

Erythropoietin improves cerebral malaria outcome in mice by attenuating brain edema and enhancing perfusion

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Target audience: scientists, physicians and pharmaceutical companies

Objectives: Malaria is a cause of morbidity and mortality with an estimated death toll close to 1 million in 2008. Cerebral malaria (CM), the most lethal complication in the course of *Plasmodium* infection, leads to death in 15-20 % of the cases. Although antimalarial drugs can kill the parasite and clear parasitemia, they often remain inefficient against CM. Thus, there is an urgent need for fast-acting adjunctive therapies that could efficiently treat this cerebral syndrome. Recently, erythropoietin (EPO) was found to reduce mortality in experimental CM¹. We previously proved the fatal role of brain ischemic edema in experimental CM and provided reliable MR markers of the disease². Here, our purpose was to decipher the mechanisms by which EPO ameliorates CM in mice infected with *Plasmodium Berghei* ANKA (PbA). To this end, we used an approach combining MRI techniques at high field (11.75T) for in vivo longitudinal cerebral studies², with immunological and histological techniques.

Methods: Animal studies were approved by our institutional committee on Ethics in animal research. Eight-week old female CBA/J mice were inoculated with parasitized red blood cells (RBC)². The animals were monitored daily for the clinical signs of CM² and parasitemia was assessed with Giemsa-stained blood smears. CM death occurs within 7-10 days after PbA infestation in the CBA/J strain. At d5 post-infestation, the animals were divided into two groups receiving either daily injections of EPO, or vehicle (sham-treated animals). The animals were imaged on an 11.75 T vertical Bruker AVANCE 500 WB wide bore MR system (Bruker, Germany) using a transmitting and receiving head resonator of 2.5 mm. The MRI follow-up started one week before PbA infestation (d-7) and extended to d20 post-infestation in EPO-treated mice. Brain T₂-weighted images were acquired using a spin-echo sequence (TE/TR= 37.1/5000 ms; matrix, 256²; FOV, 20² mm²). Angiography was performed with a 3D-gradient echo TOF sequence (TE/TR= 3.6/30 ms; flip angle, 50°; matrix, 256x192x64; FOV, 18x12x9 mm³). Cerebral perfusion was evaluated using a multi-slice perfusion imaging technique based on pseudo-continuous ASL (pCASL) as previously described³. T₂-weighted images were processed under ImageJ, while quantitative cerebral blood flow (CBF) maps were generated with a homemade software under IDL environment³. Quantitative CBF analysis was performed in different brain regions with ROIs positioned in the cortex, the caudate putamen and thalamus. Hematological data (red blood cell counts) were obtained in control animals, in EPO-treated CM mice (d8, d15 and d20 post-infestation), and in sham-treated CM mice (d8 only). Sham-treated CM mice were sacrificed on d8 (moribund stage) for brain histology (hematoxylin and eosin staining), or adhesion molecule analysis in brain tissue (E-selectin, ICAM-1, VCAM-1) with the Luminex technique. EPO-treated CM mice were sacrificed at d8, d15 or d20 for the same analyses.

Results: We observed a better outcome in EPO-treated CM mice compared to sham-treated animals, with a survival rate of 60% at d20 post-infestation. EPO-treated CM mice were explored at d8, d15 and d20 post-infestation. T₂-weighted MRI revealed an absence of brain swelling, and of parenchyma lesions typical of the cerebral syndrome² in EPO-treated CM animals, on the contrary to sham-treated CM mice. Moreover, these animals were normal at angiography, whereas sham-treated CM animals showed flow voids on angiography². Quantitative CBF maps showed higher brain perfusion in EPO-treated CM mice in comparison to sham-treated CM mice at d8, although not reaching the statistical significance (Fig1.A-B). At day 15, a large increase in CBF was measured which was significant in almost all brain regions as illustrated in the case of the caudate putamen (Fig.1B). This rise in CBF was concomitant with a significant increase in red blood cell count (Fig.1C). The analysis of adhesion molecules content in brain tissue extracts with the Luminex assay showed lower levels of E-selectin, ICAM-1, VCAM-1 in EPO-treated CM mice in comparison to sham-treated CM animals

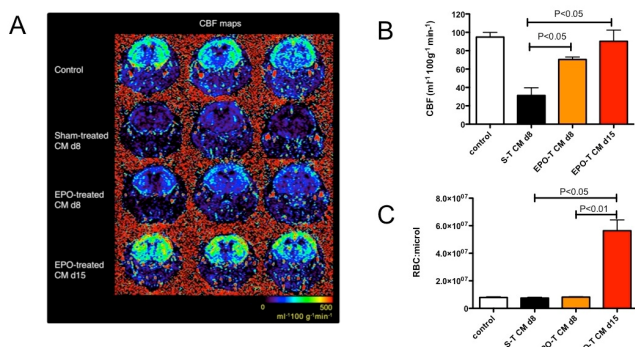


Figure 1: Impact of EPO treatment on CBF and RBC counts. (A) Typical CBF maps of a control mouse, a sham-treated CM mouse at d8, and an EPO-treated mouse at d8 and d15 after PbA infestation. (B) CBF values in the caudate putamen of control animals (n=10), of sham-treated (S-T) CM mice at d8 (n=10), of EPO-treated (EP-T) mice at day 8 (n=9) and at d15 (n=4). (C) RBC counts of control animals (n=9), of sham-treated (S-T) CM mice at d8 (n=8), of EPO-treated (EP-T) mice at day 8 (n=5) and at d15 (n=5).

Discussion and conclusion: Our results show that administration of EPO prevents brain edema in responding animals, while brain swelling is responsible for artery compression and death in sham-treated CM mice. The absence of significant brain swelling in responding mice could be partly linked to the non-erythropoietic effects of EPO, which may involve rapid multiple protective mechanisms, such as immunomodulation, protection of microvasculature, modulation of astrocyte swelling, and anti-oxidative effects. Indeed, our results indicate that EPO treatment ameliorates CBF as early as d8 post-infestation when the RBC counts in EPO-treated animals are still within the normal range. Moreover, the decrease in adhesion molecules indicates an attenuation of inflammatory processes in EPO treated mice. Our data also demonstrate that the improvement in brain perfusion at d15 is correlated to the stimulation of erythropoiesis, a process that becomes overt at d15. In addition to the enhancement of CBF, RBC proliferation could probably reduce parasitemia and counteract anemia caused by PbA. In conclusion our results demonstrate that EPO early protective effects are non hematopoietic, while long-term effects involve erythropoiesis stimulation. Our study contributes to a better understanding of EPO protective mechanisms, which is crucial for the optimization of this potential adjunctive therapy, but also for the use of alternative drugs such as the already existing synthetic EPO derivatives

References

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