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A Reader's Guide to the Bactericidal Activity of Pyrazinamide and Clofazimine Alone and in Combinations with Pretomanid and Bedaquiline

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In this issue of the *Journal*, Diacon and colleagues (pp. 943–953) assessed the 14-day extended early bactericidal activity (EBA) of pyrazinamide alone, clofazimine alone, and four combination regimens of bedaquiline in permutations with pyrazinamide, pretomanid (Pa824), and clofazimine (1). The positive treatment control was a standard regimen of rifampin, isoniazid, pyrazinamide, and ethambutol, delivered as a combination tablet (Rifafour; sanofi-aventis, Paris, France), and the study participants were treatment-naive patients with pulmonary tuberculosis with positive results from sputum-smear microscopy for acid-fast bacilli. On Day 14 of treatment, the pharmacokinetic (PK) parameters of the prescribed drugs alone and in combination were measured.

The results were clear-cut. First, each of the three experimental three-drug combinations (bedaquiline, pretomanid, and pyrazinamide; bedaquiline, pretomanid, and clofazimine; and bedaquiline, pyrazinamide, and clofazimine) and the one experimental four-drug combination (bedaquiline, pretomanid, pyrazinamide, and clofazimine) had extended EBA that was not significantly different from that of the Rifafour control regimen. Second, pyrazinamide alone had minimal EBA, as expected, and clofazimine alone provided no early activity at all. Third, the main PK parameters of each studied drug were apparently not affected by the other drugs given in combination; that is, drug–drug interactions were not apparent, and PK parameters were mostly within the expected range. For clofazimine, the peak plasma concentration after 14 days of daily administration was, on average, 0.2 μg/ml and close to its 0.25 μg/ml minimum inhibitory concentration for *Mycobacterium tuberculosis*. The peak plasma concentrations and 24-hour area under the concentration curve of bedaquiline, its M2 metabolite, and clofazimine varied six- to eightfold across regimens and among individuals, but the median exposures achieved were relatively similar across regimens; there was less variation in pretomanid and pyrazinamide exposures.

Such results are informative. Despite bedaquiline's ability to shorten the time to culture conversion in patients with multidrug-resistant tuberculosis (2, 3) and in the mouse model (4), especially when combined with pyrazinamide (5), its potent bactericidal activity does

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not become fully evident until 5–7 days have elapsed (6, 7). The present manuscript thus illustrates how this characteristic affects EBA of bedaquiline-containing combinations: none had a better EBA than the standard Rifafour combination. However, this observation applies only to the first 14 days of therapy. In longer-term studies, bedaquiline-containing combinations exert potent antimicrobial activity beyond the 14 initial days (2–4).

As a drug with delayed bactericidal activity, bedaquiline is in good company with pyrazinamide and rifampin, the drugs responsible for shortening standard tuberculosis treatment from 18 to 6 months. The relationship between EBA and later sterilizing activity is not obvious. Bactericidal drugs inflict lethal injury on actively replicating organisms during the initial days of treatment; this might not reflect or predict the more subtle metabolic inhibition induced by sterilizing activity later in therapy against nonreplicating bacilli (so-called “dormant” or “persister” populations). This emphasizes a limitation of EBA evaluations (that they better reflect early “killing” activity against metabolically active bacilli, rather than later “sterilizing” efficacy against dormant bacilli); it also illustrates why the original EBA emphasis on the first 2 days of therapy (8) has been revised to extend to 7, then 14, and now 60 days of therapy as so-called serial sputum colony count studies (9). However, even the extended EBA cannot fully predict the outcome of a treatment that lasts 6 or more months and engages a pathogen capable of marked metabolic modification in response to stresses (10).

A puzzling aspect of the current study is that the patients taking the control regimen, Rifafour, did not have a pronounced initial fall of colony-forming unit counts; this initial fall is historically considered the trademark of isoniazid alone or with other drugs (11). However, in the past, the same authors made a similar observation (12), whereas in another case, they did not (7). We and others have noted as well smaller baseline colony-forming unit counts in recent years in comparison with in the past. Whatever the cause, it emphasizes the natural variations in clinical microbiology, the questionable value of historical controls, and the caution with which EBA results should be interpreted.

Finally, a significant contribution of the current study is to demonstrate the total lack of EBA during Days 0–14 for clofazimine, despite administration of the drug with loading doses of 300 mg on Days 1–3, followed by doses of 100 mg from Day 4 to Day 14. From their extensive PK investigation on the 14th day of treatment, the authors observed that the clofazimine plasma levels were quite variable from one patient to another; these levels were also close or slightly inferior on average to the minimum inhibitory concentration of clofazimine for *M. tuberculosis*. They speculated that the lack of EBA could result from insufficient free serum drug concentrations of clofazimine. Although this is a possibility, other explanations are also reasonable. Clofazimine does provide antituberculosis activity, as demonstrated in both clinical (13, 14) and experimental murine (15) studies. The simplest explanation for the lack of clofazimine EBA is that the drug, which is highly lipophilic, needs to accumulate to a certain level in the lipid-rich cell wall of *M. tuberculosis* before being able to reach the cell membrane, where it will disrupt the respiratory chain (16). Alternatively, bacterial intracellular energy stores might need to be depleted before the effect of the drug can be observed, as has been suggested for bedaquiline (7). In addition, reactive oxygen species, thought to be responsible for the antimicrobial effect of clofazimine (17),

might take time before inducing lethal mycobacterial DNA damage. Much work remains to be done to understand how and when clofazimine exerts its antimicrobial activity. In the meantime, clofazimine remains one of the group of potent antituberculosis drugs with little or no EBA. It is a merit of the work by Diacon and colleagues that it reminds us of the limits of the EBA approach: Potent EBA is valuable, but absent or limited EBA does not predict the absence of later antituberculosis activity reliably.

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