Sulfamethoxazole Levels in HIV-Exposed Uninfected Ugandan Children

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INTRODUCTION

Malaria is highly prevalent in many areas of the world where HIV-infected children live, especially sub-Saharan Africa. Studies have shown that when HIV and malaria are present as coinfections, each disease can enhance the pathogenicity of the other.1 Moreover, as more patients are managed for HIV infection in malaria-endemic areas, understanding the impact of drugs used in HIV exposure and infection on malaria infection is important.

The World Health Organization recommends daily trimethoprim–sulfamethoxazole (TMP–SMX) prophylaxis for children of HIV-infected mothers daily starting at 4–6 weeks of age and continued until HIV infection has been excluded by an age-appropriate HIV test, after cessation of breastfeeding.2 The World Health Organization recommended three-drug fully suppressive treatment options, including AZT, NVP, or lamivudine.6 Therefore presents data from an exploratory objective. No drug adherence with TMP–SMX is needed.

METHODS

Infants aged 0–12 months from Rakai district were eligible for enrollment and followed up at the Kalisizo Hospital and Rakai Health Services Program. Subjects were followed up monthly from February 2015 to August 2015. The original study design included enrollment of HUE and HIV-uninfected, unexposed children (HUU) with a primary objective of characterizing malaria incidence, but was stopped early for futility because of low malaria incidence in the region. This report therefore presents data from an exploratory objective. No drug adherence with TMP–SMX prophylaxis for those guardians responding “yes”; “reported nonadherence” for those guardians responding “no”; or “missing self-report” for data not obtained at that visit), and heel/finger stick and venous blood collected for drug levels (SMX of TMP–SMX). To preserve sample stability, the samples were kept at −80°C immediately after collection and processing on site in Uganda. The samples were shipped back on dry ice (with temperature monitoring and no thawing). Once received, the samples were immediately stored at −80°C until use for the assay. If any samples needed to be rerun, that sample was kept at −20°C after the initial run and until use for the next run.

At all visits, children were tested for malaria using Giemsa-stained malaria thick smear. Clinical illness was managed according to Integrated Management of Childhood Illness guidelines7 and WHO malaria treatment recommendations.8 Dried blood spots were also collected for malaria polymerase

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chain reaction, performed as previously described. Maternal HIV status was determined from documented medical history. All subjects received insecticide-treated bednets to prevent malaria if they did not already have them.

**Study site.** Rakai district registers an HIV seroprevalence of 8.5% among the pregnant mothers across the various geographical populations, with an estimated 2,000 births per year (F. Nalugoda, personal communication). Rakai district is on a plateau at an altitude ranging between 750 and 900 m and has fair rainfall throughout the year, with relatively dry periods during January and February and from June through August. Peak rainfall varies from year to year, but occurs typically in March/April and October/November. Malaria is meso- to holoendemic with year-round transmission and highest intensity after the rainy seasons or in communities adjacent to lakes and other mosquito breeding sites.

**Assay for sulfas level.** Sulfas levels in serum were measured using a previously described colorimetric assay with some modifications, including adaptation to 96-well plates. Briefly, 20 μL of serum was diluted in 260 μL of water, incubated at room temperature for 5 minutes, and boiled (98°C) for 1 hour in a thermocycler to deacetylate the small portion of SMX, which is naturally acetylated in the body. The plate was then centrifuged at 2,000 rpm for 3 minutes. A 120-μL quantity of 20% p-toluenesulfonic acid in 0.2 M HCl was added to each sample and incubated for 5 minutes to precipitate serum proteins. The plate was then centrifuged at 4,000 rpm for 10 minutes. A 100-μL quantity of supernatant was recovered and combined with 20 μL of a citric acid buffer, followed by 40 μL of a 2% dimethylaminobenzaldehyde solution in ethanol, resulting in a color change that was quantified at 450 nm as a measure of sulfas levels. The assay was standardized using control serum, to which SMX of known level was added in serial dilutions. Our limit of detection was 1.9 μg/mL and, therefore, levels equal to 1.9 μg/mL or less were considered undetectable. Standard curves were included with each plate to allow for determination of sample level. Samples with values above 5.8 μg/mL were considered therapeutic for IC50 for parasite strains in this region, based on in vitro growth inhibition assay IC50 data for SMX with Plasmodium falciparum strains with known antifolate resistance mutations and known resistance mutations of F32 and K1 compared with resistance data published for Uganda, indicating a range of 2.53–5.8 μg/mL.

**Statistics.** R version 3.3.1 was used for all calculations, including summary statistics (means, standard deviations [SDs], and percentages).

**Ethics.** The study was approved by the Uganda Virus Research Institute Research and Ethics Committee, the Uganda National Council for Science and Technology, and the National Institute of Allergy and Infectious Diseases Intramural Institutional Review Board. Parents or guardians of infants and children enrolled provided written consent for the infants enrolled in this study.

