Inhibition of Isoniazid-Induced Expression of \textit{Mycobacterium tuberculosis} Antigen 85 in Sputum: Potential Surrogate Marker in Tuberculosis Chemotherapy Trials

ROBERT S. WALLIS,1* MANIJEH PHILLIPS,2 JOHN L. JOHNSON,2 LUCILÉIA TEIXEIRA,3 LIA MÁRCIA MASSINI CANEDO ROCHA,2 ETHEL MACIEL,3 LYNN ROSE,4 CHARLES WELLS,4 MOISÉS PALACI,2 REYNALDO DIETZE,3 KATHLEEN EISENACH,3 AND JERROLD J. ELLNER1

University of Medicine and Dentistry—New Jersey Medical School, Newark, New Jersey1; Case Western Reserve University, Cleveland, Ohio2; Universidade Federal do Espírito Santo, Vitória, Brazil3; and PathoGenesis Corporation, Seattle Washington4

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\textit{Mycobacterium tuberculosis} antigen 85 is induced in vitro by isoniazid (INH); its sustained induction in sputum during tuberculosis (TB) therapy predicts relapse. In this trial, rifampin or rifalazil inhibited the induction of sputum antigen 85 by INH in a dose-dependent fashion. This approach may facilitate the evaluation of new TB drugs.

Drugs for the therapy of tuberculosis (TB) differ substantially with respect to sterilizing activity, defined as the capacity to sterilize sputum rapidly and to prevent relapse (D. A. Mitchison, Letter, Am. Rev. Respir. Dis. \textbf{147}:1062–1063, 1993). Rifampin and pyrazinamide are highly active; many other drugs, such as ethambutol, are only bacteriostatic (4). Isoniazid (INH) readily kills extracellular bacilli in sputum but has little ultimate influence on TB relapse. This characteristic appears to reflect its reduced activity against stationary-phase or semidormant organisms. These differential drug effects on specific mycobacterial subpopulations give rise to the independence of sterilizing activity from early bactericidal activity (EBA), defined as the rate of decline of sputum CFU counts during the first few days of therapy. EBA primarily reflects the killing of extracellular bacilli. INH has high EBA but low sterilizing activity; conversely, rifampin and pyrazinamide have low or no EBA despite high sterilizing activity (1, 2, 6). This discrepancy between EBA and sterilizing activity limits the power of EBA as a research tool and has slowed the evaluation of new drugs for TB.

We previously examined the role of sputum \textit{Mycobacterium tuberculosis} antigen 85 as a surrogate marker in patients receiving standard short-course TB therapy (7, 8). The expression of this antigen is induced in vitro by INH (3). Its sustained induction in sputum shortly after the initiation of TB therapy predicts treatment failure or relapse, apparently indicating the lack of a sterilizing effect (7). In this study, we investigated the potential role of monitoring sputum antigen 85 in the evaluation of new TB drugs. We examined the effects of two dose levels of rifalazil (also known as KRM-1648 or benzoxazinorifamycin) on sputum \textit{M. tuberculosis} antigen 85 and CFU counts in an EBA-type trial.

*Corresponding author. Mailing address: Department of Medicine, UMDNJ-NJMS, 185 S. Orange Ave., MSB 1-503, Newark, NJ 07103-2757. Phone: (973) 972-8778. Fax: (973) 972-7790. E-mail: wallisrs@umdnj.edu.
Sputum antigen 85 values changed significantly during the 2-week protocol, as determined by repeated-measures two-way ANOVA ($P = 0.04$) (Fig. 2). However, the power of this analytic approach to identify individual arms or days giving rise to the overall effect was limited by the small sample size and the inherent variability of sputum. Therefore, the differences among the four arms were determined by one-way ANOVA for each day of the study. A significant difference was found only on day 5, although a similar trend also was observed on day 3. Values in all groups returned to baseline by the end of the treatment interval. The timing of this effect was consistent with our prior observation that the induction of antigen 85 was maximal on day 4 of therapy and was transient in subjects who ultimately were cured (7).

Data for individual subjects in each treatment arm, expressed as change from baseline to days 3 to 5, are indicated in Fig. 3. Values in subjects receiving INH-rifampin were significantly lower than values in those receiving INH-rifampin and INH-rifalazil (10 mg) ($P = 0.02$ and 0.04, respectively, as determined by Mann-Whitney rank sum test). Values in subjects receiving INH-rifampin and INH-rifalazil (25 mg) did not differ ($P = 0.13$).

These data indicate that the administration of INH results in the transient induction of $M. tuberculosis$ antigen 85 in sputum 3 to 5 days after the initiation of treatment and that this process can be inhibited by the concurrent administration of rifampin or rifalazil in a dose-dependent fashion. Monitoring of sputum antigen 85 may therefore represent an important extension to the evaluation of new TB drugs in EBA-type trials, particularly since its sustained expression is associated with relapse (7, 8). The strong activity of both rifampin and rifalazil on antigen 85 is in marked contrast to their weak EBA. This result may reflect their mechanism of action, interrupting new protein synthesis through inhibition of RNA polymerase. Alternatively, the ability to block INH-induced antigen 85 expression may be shared by other drugs with high sterilizing activity. Further studies of drug combinations such as INH-levofloxacin and INH-pyrazinamide may be helpful in dissecting this interaction.

In summary, the monitoring of sputum $M. tuberculosis$ antigen 85 may offer an important new approach to evaluating the preliminary activity and dose-response relationship of new drugs for TB.

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REFERENCES


