



14-day bactericidal activity of PA-824, bedaquiline, pyrazinamide, and moxifloxacin combinations: a randomised trial

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Summary

Background New drugs, but also shorter, better-tolerated regimens are needed to tackle the high global burden of tuberculosis complicated by drug resistance and retroviral disease. We investigated new multiple-agent combinations over the first 14 days of treatment to assess their suitability for future development.

Methods In this prospective, randomised, early bactericidal activity (EBA) study, treatment-naive, drug-susceptible patients with uncomplicated pulmonary tuberculosis were admitted to hospitals in Cape Town, South Africa, between Oct 7, 2010, and Aug 19, 2011. Patients were randomised centrally by computer-generated randomisation sequence to receive bedaquiline, bedaquiline-pyrazinamide, PA-824-pyrazinamide, bedaquiline-PA-824, PA-824-moxifloxacin-pyrazinamide, or unmasked standard antituberculosis treatment as positive control. The primary outcome was the 14-day EBA assessed in a central laboratory from the daily fall in colony forming units (CFU) of *M tuberculosis* per mL of sputum in daily overnight sputum collections. Bilinear regression curves were fitted for each group separately and groups compared with ANOVA for ranks, followed by pair-wise comparisons adjusted for multiplicity. Clinical staff were partially masked but laboratory personnel were fully masked. This study is registered, NCT01215851.

Findings The mean 14-day EBA of PA-824-moxifloxacin-pyrazinamide (n=13; 0.233 [SD 0.128]) was significantly higher than that of bedaquiline (14; 0.061 [0.068]), bedaquiline-pyrazinamide (15; 0.131 [0.102]), bedaquiline-PA-824 (14; 0.114 [0.050]), but not PA-824-pyrazinamide (14; 0.154 [0.040]), and comparable with that of standard treatment (ten; 0.140 [0.094]). Treatments were well tolerated and appeared safe. One patient on PA-824-moxifloxacin-pyrazinamide was withdrawn because of corrected QT interval changes exceeding criteria prespecified in the protocol.

Interpretation PA-824-moxifloxacin-pyrazinamide is potentially suitable for treating drug-sensitive and multidrug-resistant tuberculosis. Multiagent EBA studies can contribute to reducing the time needed to develop new antituberculosis regimens.

Funding The Global Alliance for TB Drug Development (TB Alliance).

Introduction

Tuberculosis remains a major global health problem; although recently the global incidence has fallen slightly, the number of cases remains daunting and has overwhelmed the capabilities of many health systems, especially in countries with a concomitant HIV epidemic.¹ Antituberculosis therapy relies on combinations of bactericidal and sterilising drugs that protect from development of resistance.² Crucial to containing and defeating the tuberculosis epidemic are new, shorter, and safe treatment regimens, which are affordable and practical for use in low-resource settings. Ideally, such regimens would contain new drugs able to combat tuberculosis resistant to currently available drugs, especially multidrug-resistant (MDR) tuberculosis resistant to at least isoniazid and rifampicin, and free of interactions with antiretroviral regimens.

Since 2004 several new antituberculosis drugs have entered clinical assessment; among these drugs are bedaquiline, a diarylquinoline previously known as TMC207, and PA-824, a nitroimidazo-oxazine, which

have completed initial dose-ranging monotherapy studies and have shown dose-related early bactericidal activity (EBA).³⁻⁵ Murine studies have provided evidence of the significant sterilising activity of both compounds.^{6,7} Bedaquiline has also been studied in the first 6 months of MDR-TB treatment with promising early results.^{8,9} In mouse experiments there is synergism between the first-line agent pyrazinamide and bedaquiline and between pyrazinamide and PA-824.^{10,11} Moxifloxacin and other fluoroquinolones have shown bactericidal activity approaching that of isoniazid in EBA studies¹²⁻¹⁴ and murine studies have shown a powerful sterilising effect for moxifloxacin alone¹⁵⁻¹⁷ and when combined with PA-824 and pyrazinamide.¹⁸ Although formal interaction studies have not been completed, preclinical data suggest a low likelihood of clinically significant interactions of PA-824, moxifloxacin, and pyrazinamide with antiretroviral drugs.

EBA studies assess the fall in colony forming units (CFU) of *Mycobacterium tuberculosis* in sputum of patients with smear-microscopy-positive pulmonary tuberculosis in response to treatment. These studies are most often

Published Online
July 23, 2012
[http://dx.doi.org/10.1016/S0140-6736\(12\)61080-0](http://dx.doi.org/10.1016/S0140-6736(12)61080-0)

See Online/Comment
[http://dx.doi.org/10.1016/S0140-6736\(12\)61183-0](http://dx.doi.org/10.1016/S0140-6736(12)61183-0)

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done with single drugs for proof of concept (ie, to confirm antimycobacterial activity of an agent on first use in patients with tuberculosis in closely monitored small patient groups, assess safety, and assist in identification of an appropriate dose for future studies¹⁹). Nonetheless, any new agent will be ultimately incorporated in multidrug regimens. This approach is usually assessed by substituting new agents one at a time for one constituent of the current standard treatment.^{20–23} However, when many new agents are available this stepwise process is time consuming, costly, and requires substantial resources for clinical trials. New approaches are urgently needed to expedite progress from single drugs to new combination regimens.

We describe a 14-day EBA study in treatment naive patients with sputum-microscopy smear-positive fully drug-susceptible pulmonary tuberculosis, assessing various combinations of the new antituberculosis agents bedaquiline, PA-824, and moxifloxacin and the established agent pyrazinamide with a view to developing appropriate combinations for longer-term studies, leading to a tuberculosis regimen for management of drug-susceptible and MDR disease.

