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Modulation of cerebral malaria by fasudil and other immune-modifying compounds

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ABSTRACT

Malaria continues to cause millions of deaths annually. No specific effective treatment has yet been found for cerebral malaria, one of the most severe complications of the disease. The pathology of cerebral malaria is considered to be primarily immunological. We examined a number of compounds with known effects on the immune system, in a murine model of cerebral malaria. Of the compounds tested, only fasudil and curcumin had significant effects on the progression of the disease. Although neither drug caused a reduction in parasitemia, survival of the treated mice was significantly increased, and the development of cerebral malaria was either delayed or prevented. Our results support the hypothesis that an immunomodulator efficient in preventing CM should be administered together with anti-plasmodial drugs to prevent severe malaria disease; curcumin and fasudil should be further investigated to determine efficiency and feasibility of treatment.

1. Introduction

The severe complications of *Plasmodium falciparum* infection cause high morbidity and approximately two million deaths annually. Many of these deaths are due to cerebral malaria (CM). About 7% of *P. falciparum* malaria cases progress to CM, characterized by the presence of neurological features, especially impaired consciousness (Maitland and Newton, 2005). Clinical manifestations of CM include three primary symptoms generally common to both adults and children: impaired consciousness with non-specific fever; generalized convulsions and neurological sequelae; and coma that persist for 24–72 h, initially rousable and then non-rousable (Newton and Krishna, 1998; Idro et al., 2005).

The pathogenesis of CM results from the adherence and sequestration of parasitized erythrocytes (pRBC), immune cells and platelets to vascular endothelial cells lining the small blood vessels of the brain (Coltel et al., 2004). Thus, parasite-triggered cerebral inflammation is a possible cause of death from CM. The harmful, deregulated immune response leading to CM is mainly of the Th1 type, with overproduction of some cytokines, such as IFNγ, combined with underproduction of others (e.g. IL-10) (Hunt and Grau, 2003). A preventive measure that will eliminate or reduce even a single factor associated with the cascade of events leading to CM may completely avert it (Randall et al., 2008).

Current CM treatment consists mainly of supportive therapy, such as anti-pyretics and anti-convulsants, and the administration of an effective and rapidly active parasiticidal drug, usually quinine (Golenser et al., 2006; Idro et al., 2005). As parasite resistance to quinine is increasing globally, artemisinin and its derivatives have become the drugs of choice for CM combination therapy (Idro et al., 2005; Newton and Warrell, 1998). With current treatments, mortality rates due to CM remain up to 30% (Idro et al., 2005; WHO, 2000). A main obstacle is finding a treatment that prevents the advanced stages of CM, once symptoms of impaired consciousness are observed, but before coma sets in.

Immunomodulatory compounds may represent an interesting new approach to CM treatment. The Rho kinase inhibitor fasudil (Yamaguchi et al., 2006), a drug already in clinical use, may protect endothelial cells from pRBC-induced apoptosis and damage mediated by plasmodial antigens and adhesion molecules (Taoufiq et al., 2008), by direct inhibition of glutamate-induced neurotoxicity (Yamashita et al., 2007) and by suppression of the inflammatory response (Satoh et al., 1999). In addition, fasudil inhibits contraction of cerebral arteries (Masumoto et al., 2000), leading to vascular dilation and an increase in cerebral blood flow (Yamaguchi et al., 2006; Rikitake et al., 2005). Fasudil was shown to be effective and well tolerated in phase 2 trials in patients with cerebral ischemic stroke (Shibuya et al., 2005). Ischemic stroke and cerebral malaria have common mechanistic denominators: CM can easily be considered an ischemic/reperfusion event. Therefore, a remedy for one of these diseases may be effective for treatment of the other. These ideas have been suggested in several publica-

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Table 1
Experimental compounds examined for possible effects on murine cerebral malaria.

