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Differences in early bactericidal activity during the first two days of standard anti-tuberculosis therapy in different geographic regions

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In a recent article entitled, “Comparison of time to positive and colony counting in an early bactericidal activity study of anti-tuberculosis treatment” we found a relatively lower early bactericidal activity (EBA) of combination anti-TB treatment with isoniazid (INH), rifampicin, ethambutol and pyrazinamide between days 0 and 2 in HIV-uninfected Ugandan adults with newly-diagnosed, initial episodes of smear-positive pulmonary TB.¹ The mean EBA 0-2, measured in colony forming units (CFU), from 40 patients receiving directly observed therapy (DOT) was $0.26 \pm 0.24 \log_{10}$ CFU/ml/day. This result was similar to an EBA 0-2 value of $0.28 \pm 0.42 \log_{10}$ CFU/ml/day we calculated using data from an earlier 2003 clinical trial conducted at the same site.² In comparison, previous studies done mainly in South Africa have demonstrated a higher EBA 0-2 for standard 4-drug therapy of approximately $0.5 \log_{10}$ CFU/ml/day, while the EBA 0-14 was similar to that found in our study.^{3,4} The reason for the difference in EBA during the first 2 days of therapy is not clear. Patients in our study received DOT on an inpatient basis with Good Manufacturing Practice grade medications. Medications were administered as loose tablets, and all patients received 300 mg of INH. The mean dose of INH was 5.8 ± 0.8 mg/kg (range 3.7 to 7.9), which should yield maximum EBA 0-2.⁵ CFU counts were done on 16-hour pooled overnight sputum collections using standardized methods. The mean baseline sputum colony count of $6.57 \pm 0.58 \log_{10}$ CFU/ml was similar to previous EBA studies. Baseline sputum colony count has been associated with EBA 0-2 in previous studies,^{5,6} and we did find a correlation in this study ($r_s = 0.34$, $P = 0.03$), however the relatively high mean baseline count in our patients would again predict maximal EBA 0-2. Finally, recent exposure to INH was excluded by urine INH metabolite testing (BBL Taxo INH Test Strips, BD, Sparks, MD, USA) during screening and at the time of enrollment to the ward to begin the study. All *Mycobacterium tuberculosis* (MTB) isolates from the study subjects were susceptible to INH, rifampicin, ethambutol and pyrazinamide, and sputum collection and processing methods in our study were similar to those used in the earlier studies.

Given the unexplained finding, we performed further pharmacokinetic and pharmacogenomic investigations after appropriate protocol, IRB, and consent amendments.

We focused on INH because most bactericidal activity during the first 2 days of combination therapy is believed to be due to INH.⁷ Variations in INH serum concentrations are directly determined by genetic differences in metabolism of the drug by the liver enzyme N-acetyltransferase 2 (NAT2). Individuals are either homozygous slow, heterozygous fast, or homozygous fast acetylators of INH.⁸ Donald and colleagues have previously shown that patients with fast acetylator genotypes have significantly lower EBA 0-2.⁹ We obtained NAT2 genotypes on 35 of the 41 patients included in our original study using TaqMan SNP Genotyping Assays (Applied Biosystems) and the Statistical Analysis for Genetic Epidemiology software (S.A.G.E., Release 6.3: <http://darwin.cwru.edu>). Only 3 (9%) were fast acetylators, and there were no significant differences by NAT2 status (table), excluding this as the cause for the low EBA 0-2. We also measured INH levels in the 7 patients who enrolled after the protocol amendment approval. Six of the seven patients had 2 hour INH levels greater than 2 (range 1.8-4.3), which has previously been shown to produce maximum INH EBA.⁹

The EBA 0-2 of 0.26 log₁₀ CFU/ml/day in our study was lower than expected given the adequate INH exposure demonstrated by NAT2 genotype and plasma INH levels. We were unable to identify the cause of the lower EBA 0-2. Other microbial and patient factors such as slow bacillary multiplication in our patients due to more chronic disease, or differences in MTB strains in different geographic regions may have influenced the kinetics of initial mycobacterial killing.¹⁰⁻¹²

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Table

EBA 0-2 and N-acetyltransferase 2 (NAT2) status during combination isoniazid, rifampin, pyrazinamide and ethambutol therapy

NAT2 Status	n	Mean EBA 0-2 * (SD)	P-value, t-test (NAT2 status vs slow)
Homozygous slow	19	0.22 (0.27)	-
Heterozygous fast	13	0.30 (0.18)	0.32
Homozygous fast	3	0.32 (0.25)	0.53
Combined homozygous and heterozygous fast	16	0.31 (0.19)	0.27

* log₁₀ CFU/ml/day