Methods

Trial design and patients

We did a phase 2A, partially double-blinded, randomised trial assessing EBA, safety, tolerability, and pharmacokinetics of bedaquiline alone, bedaquiline-PA-824,

bedaquiline-pyrazinamide, PA-824-pyrazinamide, and PA-824-moxifloxacin-pyrazinamide over 14 days of treatment in groups of 15 treatment-naive patients with pulmonary tuberculosis without complicating factors. We also included a sixth group of ten patients who were randomised as a positive control to receive standard tuberculosis treatment with isoniazid-rifampicin-pyrazinamide-ethambutol (Rifafour e-275 [Sanofi-Aventis, Midrand, South Africa]) in accordance with South African Department of Health guidelines (panel 1). The study was done between Oct 7, 2010, and Aug 19, 2011. The trial was approved by the Medicines Control Council of South Africa and the independent ethics review committees of the clinical sites and was undertaken according to the principles of Good Clinical Practice guidelines and the Declaration of Helsinki. All patients provided written informed consent before study enrolment.

Patients were recruited from outpatient clinics in Cape Town, South Africa, and were admitted to hospital for the study duration at one of two centres (Task Applied Science, Intercare, Cape Town, or the Centre for Tuberculosis Research Innovation, University of Cape Town Lung Institute, Cape Town). Eligible patients were aged 18 to 65 years inclusive with a bodyweight from 40 kg to 90 kg inclusive. Participants needed to be sputum-microscopy smear-positive (at least 1+ on the WHO—International Union Against Tuberculosis and Lung Disease scale),²⁴ have a chest radiograph consistent with tuberculosis, and be able to produce at least 10 mL of sputum estimated from spot assessments. Patients not free of disease complications or concomitant illness that might compromise their safety or the interpretation of trial endpoints were excluded, as were patients with diabetes who required insulin treatment, patients with any history or signs of significant cardiac arrhythmia on electrocardiography (ECG), and patients with a history or evidence of lens opacity on slit-lamp examination. Patients with HIV infection participated if their CD4+ count was >300 cells/ μ L and they were not on antiretroviral treatment. After hospital discharge, patients were referred to their local tuberculosis clinic to complete a standard course of antituberculosis chemotherapy and returned for follow-up visits at the site 14 days, 28 days, and 90 days after discharge.

Randomisation and masking

Participants were assigned a study-generated participant-identification code ensuring anonymity. Treatment allocation was undertaken centrally with a computer-generated randomisation sequence to the effect that no person at site level had access to the treatment codes. Masking of patients and site staff was only partial because groups receiving PA-824 required more intensive ECG monitoring than did groups receiving bedaquiline. Trial personnel were thus masked within the bedaquiline and PA-824 containing groups; laboratory staff assessing microbiological endpoints were fully

For the study protocol see http://www.tballiance.org/downloads/NC-001_J-M-Pa-Z_Working_Protocol.pdf

Panel 1: Treatment groups

Bedaquiline

Bedaquiline 400 mg a day, but preceded by a dose of 700 mg on treatment day 1, 500 mg on treatment day 2, and 400 mg a day thereafter and accompanied by a pyrazinamide placebo

Bedaquiline-PA-824

Bedaquiline given in doses as in the bedaquiline alone group, but accompanied by PA-824 200 mg a day

Bedaquiline-pyrazinamide

Bedaquiline given in doses as in the bedaquiline alone group but accompanied by pyrazinamide 25 mg/kg bodyweight (range 20–30 mg/kg) a day

PA-824-pyrazinamide

PA-824 200 mg a day accompanied by pyrazinamide 25 mg/kg (range 20–30 mg/kg) bodyweight a day

PA-824-moxifloxacin-pyrazinamide

PA-824 200 mg a day, pyrazinamide 25 mg/kg (range 20–30 mg/kg) a day, and moxifloxacin 400 mg a day

Isoniazid-rifampicin-pyrazinamide-ethambutol

Rifafour e-275 dosed according to bodyweight: 30–37 kg two tablets; 38–54 kg three tablets; and 55–70 kg four tablets. Each tablet of Rifafour e-275 contains isoniazid 75 mg, rifampicin 150 mg, pyrazinamide 400 mg, and ethambutol 275 mg

masked. Treatment allocation remained masked until the database was locked.

Procedures

Sputum specimens were collected for 16 h overnight for 2 days before, and each day after, treatment initiation; collections were completed before administration of the next day's therapy. Sputum for CFU counts of *M tuberculosis* and measurement of time to positivity (TTP) in liquid culture medium (BACTEC MGIT 960, Becton Dickinson, Woodmead, South Africa) were subject to laboratory processing centrally as described previously^{3,25} in the Department of Medical Biochemistry, Faculty of Health Sciences, Stellenbosch University, Cape Town, South Africa. Cultures from baseline and the last available overnight sputum collections were tested for susceptibility to first-line drugs (MGIT SIRE kit, Becton Dickinson) and the minimum inhibitory concentrations

(MIC) of bedaquiline, PA-824, and moxifloxacin were established for all isolated strains with agar proportion methods. *M tuberculosis* speciation was done by PCR.²⁶ For patients receiving bedaquiline the full pharmacokinetic profile of the drug and its metabolite was determined on treatment day 14 only; in patients receiving PA-824 containing drug combinations the pharmacokinetics of PA-824, pyrazinamide, and moxifloxacin were studied on treatment days 1, 8, and 14.