**RESULTS**

**Demographics.** Seventy HUE subjects were enrolled, aged 3–12 months while on study with a mean duration on study of 11.78 weeks (SD 6.84). Mean age at enrollment was 6.76 (2.37) years (Table 1). Demographics, malaria incidence, breastfeeding, and bednet use for HUE subjects are also summarized in Table 1. Parallel data for the HUU group are presented in Supplemental Table 1.

| Category               | Subcategory | Subjects (N = 70) |
|------------------------|-------------|-------------------|
| Age at enrollment (month) | Range | Mean (SD) |
| Range                  | 6.76 (2.37) |
| Age group (month)      | Range | Mean (SD) |
| 3–4                    | 14 (20%) |
| 5–8                    | 36 (51.4%) |
| 9–12                   | 20 (28.6%) |
| Gender                 | Male   | 50 (42.9%) |
| Malaria episodes       | PCR    | 2 (0.71%) |
| Bednet use†            | 66 (89.13%) |

**DISCUSSION**

In our study, adherence to TMP–SMX was reported in only 56% of clinical visits. Even when adherence with TMP–SMX therapy was reported, 33% of concurrent drug levels of SMX were below IC50s for P. falciparum with some, but not all, of the already reported antifolate resistance mutations that exist in Uganda (Figure 2).

Previous studies have shown over time that HIV-infected and exposed children on TMP–SMX prophylaxis have reduced clinical malaria burden, and the degree of the effect likely depends on transmission intensity and preexisting antifolate resistance mutation prevalence in the region. Few prior studies have examined TMP–SMX levels in this context. A recent study of 136 West African children on ARV and TMP–SMX prophylaxis suggested that overall TMP–SMX levels in children dosed according to WHO recommendations were lower than those achieved in adults, although the relevance of this to preventing infections or driving drug resistance requires further study. Trimethoprim–sulfamethoxazole prophylaxis impact on the development of malaria-specific immunity in children requires further study. Trimethoprim–sulfamethoxazole also has activity that surpasses expected antimicrobial effects in bacterial and fungal infections in HIV-exposed and infected patient populations.

Both in malaria and other infections, TMP–SMX prophylaxis studies should include more extensive drug-level assessment as a reflection of reported adherence, especially given how widely it is now being used.

Weaknesses of our study include that we were not able to perform more detailed pharmacokinetics of TMP–SMX on these children. In addition, we acknowledge certain caveats in attempting to interpret available in vitro P. falciparum study data for antifolates to clinical efficacy. First, we know that in vitro, indications of antifolate resistance do not directly

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**SUPPLEMENTAL TABLE 1.**

Demographic information and malaria episodes for HIV-uninfected, exposed (HUE) children enrolled on study

| Category               | Subcategory | Subjects (N = 70) |
|------------------------|-------------|-------------------|
| Age at enrollment (month) | Range | Mean (SD) |
| 3–4                    | 14 (20%) |
| 5–8                    | 36 (51.4%) |
| 9–12                   | 20 (28.6%) |
| Gender                 | Male   | 50 (42.9%) |
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† The figures indicated represent subject responses at their last study visit.
translate to clinical failure—one reason being that in vitro assays are not able to account for the host immunity, which in malaria, is associated with control of drug-resistant parasite strains. Moreover, interpretation of *P. falciparum* parasite strain in vitro susceptibility to antifolate medications in general is well known in its complexity because of the impact that exogenous folate supplementation can have on varying parasite strains. In parallel, we know that patients will have varying degrees of underlying nutrition, which makes direct extrapolation of these assays more difficult. And although many other studies have examined TMP–SMX impact on malaria in children, we did not observe robust transmission in either group to draw any comparable conclusion in this study (Table 1, Supplemental Table 1). However, with all those caveats taken into account, it is still concerning to note that if we further adjusted our random SMX levels to account for the amount of protein-bound (70%) as opposed to free (active) drug (30%), levels are considerably lower than IC50s.

Although TMP–SMX was not originally intended to treat or provide prophylaxis against malaria, the HIV pandemic in areas...
of malaria endemicity presents continued questions surrounding TMP–SMX impact on malaria. Further studies are required.

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REFERENCES

1. Plateau C, Le Loup G, Pialoux G. 2011. Consequences of HIV infection on malaria and therapeutic implications: a systematic review. Lancet Infect Dis 11: 541–556.

2. World Health Organization. 2014. Guidelines on Post-Exposure Prophylaxis for HIV and the Use of Co-Trimoxazole Prophylaxis for HIV-Related Infections among Adults, Adolescents and Children Recommendations for a Public Health Approach—December 2014 Supplement to the 2013 Consolidated ARV Guidelines. Available at: http://www.who.int/hiv/pub/guidelines/avvs2013supplement_dec2014/en/. Accessed October 13, 2015.

