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Resistance of a Rodent Malaria Parasite to a Thymidylate Synthase Inhibitor Induces an Apoptotic Parasite Death and Imposes a Huge Cost of Fitness

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Abstract

Background: The greatest impediment to effective malaria control is drug resistance in *Plasmodium falciparum*, and thus understanding how resistance impacts on the parasite's fitness and pathogenicity may aid in malaria control strategy.

Methodology/Principal Findings: To generate resistance, *P. berghei* NK65 was subjected to 5-fluoroorotate (FOA, an inhibitor of thymidylate synthase, TS) pressure in mice. After 15 generations of drug pressure, the 2% DT (the delay time for proliferation of parasites to 2% parasitaemia, relative to untreated wild-type controls) reduced from 8 days to 4, equalling the controls. Drug sensitivity studies confirmed that FOA-resistance was stable. During serial passaging in the absence of drug, resistant parasite maintained low growth rates (parasitaemia, 15.5%±2.9, 7 dpi) relative to the wild-type (45.6%±8.4), translating into resistance cost of fitness of 66.0%. The resistant parasite showed an apoptosis-like death, as confirmed by light and transmission electron microscopy and corroborated by oligonucleosomal DNA fragmentation.

Conclusions/Significance: The resistant parasite was less fit than the wild-type, which implies that in the absence of drug pressure in the field, the wild-type alleles may expand and allow drugs withdrawn due to resistance to be reintroduced. FOA resistance led to depleted dTTP pools, causing thymineless parasite death via apoptosis. This supports the tenet that unicellular eukaryotes, like metazoans, also undergo apoptosis. This is the first report where resistance to a chemical stimulus and not the stimulus itself is shown to induce apoptosis in a unicellular parasite. This finding is relevant in cancer therapy, since thymineless cell death induced by resistance to TS-inhibitors can further be optimized via inhibition of pyrimidine salvage enzymes, thus providing a synergistic impact. We conclude that since apoptosis is a process that can be pharmacologically modulated, the parasite's apoptotic machinery may be exploited as a novel drug target in malaria and other protozoan diseases of medical importance.

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Introduction

Malaria, caused by protozoan parasite of the genus *Plasmodium* is the most widespread parasitic disease, with malaria endemic regions encompassing approximately 40% of the global human population. Traditionally, four *Plasmodia* species cause human malaria, *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*, but recently the primate parasite *P. knowlesi* was established as the fifth causative agent [1]. The global malaria situation is being exacerbated by the fact that *P. falciparum*, which causes about 90% of all global malaria cases, has rendered most of the classical antimalarials ineffective. More worrying are recent reports from Southeast Asia region, specifically near the Thai-Cambodia border indicating that resistance to the artemisinin-based combination therapies (ACTs), the only fully effective class of antimalarial drugs against falciparum malaria, is imminent [2]. Whereas the rising incidence in malaria morbidity and mortality is largely associated with drug failure following resistance, it is also possible that resistance induces an alteration of the intrinsic parasite traits that may influence parasite fitness (growth and multiplication) and virulence (harm to the host following infection), thus impacting on malaria mortality and morbidity [3,4]. Drug pressure, the force that mainly drives antimalarial drug resistance through a population is a function of antimalarial drug use [5,6]. While exposure of parasites to sub-curative drug doses facilitate the evolution of 'classical drug resistance' (point mutations, overexpression of target proteins), it can also engender genetically-encoded parasite traits that could influence parasite survival in a drug environment [7].

There are very few reports on how development of resistance in field populations of malaria parasites impacts on parasite fitness, and indeed studies on the fitness of drug-resistance genes of