Safety assessments included daily history, vital signs, physical examination, and monitoring for adverse events; the latter also comprised full blood counts, coagulation studies, clinical chemistry, and urinalysis. 12-lead ECGs were done in the morning and evening before treatment and on days 1, 3, 8, and 14; we assessed rhythm disturbances and changes from the baseline QT interval corrected by Friderica's method (QTcF) and Bazett's method (QTcB).^{27,28} Ophthalmological assessments

	Bedaquiline (n=15)	Bedaquiline-pyrazinamide (n=15)	Bedaquiline-PA-824 (n=15)	PA-824-pyrazinamide (n=15)	PA-824-moxifloxacin-pyrazinamide (n=15)	Isoniazid-rifampicin-pyrazinamide-ethambutol (n=10)	All patients (n=85)	p value
Males	11 (73%)	12 (80%)	11 (73%)	11 (73%)	12 (80%)	6 (60%)	63 (74%)	0.906
Mixed ethnicity	8 (53%)	10 (67%)	8 (53%)	11 (73%)	9 (60%)	3 (30%)	49 (58%)	0.399
Age (years)	31.27 (11.60)	29.13 (8.67)	33.33 (8.47)	29.73 (8.93)	28.33 (9.34)	27.00 (6.63)	30 (9.13)	0.559
BMI (kg/m ²)	18.91 (2.99)	18.32 (3.48)	19.95 (3.46)	18.80 (2.47)	19.15 (2.11)	18.16 (1.57)	18.92 (2.80)	0.905
CFU (log ₁₀ /mL sputum)	5.956 (1.060)	5.911 (0.739)	6.680 (0.719)	5.910 (1.045)	5.835 (1.101)	5.645 (0.625)	6.002 (0.946)	0.09
TTP (h)	110.900 (21.756)	99.067 (13.376)	100.300 (37.864)	103.050 (30.852)	96.883 (20.779)	110.150 (25.993)	103.153 (26.029)	0.284

Data are number (%) or mean (SD). CFU or TTP values derived from the mean of CFU counts or TTP respectively on 2 consecutive days before the start of treatment. BMI=body mass index. CFU=colony forming units. TTP=time to positivity.

Table 1: Baseline characteristics of study participants

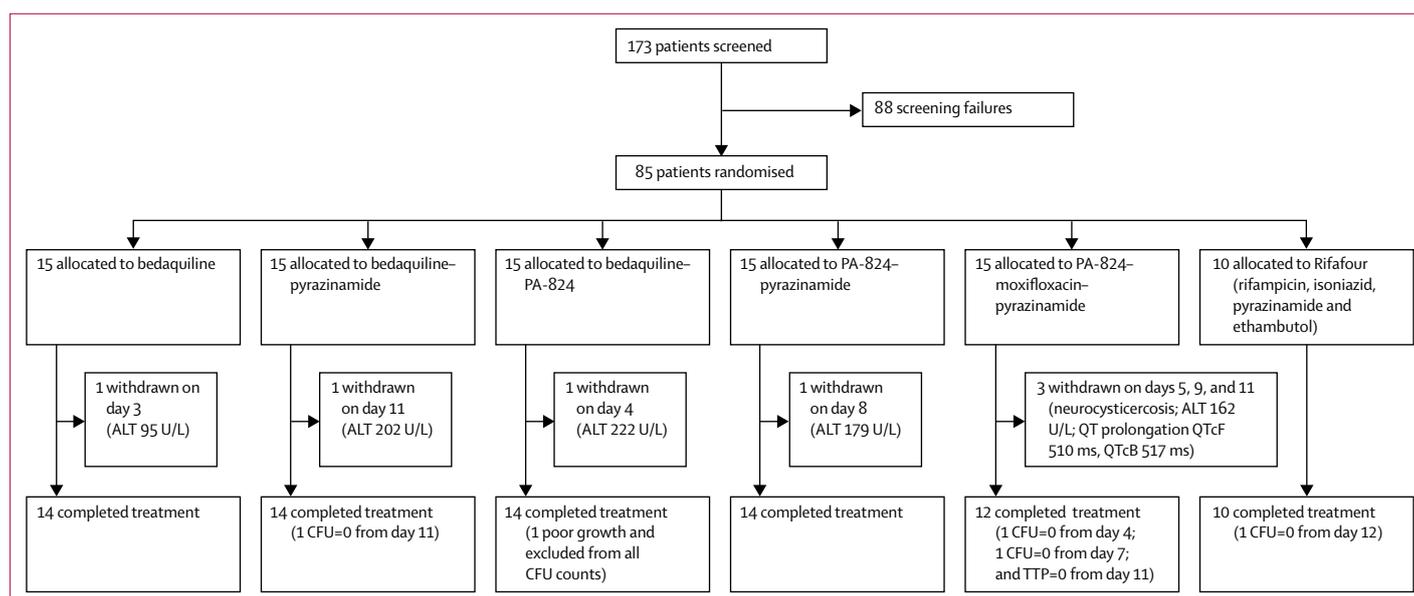


Figure 1: Study profile

ALT=alanine aminotransferase. CFU=colony forming unit. QTcF=QT interval corrected by Friderica's method. QTcB=QT interval corrected by Bazett's method.

included fundoscopy, slit-lamp examination, and assessment of visual acuity before and after completion of investigational treatment. Study participants were withdrawn if they had aspartate aminotransferase or alanine aminotransferase concentration of three or more times the upper limit of normal and also if they had a QTcB or a QTcF interval of greater than 500 ms or an increase from baseline of greater than 60 ms present on repeated ECG that resulted in an interval exceeding 430 ms for men and 450 ms for women.

