Lack of resistance of Plasmodium falciparum to dihydroartemisinin in Uganda based on parasitoldogical and molecular assays

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Lack of resistance of *Plasmodium falciparum* to dihydroartemisinin in Uganda based on parasitological and molecular assays

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Introduction

- Artemisinin-based combination therapy is now standard treatment for falciparum malaria. However, this regimen is threatened by resistance to artemisinins, manifest as delayed clearance of parasitemia after therapy, in southeast Asia.

- Artemisinin resistance in southeast Asia is associated with increased parasitemias in culture, compared to those in sensitive parasites, 72 hours after a 6 hour pulse with 700 nM dihydroartemisinin (DHA), and with propeller domain polymorphisms in the *Plasmodium falciparum* kelch (K13; PF3D7_1343700) gene.

- Given that artemether/lumefantrine has been adopted as standard therapy for malaria within the last decade in Uganda, we characterized artemisinin sensitivity in fresh *P. falciparum* isolates from Kampala using *ex vivo* ring-stage survival and IC50 assays. We also assessed the K13 gene for polymorphisms.

Methods

**Fig. 1. Study site - Kampala, UG.**

Parasite isolate collection, filter papers (N=53)

Parasite isolate collection, filter papers for K13, pfcrt and pfmdr1 genotyping (N = 15)

*Ex vivo* IC50 assays (N = 15)

*Ex vivo* ring-stage survival assays (N = 43)

- gDNA from filter papers for K13, pfcrt and pfmdr1 genotyping

- Long term cultures to test for recrudescence (N = 12)

53 fresh *P. falciparum* isolates were collected from patients diagnosed with malaria from May-August 2014, at Mulago Hospital, Kampala.

Parasite IC50s to DHA were determined by a standard 72 h *ex vivo* microplate assay using HRP2 detection.

Parasite susceptibility to DHA was assessed in the *ex vivo* ring-stage survival assay as described. Survival rates were expressed as the proportion of parasites in the 6 h, 700 nM DHA-pulsed cultures relative to DMSO controls, at the end of the 72 hour assay. Twelve cultures exposed to DHA were allowed to grow for 30 d to test for recrudescence.

*K13* propeller-encoding domains (codons 440-726) were dideoxy sequenced. Polymorphisms in pfcrt and pfmdr1 were assessed with multiplex ligation detection reaction-fluorescent microsphere assays as previously described.

**Results**

**Fig. 2. Ring-stage survival assay.**

- Ring-stage survival was 0% in 40/43 cultures
- Ring-stage survival ranged 0.7 - 1.9% in 3/43 cultures
- No association with survival and SNPs in K13, pfcrt or pfmdr1
- Parasites reemerged from 10/12 long-term cultures after DHA pulse

**Fig. 3. Prevalences of wild-type, mixed, and mutant sequences at the indicated positions for all 53 isolates.**

**Fig. 4. Ex vivo IC50 values for DHA from 15 isolates. The geometric mean IC50 value was 1.6 nM.**

**Fig. 5. Two kelch (K13) polymorphisms were detected from our samples. Numbered boxes indicate the six blades comprising the propeller domain of the kelch 13 protein.**

Summary and Conclusions

K13 mutations were found in 2/53 parasite isolates from Kampala, but were not mutations associated with resistance in SE Asia.

RSA and IC50 data showed that parasites remain highly sensitive to DHA *in vitro*.

The results of this study, as well as findings from other studies, suggest that artemisinin resistance is not yet a problem in Uganda. The polymorphic nature of K13 in Africa and altered ACT partner drug sensitivity in Uganda indicate the continued need for surveillance of ACT efficacy in the region.

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References