The anti-inflammatory effects of cotrimoxazole prophylaxis for people living with HIV in sub-Saharan Africa

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Cotrimoxazole prophylaxis reduces morbidity and mortality among people living with HIV in sub-Saharan Africa, regardless of age, degree of immunosuppression, disease stage or duration of antiretroviral therapy (ART)[1-6]. Long-term cotrimoxazole is therefore included in World Health Organization recommendations for people living with HIV in areas with a high prevalence of severe bacterial infections and/or malaria[7]. It is surprising that cotrimoxazole confers such clinical benefits, given the high prevalence of antimicrobial resistance among its key target pathogens in sub-Saharan Africa[8]. The conundrum of how cotrimoxazole works has led to recent interest in assessing its impact on immune activation, which is a cardinal feature of HIV infection. Soluble and cellular markers of immune activation have been associated with morbidity and mortality independently of CD4 count in numerous studies across diverse settings[9-12]. The degree of immune activation is greater among people in sub-Saharan Africa than in Europe, even in the absence of HIV infection (e.g.[13]), and is particularly pronounced in those presenting with advanced HIV disease[14]. Although ART reduces immune activation, it does not completely normalize biomarkers, which remain predictive of morbidity and mortality among ART-treated cohorts[11, 15, 16]. There is therefore substantial interest in evaluating interventions to modulate the inflammatory milieu in order to improve clinical outcomes among people living with HIV.

Cotrimoxazole comprises two antibiotics, trimethoprim and sulfamethoxazole, which sequentially inhibit microbial folate synthesis. Cotrimoxazole was first shown to have *in vivo* immunomodulatory properties in 1970[17]. Mice treated with trimethoprim retained skin grafts longer than untreated animals and to a similar extent to mice treated with the immunosuppressive drug azathioprine, which also has a pyrimidine ring structure[17]. Cotrimoxazole has been shown to confer clinical benefits in conditions such as rheumatoid arthritis[18] and granulomatosis with polyangiitis (formerly Wegener's granulomatosis)[19], partly due to anti-inflammatory properties, although its mechanism of action has remained largely uncharacterized. We recently reported findings from a substudy of the ARROW trial

(Clinical Trial Registry: ISRCTN44723643) in Zimbabwe and Uganda, in which HIV-positive children randomized to continue long-term cotrimoxazole had lower systemic inflammatory markers than those who stopped prophylaxis[20]. In this issue of *Journal of Infectious Diseases*, a study by Kyosiimire-Lugemwa and colleagues provides further evidence for anti-inflammatory effects of cotrimoxazole among people living with HIV in sub-Saharan Africa.

Kyosiimire-Lugemwa and colleagues report results from a subgroup of participants enrolled in the COSTOP trial in Uganda (Clinical Trial Registry: ISRCTN44723643). COSTOP was a randomized, double-blind, placebo-controlled non-inferiority trial of stopping or continuing cotrimoxazole among ART-treated HIV-positive adults with CD4 counts ≥250 cells/uL[21]. The main trial found that continuing cotrimoxazole reduced bacterial infections and malaria but increased adverse haematological events (mainly neutropenia)[21]. The current study sought to investigate whether systemic inflammation was lower among those continuing cotrimoxazole. The subgroup of 172 participants were predominantly females in their early 40s, who had been on ART for several years (virtually all on non-nucleoside reverse-transcriptase inhibitor-based regimens); most had WHO stage III and IV disease but well-preserved CD4 counts (median at randomization >500 cells/uL). Viral loads were not reported in either arm of the study. Overall, the trajectories of CRP and IL-6 over 12 months post-randomization differed significantly between placebo and cotrimoxazole arms. There were no consistent differences between arms in the proportions of CD4+ or CD8+ T cells expressing markers of activation (CD38 and HLA-DR), which are associated with poor prognosis among people living with HIV in high-income settings (although less so in sub-Saharan Africa)[22, 23].

A major strength of this study was that it was specifically designed and powered to detect differences in biomarkers within a randomized, placebo-controlled design using intention-to-

treat analysis. There was a baseline imbalance in CRP between cotrimoxazole and placebo arms despite independent randomization of this subgroup, which complicates interpretation of the findings, although baseline values were taken into account in analyses. Overall, the authors interpret their findings as providing evidence of anti-inflammatory benefits of cotrimoxazole in adults living with HIV. This is similar to recent findings published by our own group from Zimbabwean children enrolled in the ARROW trial in the same setting, in which children randomized to continue versus stop long-term cotrimoxazole showed reductions in CRP and IL-6 over two years of follow-up[20]. The finding that CRP and IL-6 are the key inflammatory markers influenced by cotrimoxazole in both studies is potentially important, since these are the biomarkers most consistently associated with mortality in both adults and children[9-12, 24].