Statistical analyses

The sample size of 15 patients per group accorded with previous phase 2 studies allowing for up to three drop-outs per group.¹⁹ The primary efficacy endpoint was the EBA over 14 days measured by the daily rate of change of log₁₀ CFU in sputum (EBA_{CFU} 0–14). The slopes could be described by linear, bilinear, or multiple regression depending on which method fitted the data best. On the basis of the slopes, the EBA parameters were established as weighted averages over the study period for each individual patient and then averaged to obtain the value for each treatment group. In the case of dropouts, their data were included in the analyses as long as enough points were recorded to allow curve fitting. Secondary endpoints included the EBA_{CFU} days 0–2, EBA_{CFU} days 2–14, EBA_{CFU}

days 0–7, and EBA_{CFU} days 7–14. EBA_{TTP} was assessed in an analogous fashion from the daily prolongation of TTP over the relevant time period. The study was not powered for difference testing; only exploratory comparisons between experimental groups with one-way ANOVA were attempted and no statistical comparison was made with the EBA of the control group on standard treatment.

This trial is registered, NCT01215851.

Role of the funding source

The Global Alliance for TB Drug Development (TB Alliance) was involved in study design, data collection, data analysis, and participated in data interpretation and writing of this report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Most patients were male and of mixed ethnicity. Mean age was 30·00 (SD 9·13) years and mean body mass index 18·92 (2·80) kg/m². These characteristics as well as baseline log₁₀CFU and TTP values did not differ significantly between treatment groups. Six patients were co-infected with HIV (table 1).

One patient receiving bedaquiline-PA-824 was excluded from the analysis owing to very poor and

	Days 0–14	Days 0–2	Days 0–7	Days 2–14	Days 7–14
Bedaquiline	14 (0·061 [0·068])	15 (–0·022 [0·121])	14 (0·043 [0·074])	14 (0·076 [0·069])	14 (0·123 [0·097])
Bedaquiline-pyrazinamide	15 (0·131 [0·102])	15 (0·079 [0·167])	15 (0·106 [0·119])	15 (0·143 [0·109])	15 (0·152 [0·120])
Bedaquiline-PA-824	14 (0·114 [0·050])	14 (0·114 [0·149])*	14 (0·114 [0·089])	14 (0·114 [0·047])	14 (0·114 [0·069])
PA-824-pyrazinamide	14 (0·154 [0·040])*	15 (0·170 [0·082])*	14 (0·155 [0·040])*	14 (0·148 [0·043])*	14 (0·124 [0·080])
PA-824-moxifloxacin-pyrazinamide	13 (0·233 [0·128])*†‡	15 (0·315 [0·133])*†‡§	12 (0·225 [0·091])*†‡	13 (0·222 [0·130])*†‡	13 (0·175 [0·146])
Isoniazid-rifampicin-pyrazinamide-ethambutol	10 (0·140 [0·094])	10 (0·177 [0·188])	10 (0·162 [0·124])	10 (0·135 [0·103])	10 (0·136 [0·102])

Data are number (mean [SD]). The rate of change in log₁₀CFU per mL of sputum per day of *Mycobacterium tuberculosis* was expressed with the weighted average of slopes from the bilinear regression by treatment group. Pairwise comparisons (p<0·001) with Holm’s method, following ANOVA for ranks for the rates of change are shown. CFU=colony forming units. *Group superior to bedaquiline. †Group superior to bedaquiline-pyrazinamide. ‡Group superior to bedaquiline-PA-824. §Group superior to PA-824-pyrazinamide.

Table 2: Bactericidal activity of agents and regimens determined by growth of log₁₀CFU of *Mycobacterium tuberculosis* on solid media

	Days 0–14	Days 0–2	Days 0–7	Days 2–14	Days 7–14
Bedaquiline	14 (5·414 [3·523])	15 (2·043 [5·945])	14 (4·625 [3·600])	14 (5·808 [3·621])	14 (7·466 [4·806])
Bedaquiline-pyrazinamide	15 (9·970 [6·987])	15 (12·393 [10·475])	15 (11·115 [8·299])*†‡	15 (9·556 [6·753])	15 (9·117 [6·674])
Bedaquiline-PA-824	15 (5·855 [2·785])	15 (3·941 [9·156])	15 (3·948 [3·968])	15 (6·174 [4·117])	15 (6·761 [7·089])
PA-824-pyrazinamide	14 (8·805 [3·468])	15 (10·243 [5·982])*	15 (10·243 [5·982])*†‡	14 (8·424 [3·485])	14 (6·520 [4·887])
PA-824-moxifloxacin-pyrazinamide	13 (18·482 [22·582])*†‡§	15 (21·018 [11·506])*†‡§	14 (19·396 [13·024])*†‡§	13 (18·154 [24·888])*†‡§	13 (16·775 [34·957])
Isoniazid-rifampicin-pyrazinamide-ethambutol	10 (11·841 [3·932])	10 (25·492 [12·360])	10 (19·557 [7·160])	10 (9·566 [5·064])	10 (8·874 [5·534])

Data are number (mean [SD]). The rate of change in the lengthening of time (h) to positive signal in liquid culture media per day for *Mycobacterium tuberculosis* with the weighted average of slopes from the bilinear regression by treatment group. Pairwise comparisons (p<0·001) with Holm’s method, following ANOVA for ranks for the rates of change are shown. *Group superior to bedaquiline. †Group superior to bedaquiline-pyrazinamide. ‡Group superior to bedaquiline-PA-824. §Group superior to PA-824-pyrazinamide.

