice that were ECM positive on dpi 6. "High dose" infected animals showed mild symptoms on dpi 6 but recovered quickly and survived past the time point of ECM onset.
- Analysis of brain and spleen tissue of PbA infected animals ±DOX treatment showed that the general activity and the antigen-specific cytotoxicity of immune cells was significantly reduced after DOX treatment. These findings correlate to observations in earlier studies, leading to the assumption that protection of mice infected with the reference dose of 5e4 iRBC upon DOX treatment were due to a combination of DOX-mediated parasite reduction AND immunoregulation.
- Pilot data indicate that treatment of PbA infected mice (5e4 iRBC) with sub-anti-microbial doses of DOX that do not affect the parasite burden is sufficient to protect from detrimental excessive inflammatory processes in the brain.

CONCLUSION

The analysis of DOX-mediated protection of PbA infected mice from experimental CM strongly suggest that DOX induces strong immune modulatory properties that are sufficient to prevent ECM even in conditions of elevated parasitemia, which were lethal for non-treated mice. These observations might be valuable for the treatment options of human CM and help reducing mortality.

REFERENCES

¹Schmidt KE, Kuepper JM, Schumak B, Alferink J, Hofmann A, Howland SW, Rénia L, Limmer A, Specht S, Hörauf A. Doxycycline inhibits experimental cerebral malaria by reducing inflammatory immune reactions and tissue-degrading mediators. PLoS One. 2018 Feb 13;13(2):e0192717.

FUNDING