Compound	Mouse	Treatment schedule			Treatment effect
		Dose	Days p.i.	Method	
Fasudil	ICR	10 or 25 mg/kg/d	1–9	Intra-peritoneal injection	No significant effect on parasitemias or survival rates, treated vs. control mice
	ICR[a]	10 or 25 mg/kg/d	1–9	Intra-peritoneal injection	No significant effect was seen on parasitemias or survival in the 10 mg/kg/d group. 25 mg/kg/d reduced CM to 40% (vs. 100% in the control group) and prolonged survival from a median of 7 days to one of 12 days
	C57Bl/6	25 mg/kg/d	1–9	Intra-peritoneal injection	No significant effect on parasitemias or survival rates, treated vs. control mice
Curcumin	C57Bl/6	50 mg/kg/d	0–5	Gavage	No CM in the treated group; 60% CM in the control group
	ICR	25 or 2 × 25 mg/kg/d	0–5	Gavage	63% and 50% CM vs. 56% (control); significantly increased survival in the 50 mg/kg/d group (mean days of death – day 9, control, vs. day 11 and day 23 p.i. in the treated groups)
	ICR	50 or 100 mg/kg/d	0–5	Gavage	67% and 60% CM vs. 77% (control); toxicity leading to mouse death observed in both treated groups (25% and 38%)
Iodine	ICR	1%, 0.5%, 0.25% or 0.13% iodine v/v[b] in 0.1 ml saline, twice a day	1–5	Gavage[c]	No positive effect on CM. Survival was negatively affected in groups administered 1% or 0.5% iodine: 40% and 13% of treated mice succumbed due to drug toxicity, respectively. No significant effect on parasitemia or survival was seen in the remaining groups
NDGA	ICR	12.5 or 25 mg/kg	1–4	Intra-peritoneal injection	70% and 100% CM, respectively, vs. control 80% CM. No effect on survival was seen.
PADMA-28	ICR	167 mg/kg/d followed by 67 mg/kg/d	0–2, 4–20, every other day	Intra-peritoneal injection	80% CM vs. 70% in the control group; a slight effect on survival was seen on days 10–18 p.i. ($p < 0.0001$).
Thalidomide	C57Bl/6	2 × 20 or 2 × 60 mg/kg/d	0–5	Intra-peritoneal injection	Treatment postponed death from CM by two days; no significant effect on survival was seen
Cannabidiol	C57Bl/6	$2 \times 5^{-2} \times 10$ mg/kg/d	0–5	Intra-peritoneal injection	No effect on the rate of CM (90–100% in control and treated groups) or survival
Melatonin	C57Bl/6	2 × 5 mg/kg/d	0–5	Intra-peritoneal injection	No effect on the rate of CM (90–100% in control and treated groups) or survival
N-acetylcysteine amide (AD4)	C57Bl/6	2 × 50 mg/kg/d	0–5	Intra-peritoneal injection	No effect on the rate of CM (90–100% in control and treated groups) or survival

Note: Mice were infected with 5×10^4 PbA-infected erythrocytes, and treated as described post-infection (p.i.).
[a] Mice were infected with PbA-GFP.
[b] Original formulation: 2% iodine by weight (Magnascent™ Iodine, Magnascent, Bedford, Texas, USA).
[c] Mice underwent overnight fasting before drug administration.

tions (Gallien et al., 2007; Penet et al., 2005; Sanni et al., 2001). In view of their in vitro results and the known in vivo effects in human pathologies, Taoufiq et al. (2008) suggested fasudil as an immunomodulator which may alleviate cerebral malaria.

Curcumin, a polyphenolic organic molecule derived from turmeric, has anti-inflammatory properties as well as in vitro antiplasmodial activity. Curcumin inhibits activation of nuclear factor kappaB (NFκB) by IL-1 and TNFα (Brennan and O'Neill, 1998), inhibits production of TNFα and NO· by macrophages, and prevents induction of iNOS gene expression by suppression of IFNγ and IL-12 production (Gao et al., 2004; Pan et al., 2000). Artemisinin-type drugs have been shown to bind to and inhibit the key plasmodial enzymes sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA), an ATP-coupled Ca^{2+} ion pump involved in metabolic arrest, and histone acetyltransferase (HAT) (Golenser et al., 2006; Eckstein-Ludwig et al., 2003); curcumin displays good and optimal binding to HAT and SERCA, respectively (Singh and Misra, 2009; Cui et al., 2007). Curcumin has been shown to cause death of *P. falciparum* parasites by production of reactive oxygen species (Cui et al., 2007), and to reduce blood parasitemia in a murine model of malaria (Reddy et al., 2005). Therefore, we decided to examine the effect of curcumin on cerebral malaria.

Several additional compounds, chosen on the basis of known effects on the immune system or malaria-related pathologies, were examined for possible effects on CM development. Treatment to reduce thyroid activity caused by plasmodial infection leads to lower parasitemias and increased survival: this effect is mediated by thyroxine (Shoemaker et al., 1974). Thyroxine, the main thyroid hormone, contains four iodine atoms; therefore, we examined the effect of iodine on CM development. Nordihydroguaiaretic acid (NDGA) and the herbal formulation PADMA-28 display antioxidant properties, as well as suppression of IFNγ-mediated inflammatory responses (Kim et al., 2008; Im and Han, 2007; Jeon et al., 2005; Neurauter et al., 2004; Barak et al., 2004; Combe, 2001; Moeslinger et al., 2000; Brennan and O'Neill, 1998; Carman-Krzan and Wise, 1993), making them interesting candidates for CM treatment. Thalidomide, developed as a nonbarbiturate sedative agent, was removed from the market in 1961 because of teratogenic effects. However, it was later approved in 1998 for human use by the United States FDA, under strict controls. Thalidomide has multiple effects, including modulation of cytokine production (e.g. reduced TNFα production) and is currently being evaluated for possible use against inflammatory and neoplastic conditions (Combe, 2001). An earlier report claims that *Plasmodium berghei* ANKA (PbA)-infected CBA mice treated with thalidomide showed an increase in macrophage phagocytic index, and in survival (Muniz-Junqueira et al., 2005). *Cannabis sativa* preparations have been used as therapy for various medical conditions (Wade et al., 2003). Pa-