Table 3: Activity of agents and regimens determined by time (h) to positivity in liquid culture

inconsistent culture growth (figure 1). By contrast with previous EBA studies negative CFU results were reported in four patients and in one of these patients a negative TTP result was also noted; two of these participants were on PA-824-moxifloxacin-pyrazinamide, including the one with a negative TTP, and one each on bedaquiline-pyrazinamide and standard treatment. To allow negative cultures to reflect in the slope and therefore in the EBA calculations negative cultures on solid media were allotted the lower limit of detection, which is a count of $\log_{10}\text{CFU}=1$; similarly, a liquid culture without growth at 42 days remained in the analysis with a value of 1008 h, this being the longest period MGIT cultures are routinely continued.

The values of patients that dropped out were included as long as they were available. Empirical curve fitting identified the best method to describe the data as bilinear regression with a change in slope estimated for each group separately. These inflection points were for 6.5 days for bedaquiline; 3.5 days bedaquiline-pyrazinamide; 4.5 days for bedaquiline-PA-824; 8.5 days for PA-824-pyrazinamide; and 6.5 days for PA-824-moxifloxacin-pyrazinamide (tables 2, 3; figures 2, 3). Figures 2 and 3 display diverging patterns of activity in the first week while in the second week the treatments showed similar activity slopes. The EBA_{CFU} days 0–14, the primary endpoint, was highest with PA-824-moxifloxacin-pyrazinamide (0.233 $\log_{10}\text{CFU}$ per mL of sputum per day). This combination showed the most rapid onset of activity with a slight deceleration at 8.5 days and appeared more efficacious than all other groups measured by both EBA_{CFU} and EBA_{TTP} . As previously seen, the onset of activity of bedaquiline was delayed with an inflection point at 6.5 days. The activity of this drug seemed to set in earlier after the addition of pyrazinamide resulting in greater activity of bedaquiline and pyrazinamide than bedaquiline alone. The addition of PA-824 to bedaquiline seemed to have little if any effect on the activity of bedaquiline, albeit without causing clear antagonism. In the absence of groups with pyrazinamide alone and PA-824 alone for direct comparisons the contributions of the single drugs are difficult to assess. The activity of PA-824-pyrazinamide was significantly greater than that of bedaquiline over the first 7 days (tables 2,3).

All patients had infection with *M tuberculosis* strains that were susceptible to the experimental compounds at baseline and at the end of treatment period. Minimum inhibitory concentrations ranged from $<0.03 \mu\text{g/mL}$ to $0.06 \mu\text{g/mL}$ for bedaquiline, from $<0.1 \mu\text{g/mL}$ to $0.2 \mu\text{g/mL}$ for PA-824, and from $<0.125 \mu\text{g/mL}$ to $0.5 \mu\text{g/mL}$ for moxifloxacin. Minimum inhibitory concentrations did not seem to increase over the 14 days of drug treatment. The pharmacokinetic results will be presented in greater detail in a further report. No clinically significant drug–drug interactions occurred.

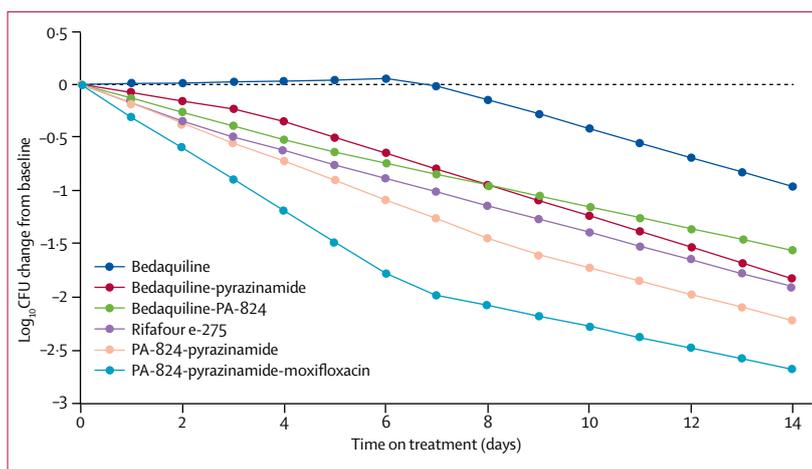


Figure 2: Bilinear regression showing the fall in mean $\log_{10}\text{CFU}$ from baseline
CFU=colony forming unit.

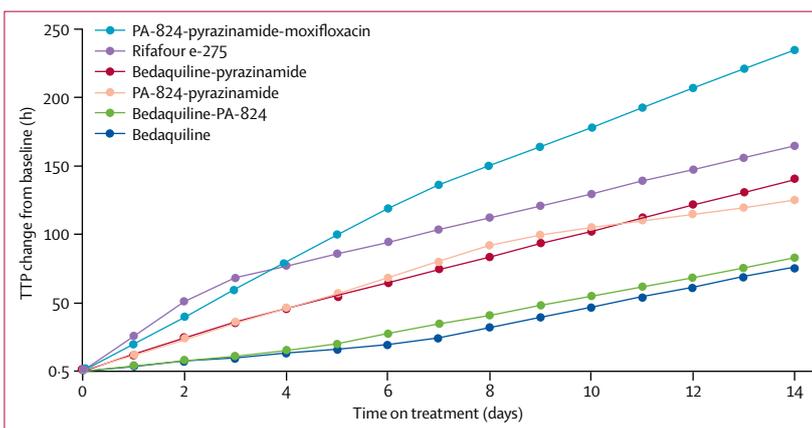


Figure 3: Bilinear regression showing the mean daily increase from baseline in time (h) to positivity (TTP) in liquid culture
TTP=time to positivity.