3. Bukwera-Djangarembozi M et al., 2014. A randomized trial of prolonged co-trimoxazole in HIV-infected children in Africa. N Engl J Med 370: 41–53.

4. Homsy J, Dorsey G, Arinaitwe E, Wanzira H, Kakuru A, Bigira V, Muhindo M, Kamya MR, Sandison TG, Tapper JW. 2014. Protective efficacy of prolonged co-trimoxazole prophylaxis in HIV-exposed children up to age 4 years for the prevention of malaria in Uganda: a randomised controlled open-label trial. Lancet Glob Health 2: e727–e736.

5. Ugandan Ministry of Health. 2012. The Integrated National Guidelines on ART, PMTCT, and IYCF. Available at: http://sustainuganda.org/content/integrated-national-guidelines-art-pmtct-and-lycf-2012. Accessed January 1, 2013.

6. WHO. 2015. Consolidated Guidelines on the Use of Anti-retroviral Drugs for Treating and Preventing HIV Infection: What’s New. Geneva, Switzerland: World Health Organization. Available at: http://www.who.int/hiv/pub/avr/policy-brief-avr-2015/en/. Accessed March 4, 2016.

7. WHO, 2006–2011. Documents. Integrated Management of Childhood Illness. Geneva, Switzerland: World Health Organization. Available at: http://www.who.int/maternal_child_adolescent/documents/imci/en/. Accessed April 23, 2015.

8. WHO. 2015. Guidelines for the Treatment of Malaria, 3rd edition. Geneva, Switzerland: World Health Organization. Available at: http://www.who.int/malaria/publications/atoz/9789241549127/en/. Accessed May 1, 2015.

9. Hobbs CV et al., 2016. Malaria in HIV-infected children receiving HIV protease-inhibitor-compared with non-nucleoside reverse transcriptase inhibitor-based antiretroviral therapy, IMPAACT P1088s, substudy to P1060. PLoS One 11: e0165140.

10. Hobbs CV, Anderson C, Neal J, Sahu T, Conteh S, Voza T, Langhorne J, Borkowsky W, Duffy PE. 2017.Trimethoprim-sulfamethoxazole prophylaxis during live malaria sporozoite immunization induces long-lived, homologous, and heterologous protective immunity against sporozoite challenge. J Infect Dis 215: 122–130.

11. Surveys MI, 2014–2015. Malaria Indicator Survey (MIS). Available at: http://www.malariareports.org/MIReports/MI2015Available at: http://www.malariareports.org/MIS/. Accessed August 23, 2016.

12. Harrington WE, Mutabingwa TK, Muehlenbachs A, Sorensen B, Bolla MC, Fried M, Duffy PE. 2009. Competitive facilitation of malaria to pyrimethamine, sulfadoxine, trimethoprim and sulfamethoxazole, singly and in combination. Trans R Soc Trop Med Hyg 81: 238–241.

13. Petersen E. 1987. In vitro susceptibility of Plasmodium falciparum malaria parasites in pregnant women who receive preventive treatment. Proc Natl Acad Sci USA 106: 9027–9032.

14. Pressiat C et al., 2017. Suboptimal cotrimoxazole prophylactic malaria in HIV-infected children. S Afr Med J 96: 629–635.

15. Mbogo GW et al., 2014. Temporal changes in prevalence of molecular markers mediating antimalarial drug resistance in a high malaria transmission setting in Uganda, Am J Trop Med Hyg 91: 54–61.

16. Zar HJ, Langdon G, Apollis P, Eley B, Hussey G, Smith P. 2006. Oral trimethoprim-sulphamethoxazole levels in stable HIV-infected children. S Afr Med J 96: 627–629.

17. Pressiat C et al., 2017. Suboptimal cotrimoxazole prophylactic concentrations in HIV-infected children according to the WHO guidelines. Br J Clin Pharmacol 85: 2729–2740.

18. Sibley CH, Hyde JE, Sims PF, Ploew CV, Kublin JG, Mberu EK, Cowman AF, Winstanley PA, Watkins WM, Nzila AM, 2001. Pyrimethamine-sulfadoxine resistance in Plasmodium falciparum: what next? Trends Parasitol 17: 582–588.

19. Wang P, Read M, Sims PF, Hyde JE, 1997. Sulfadoxine resistance in the human malaria parasite Plasmodium falciparum is determined by mutations in dihydroprotoetate synthetase and an additional factor associated with folate utilization. Mol Microbiol 23: 979–986.

20. Drugbank, 2017. Sulfamethoxazole. Available at: https://www.drugbank.ca/drugs/DB01015. Accessed October 12, 2017.