Since both COSTOP and ARROW explored mechanisms underlying these findings, we now have an emerging understanding of how cotrimoxazole may continue to benefit people living with HIV when used long-term. Kyosiimire-Lugemwa and colleagues hypothesized that the reduced systemic inflammation arose from cotrimoxazole-mediated reductions in sub-clinical bacterial carriage in the gut, in addition to the reduction in symptomatic infections observed in the trial[21]. Enteropathogen carriage can drive both local activation of mucosal immune cells in the gut and increased intestinal permeability, which in turn can lead to microbial translocation and systemic immune activation[25, 26]. Although direct detection of enteropathogens was not undertaken, a range of biomarkers associated with intestinal damage and microbial translocation were measured. Contrary to their hypothesis, none of the markers of enteropathy or microbial translocation differed significantly between cotrimoxazole and placebo groups, although weak positive correlations were evident between soluble CD14 and both CRP and IL-6, consistent with previous observations[27]. The study quantified total lipopolysaccharide (LPS), a component of the outer membrane of gram-negative bacteria which is elevated in HIV infection and can drive local and systemic

immune activation via the pathogen recognition receptor toll-like receptor 4 (TLR4)[25, 28]. Although plasma LPS is a frequently used measure of intestinal microbial translocation[25, 28, 29], it has limitations as a biomarker since systemic levels are not necessarily gut-derived and because it is only one of many potential microbial components that could drive immune activation. For example, in addition to having important gram-negative activity, gram-positive bacteria, mycobacteria, fungi and protozoa are also susceptible to cotrimoxazole[8]. The TLR2 ligand peptidoglycan derived from gram-positive bacteria in the stool of HIV-positive subjects has previously been shown to activate T cells to a greater extent than LPS[30], but was not measured in this study. Furthermore, there are differences in the pro-inflammatory properties of LPS sub-types in HIV-positive individuals depending on the degree of LPS acylation[29], that were not explored. Despite these limitations, Kyosiimire-Lugemwa and colleagues confirm that levels of circulating LPS and host-derived LPS-regulating molecules (lipopolysaccharide binding protein and sCD14) did not explain the reductions in systemic inflammatory mediators that were seen with continuation of cotrimoxazole prophylaxis.

In ARROW, reductions in plasma inflammatory markers among children continuing versus stopping cotrimoxazole were not explained by differences in symptomatic infections, HIV disease progression, nutritional state or sub-clinical carriage of *Enterobacteriaceae* between groups[20]. Similar to the study by Kyosiimire-Lugemwa and colleagues, we found no significant difference between groups in plasma sCD14 over the period of follow-up, but LPS was not measured[20]. We went on to explore sub-clinical mechanisms in the gut that could contribute to cotrimoxazole-mediated reductions in systemic inflammation, including the gut microbiome which was also posited as a mechanism that could affect systemic inflammation in the current study. Stool levels of myeloperoxidase, an anti-microbial reactive oxygen species predominantly released by neutrophils, were lower in children continuing versus stopping cotrimoxazole and were positively correlated with the relative abundance of

gastrointestinal viridians group Streptococci, which were suppressed in the children continuing cotrimoxazole[20]. *In vitro* cotrimoxazole treatment also reduced production of the neutrophil chemoattractant IL-8 by a colonic epithelial cell line[20]. Finally, cotrimoxazole directly suppressed pro-inflammatory cytokine production by innate immune cells in whole blood culture experiments[20]. Collectively, these findings demonstrate that cotrimoxazole influences multiple direct and indirect pathways that likely underlie chronic inflammation during HIV infection.

Trials of plausible anti-inflammatory agents among people living with HIV have shown mixed results to date[31-38] and none are in routine clinical use. Two randomized trials now indicate that cotrimoxazole has some anti-inflammatory benefits for adults and children in sub-Saharan Africa, where interventions to reduced mortality and morbidity are most needed. Although the effect size in both studies is small, the results are highly consistent and can now be understood mechanistically through effects on the microbiome, gut epithelium and innate immune cells. Even modest reductions in inflammatory markers could have enormous impact at scale and with long-term use in areas of high HIV prevalence. For example, in the ARROW trial, children stopping versus continuing cotrimoxazole had almost 2-fold higher CRP concentrations at week 48, which translated into an 18% (95% confidence interval 6-32%) estimated increased risk in adverse clinical outcomes (death, hospitalization or poor immune reconstitution)[20]. Cotrimoxazole has the advantage over other potential anti-inflammatory agents of already being recommended long-term in these settings to prevent infections, although coverage remains poor[8]. Cotrimoxazole should be a priority intervention for people living with HIV in sub-Saharan Africa, since it reduces morbidity and mortality, is inexpensive and is well-tolerated. Emerging evidence of its novel anti-inflammatory benefits provides a further justification for improving coverage of this evidence-based and highly feasible intervention, which could confer major health gains in areas of high HIV prevalence.

Conflicts of interest

CDB and AJP were co-investigators on the immunology substudy of the ARROW trial

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