Adverse events were reported in more than half of all patients; most were mild and were not related to the investigational drugs (table 4). Seven patients were withdrawn due to adverse events: five with increased alanine aminotransferase in excess of triple the upper limit of normal, one patient receiving PA-824-moxifloxacin-pyrazinamide had an increase of 60 ms or more in QTcB and QTcF intervals on day 5 that met withdrawal criteria, and one patient had an episode of altered consciousness due to newly diagnosed neurocysticercosis. All these events, except the latter case, were asymptomatic and the patients were withdrawn according to prespecified criteria. One patient on bedaquiline-PA-824 and one patient on PA-824-pyrazinamide had minor lens opacities detected at the 90-day follow-up visit, but on specialist consultation these were classed as common findings in patients with normal lenses and not clinically relevant. No deaths occurred.

	Bedaquiline (n=15)	Bedaquiline- pyrazinamide (n=15)	Bedaquiline- PA-824 (n=15)	PA-824- pyrazinamide (n=15)	PA-824- moxifloxacin- pyrazinamide (n=15)	Isoniazid-rifampicin- pyrazinamide- ethambutol (n=10)
Skin or subcutaneous disorders	0	0	0	1 (6.7%)	1 (6.7%)	0
Gastrointestinal disorders	2 (13%)	0	0	0	2 (13%)	2 (20%)
Headache	0	1 (7%)	1 (7%)	0	1 (7%)	0
Hyperthermia	0	0	1 (7%)	0	0	0
Arthralgia	0	0	0	0	0	1 (10%)
Lenticular opacities	0	0	1 (7%)	1 (7%)	0	0
ECG, prolonged QTc interval	0	0	0	0	1 (7%)	0
ALT increased	0	0	2 (13%)	1 (7%)	1 (7%)	0
ALT and AST increased	0	1 (7%)	0	0	0	0
Serum amylase increased	1 (7%)	0	0	0	0	0
Total	3 (20%)	2 (13%)	5 (33%)	3 (20%)	6 (40%)	3 (30%)

Data are number (%). ECG=electrocardiography. QTc=QT corrected. ALT=alanine aminotransferase. AST=aspartate aminotransferase.

Table 4: Adverse events

Panel 2: Research in context

Systematic review

We searched PubMed for studies of early bactericidal activity (EBA) published since 1945 with the search terms “bactericidal activity”, “quantification of mycobacteria in sputum”, and “assessment of antituberculosis drug efficacy”. Only studies published in English, German, French, Spanish, or Italian were included. Cross referencing identified potentially relevant early studies. Formal studies of EBA of antituberculosis agents have been done since 1980 and measure the activity of an agent by the daily fall in colony forming units of *Mycobacterium tuberculosis* in sputum confirming that the relevant agent does indeed kill mycobacteria. Most studies were done over the first two treatment days but more recently it has become apparent that some newer agents, notably bedaquiline, PA-824, and delamanid, like the established agent pyrazinamide, have a delayed onset of action that might not be evident for several days. Most recent studies have been undertaken over 14 days. The response to treatment is also often biphasic with greater activity during the first 2 days of treatment. Single agents are most often used to establish efficacy, assess the optimum dose, and tolerability and toxic effects. Drug combinations have seldom been studied.

Interpretation

Our study provides reassurance that the novel combination of PA-824, moxifloxacin, and pyrazinamide can be explored over a longer period of time in patients with pulmonary tuberculosis. A regimen not containing isoniazid and rifampicin would represent a substantial step towards constructing a new regimen with low interaction potential with antiretroviral regimens, which is suitable for both fully drug-susceptible tuberculosis as well as tuberculosis resistant to isoniazid and rifampicin.

Discussion

Our study reports the first multiple agent combination EBA study for development of a next generation tuberculosis treatment regimen (panel 2). The study has at least three important findings. First, the new three-drug combination PA-824-moxifloxacin-pyrazinamide has a 14-day antituberculosis activity in sputum at least comparable with that of the current standard regimen for drug-susceptible tuberculosis. This drug combination relies neither on isoniazid nor rifampicin and

thus has the potential to treat patients with tuberculosis irrespective of sensitivity to isoniazid or rifampicin. A regimen without rifamycins would greatly simplify concomitant drug treatment especially with antiretroviral agents. Second, pyrazinamide, a drug largely free of early activity, seems to increase the activity of bedaquiline in the first week of treatment. This two-drug combination could become an important building block of future regimens. Third, and perhaps most importantly, this study supports the use of murine studies for selecting drug combinations to take forward into early clinical trials in human beings. In this study the bactericidal curves of all treatment groups closely mirror the murine data of the combinations tested.^{7,10,18} If the predictive value of murine studies for the activity of combinations is confirmed by further 14-day EBA studies, this strategy can accelerate the time needed to develop new treatment regimens. Broadly similar results were obtained using both of the methodologies for measuring bactericidal activity applied in this study.

The 14-day activity of PA-824-moxifloxacin-pyrazinamide in our study is virtually identical to that of isoniazid-rifampicin-pyrazinamide-streptomycin reported by Jindani and colleagues²⁹ (EBA_{CFU} of PA-824-moxifloxacin-pyrazinamide: 0.233; isoniazid-rifampicin-pyrazinamide-streptomycin: 0.232). The positive control group in our study was not powered for statistical comparison. Compared with the pooled 14-day activity of the positive controls receiving isoniazid-rifampicin-pyrazinamide-ethambutol in the present and a very recent EBA study at the same centres (isoniazid-rifampicin-pyrazinamide-ethambutol: EBA_{CFU} 0.140 and EBA_{TPP} 12.528, n=18), PA-824-moxifloxacin-pyrazinamide (EBA_{CFU} 0.233 and EBA_{TPP} 18.482, n=13) seems at least as active as isoniazid-rifampicin-pyrazinamide-ethambutol, while PA-824-pyrazinamide (EBA_{CFU} 0.154 and EBA_{TPP} 8.805, n=14) and bedaquiline-pyrazinamide (EBA_{CFU} 0.131 and EBA_{TPP} 9.970, n=15) have comparable 14-day activity.⁵ The

high activity of PA-824-moxifloxacin-pyrazinamide in the first week of treatment probably reflects the activity of moxifloxacin, which has been shown to possess high EBA_{CFU_0-2} in several studies^{12,14} and will therefore probably contribute to the prevention of drug resistance in companion drugs.³⁰ A very important aspect of this study is the increased activity of two-drug combinations containing pyrazinamide. In two previous EBA studies this drug had negligible, if any, bactericidal activity in the first days of treatment.^{29,31} Similarly, bedaquiline had little discernible early activity in this and other studies³ but, with the addition of pyrazinamide, activity occurs much earlier and at much increased magnitude. Pyrazinamide also seemed to augment PA-824 activity, which was not measured in this study but was quantified recently at the same centres.^{4,5} With the addition of pyrazinamide the 14-day EBA_{CFU} of PA-824 increased from 0.098 to 0.154 and the EBA_{TTP} increased from 4.494 to 8.805. In smaller numbers of patients, Jindani and colleagues²⁹ showed that the EBA_{CFU} of pyrazinamide alone during days 0 to 2 was 0.044, but increased to 0.113 from days 2 to 14. Similar to this study, the addition of pyrazinamide to isoniazid-streptomycin and isoniazid-rifampicin-pyrazinamide regimens led to a significant improvement in early activity ($p < 0.05$ for the period 0–2 days). While the underlying mechanisms need clarification, these observations on the important role played by pyrazinamide raise concerns about currently reported high rates of pyrazinamide resistance in patients with MDR tuberculosis.⁹

Could the combination moxifloxacin, bedaquiline, PA-824, and pyrazinamide be even more active? Bedaquiline, PA-824, and moxifloxacin all have at least some potential to cause disturbances of cardiac rhythm and more data are needed before such a regimen can enter clinical trials. However, in previous studies over 2 months a fluoroquinolone has been substituted for either isoniazid²³ or ethambutol^{22,21} and in these studies no additional adverse cardiac events seemed to accrue. We could not assess whether the increased activity of PA-824-moxifloxacin-pyrazinamide, bedaquiline-PA-824, and PA-824-pyrazinamide persisted beyond 14 days, thus improving sterilising activity and providing cure within a shorter time. This factor remains to be assessed in phase 2B and phase 3 studies with inclusion of more HIV-positive patients to confirm the expected good compatibility with antiretroviral regimens.

Limitations of our study and other similar studies of EBA should be mentioned. The relatively small number of patients enrolled allows quick and relatively cost-effective proof of concept, but results of individuals can substantially influence the overall results. Consistent EBAs have been reported by different research groups in different populations for established agents such as isoniazid, rifampicin, and ofloxacin^{19,25} and the EBA of PA-824 and control regimens has been measured with great consistency in recent studies.^{4,5} Comparisons

between groups and studies remain difficult because of limited sample sizes. The inclusion of several new drugs into new combinations in early clinical testing might accelerate the development of new regimens but limits the ability to assign adverse events to a particular agent. The low interaction potential with antiretroviral drugs needs confirmation in studies including patients with HIV co-infection receiving antiretroviral treatment. Finally, the value of EBA studies over 14 days for predicting the ability of a regimen to prevent relapse of pulmonary TB is uncertain.

Since moxifloxacin and PA-824 have shown great potential in murine studies to aid sterilisation of lesions, PA-824-moxifloxacin-pyrazinamide can now be investigated in clinical trials for a longer duration. Bedaquiline-PA-824 and PA-824-pyrazinamide showed activity comparable with isoniazid-rifampicin-pyrazinamide-ethambutol and could become important building blocks of future regimens. A regimen not containing isoniazid and rifampicin would represent a substantial step towards a new regimen with low interaction potential suitable for both fully drug-susceptible and MDR tuberculosis. With this study the path to the construction of new regimens becomes clearer.

Contributors

AHD contributed to the execution of the study, interpretation, and report writing. RD, FvG-B, and GS contributed to patient management. AHD, PRD, CvN, HW, CMM, and MKS contributed to the study design. AV contributed microbiology data. CMM, MKS, DE, CvN, and PB contributed to the data analysis. AHD, CMM, MKS, PB, DE, CvN, PRD, and HW contributed to the data interpretation. PB contributed to the writing of the statistical concepts. HW contributed to the writing of the pharmacokinetic and pharmacodynamic data. AHD, RD, FvG-B, GS, PRD, CvN, DE, HW, CMM, and MKS contributed to the writing of the report.

Conflicts of interest

PRD has acted as a consultant to Otsuka and Tibotec regarding the development of antituberculosis agents. All other authors declared no conflicts of interest.

Acknowledgments

We thank Joanna Breitstein for assistance with preparing and submitting the report.

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Treatment of tuberculosis: have we turned the corner?



The number of multidrug-resistant (MDR) tuberculosis cases officially reported to WHO increased from 29 000 to 53 000 between 2008 and 2010,¹ still representing only 18% of the estimated 290 000 patients potentially identifiable if drug susceptibility testing was done in all notified cases of tuberculosis.¹ A recent study done in Belarus² showed a new global record for prevalence of MDR tuberculosis with 35.3% of new patients and 76.5% of previously treated patients diagnosed with the disease. This finding clearly shows how far case mismanagement can affect the chances to control (and eventually eliminate) the disease.^{1,2} Unfortunately, since only a quarter of patients with tuberculosis are treated according to established standards and the proportion of treatment success does not exceed 50%,¹ extensively drug-resistant (XDR) tuberculosis has already been reported in 77 countries and totally drug resistant cases (ie, *Mycobacterium tuberculosis* strains with resistance to all known drugs) have been recently described in Italy, Iran, and India.³

The urgent need for new drugs is obvious. The question the tuberculosis community is anxiously posing is whether, in addition to existing drugs,⁴ the most promising compounds in the development pipeline (delamanid, bedaquiline, and PA-824) are as effective as preliminary studies suggested.¹ Gler and colleagues⁵ and Diacon and colleagues⁶ provided part of the answer when they reported that delamanid in combination with a background regimen developed according to WHO guidelines,⁷ is associated with an increase in sputum-culture conversion at 2 months in patients with MDR tuberculosis.

In *The Lancet*, Andreas Diacon and colleagues⁸ point to a new direction in tuberculosis treatment with a universal regimen that would be equally effective against *Mycobacterium tuberculosis* susceptible and MDR strains. Diacon and colleagues assessed the 14-day early bactericidal activity (EBA) of PA-824-moxifloxacin-pyrazinamide. The mean 14-day EBA of this combination (n=13; 0.233 [SD 0.128]) was significantly higher than that of bedaquiline alone (n=14; 0.061 [0.068]), bedaquiline-pyrazinamide (n=15; 0.131 [0.102]), bedaquiline-PA-824 (14; 0.114 [0.050]), but not PA-824-pyrazinamide (n=14; 0.154 [0.040]), and was comparable with standard treatment (ie, rifampicin,

isoniazid, and pyrazinamide with streptomycin or ethambutol; n=ten; 0.140 [0.094]), as reported previously.^{8,9} Importantly, the addition of pyrazinamide increased the activity of bedaquiline and PA-824.

Diacon and colleagues' study makes several important contributions to the existing body of knowledge. First, treatments seem to be well tolerated and safe, although their study design and sample size does not allow assignment of adverse events to a specific agent. The exclusion of tuberculosis patients with comorbidities and the poor sample-size-related inferential strength are methodologically justified by the early clinical research phase and underline the need for further trials that enrol more heterogeneous and larger cohorts.

Second, the new experimental regimen PA-824-moxifloxacin-pyrazinamide does not include three of the current four first-line drugs, yet still retained activity at least comparable with the current standard WHO category I regimen over the first 2 weeks of treatment. Third, the new regimen seems to have a low potential for interactions with antiretrovirals. New regimens without rifampicin could be beneficial for HIV positive or negative individuals at risk of drug-drug interactions. The lack of activation of the nuclear receptor PXR by rifamycins slows down the transcription of the cytochrome CYP3A4, allowing safe administration of several medicines (eg, prednisolone, hormonal contraceptives) in patients with serious disorders.¹⁰ Furthermore, a major advantage

Published Online
July 23, 2012
[http://dx.doi.org/10.1016/S0140-6736\(12\)61183-0](http://dx.doi.org/10.1016/S0140-6736(12)61183-0)

See Online/Articles
[http://dx.doi.org/10.1016/S0140-6736\(12\)61080-0](http://dx.doi.org/10.1016/S0140-6736(12)61080-0)



of the new rifampicin-sparing regimens will rely on averting the pharmacological interactions between rifampicin and protease inhibitors, CCR-5 receptor antagonists, and non-nucleoside reverse transcriptase inhibitors.¹¹ Fourth, the new regimen seems to have the characteristics necessary to treat both drug-susceptible as well as isoniazid-resistant and rifampicin-resistant tuberculosis cases.

However, two pivotal issues remain to be addressed. First, this novel approach of regimen development will require a careful assessment of toxic effects in future studies. This kind of assessment is of crucial importance before embarking on regulatory approval and, particularly, if one or more novel, unapproved compounds are included in the regimen. Second, in future trials it would be ideal to develop and adopt biomarkers or surrogate markers able to rapidly detect the microbiological efficacy of a new single drug or of a new combination of antimycobacterial drugs, following the successful use of this approach in other infectious diseases such as HIV/AIDS and hepatitis C virus infection.

Although EBA-based studies allow investigation of the sterilising activity of available antituberculosis drugs (assessing the ability to prevent the emergence of resistant strains, in the early stages of the treatment and when the load of viable mycobacteria is elevated), they might be affected by intrinsic unpredictability (related to selected patients' clinical features and sputum-sampling methods).¹² Furthermore, the long-term effect (ie, the reduced rate of relapses) of the regimens cannot be directly investigated.

The next question to pose is whether the new drugs could rapidly treat individuals with latent infection as well as 90% of patients with XDR tuberculosis within 2 months. These outcomes need to be achieved by the new antituberculosis regimens to embark on the elimination phase, which WHO is currently debating.¹³

The rational use of antibiotics has attracted major attention and was selected as the topic of the 2011 World Health Day.¹⁴ Mistakes that have been made with the most effective drugs we have to treat tuberculosis (rifampicin and fluoroquinolones) should be kept in mind.¹⁵ The international community has

the chance to prevent the misuse of new drugs and regimens. To protect the investment in these drugs, the rational use of antibiotics within strengthened health systems is necessary to avoid the real risk of losing these new agents in a time shorter than that needed to develop them.

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We declare that we have no conflicts of interest.

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