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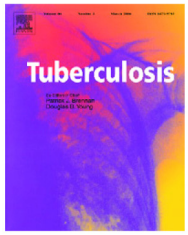

The early bactericidal activity of anti-tuberculosis drugs: a literature review

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Summary
Quantification of mycobacteria in sputum from pulmonary tuberculosis patients has been used to evaluate patient's response to treatment since the earliest days of anti-tuberculosis chemotherapy. More recently the early bactericidal activity (EBA) of anti-tuberculosis agents, measured as the fall in viable colony forming units of *Mycobacterium tuberculosis* in sputum early in therapy, has been shown to be an objective, repeatable measure of the ability of an agent to kill the metabolically active bacilli found in the sputum of patients with sputum microscopy smear-positive pulmonary tuberculosis. EBA offers an opportunity to rapidly demonstrate that a new agent has a detectable anti-tuberculosis effect in a relatively small number of patients, what the most appropriate dose is to take forward to more extensive clinical trials and allows the study of the relationship between pharmacokinetics and bactericidal activity and toxicity of the relevant agent.

Introduction

This paper reviews literature related to the early bactericidal activity (EBA) of anti-tuberculosis drugs and the place of EBA studies in anti-tuberculosis drug evaluation. The literature reviewed was identified from an existing literature collection and by a search of PubMed using keywords anti-tuberculosis drugs, anti-tuberculosis chemotherapy, early bactericidal activity, evaluation of new drugs. Further cross-references were obtained from bibliographies of identified papers; English, French and German language papers were reviewed and those in other languages if accompanied by an English summary.

Following the work of Fenner et al accurate quantification of mycobacteria in biological specimens, especially those from experimental animals, became possible.[1] With the introduction of the first anti-tuberculosis agents similar techniques were applied to sputum from pulmonary tuberculosis patients to evaluate response to chemotherapy. During early streptomycin (SM) trials considerable importance was attached to the "fall and rise" in the number of acid-fast bacilli (AFB) and viable colony forming units (CFU) of *Mycobacterium tuberculosis* in sputum as heralding the onset of drug-resistance.[2,3,4] By the late 1950's the technique was an accepted means of demonstrating the efficacy of new agents, even if a rather "rough guide".[5,6] One of the first attempts to formally evaluate and compare one drug's efficacy with another in a randomized trial quantifying the decline in AFB in sputum in response to therapy was in 1969; the Wissenschaftliche Arbeitsgemeinschaft für die Therapie von Lungenkrankheiten compared thiocarlide to para-amino salicylic acid (PAS) and bed-rest in 29 pulmonary tuberculosis patients over 6-weeks and found PAS more effective than thiocarlide, and thiocarlide

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more effective than bedrest.[7] Schütz et al from the same group then studied monotherapy with rifampicin (RMP), ethambutol (EMB) and PAS in 23 patients with previously treated cavitating pulmonary tuberculosis.[8] RMP and EMB were equivalent, but both were more effective than PAS. Hobby et al (1973) recognized the technique as valuable to study drugs and regimens as opposed to determining the prognosis of individual patients and stated "For the first time, it offers a means for precise evaluation of rates of sterilization by various chemotherapeutic regimens".[9]

The modern era of EBA studies commenced in 1980 with the groundbreaking study of Jindani et al.[10] This study described the evaluation of 27 anti-tuberculosis drugs and regimens in 124 African pulmonary tuberculosis patients with sputum microscopy smear-positive disease using enumeration of viable CFU in sputum before treatment and every second day thereafter for the first 14 treatment days. This paper provided a wealth of insights into the early actions of anti-tuberculosis agents and influenced later study design.

Following on the findings of this study EBA was defined as "The fall in counts/mL sputum/day during the first 2 days of treatment".[12] In this review the suffix $\log_{10}$cfu/mL sputum/day is omitted and the abbreviation EBA will be followed by numbers indicating the days over which the study was conducted or analyzed; EBA 0–2, EBA 0–5, 0–7 or 0–14, or 2–5, 2–7 or 2–14.

Noteworthy details include:

- A significant difference between the fall in viable counts over the first two treatment days compared to the subsequent 12 days; differences between different drugs and regimens were also most significant during this period. All calculations and comparisons thus involve the periods 0–2 and 2–14 days.
- The activity of isoniazid (INH) was dominant early in therapy and overwhelmed that of other drugs when added to combinations. Any regimen to which INH was added achieved a higher EBA, but never higher than INH alone. When RMP was added to single drugs, other than INH, activity increased, but when added to 2 or 3 drug regimens there was no further increase and possibly a decrease in activity. The single instance when the addition of other drugs to an INH containing combination increased activity was with SM/INH and SM/INH/RMP; the addition of pyrazinamide (PZA) increased the mean EBA 0–2 from 0.415 to 0.742.
- The effect of combined regimens on the EBA 2–14 was examined in greater depth in a later review of the data.[11] In multiple regression analysis RMP and SM added to combined regimens had significant effects, but not INH or PZA. EMB (dose 25 mg/kg), by contrast, had an effect in the reverse direction suggesting antagonism of activity of other drugs. From day 2 onward bactericidal activity is greater when RMP is added to other regimens, with or without INH, with an overall mean of 0.140 compared to that of INH, without RMP, of 0.089. There is also a suggestion that killing accelerated only after day 6 in patients receiving RMP or INH and RMP, but not INH. The EBA 2–14 for INH and RMP alone was 0.112 and 0.096 respectively, but 0.201 for INH and RMP combined. INH and RMP together appear to achieve greater killing than either drug alone, particularly from day 6 to day 14.
- A dose related response was shown for RMP and EMB, but not INH. With regard to RMP this suggested that the highest RMP dose (10–12 mg/kg), generally used in contemporary regimens, might be closer than usually appreciated to the minimal effective dose.
- The collection of pooled 12-hour overnight sputum samples and the use of selective media to prevent contaminant overgrowth rather than preliminary decontamination with an alkali such as sodium hydroxide. Sputum was collected on two successive days before the start of chemotherapy.
- Resistance to INH, not present before the study, was detected after the study in only 1 patient who received monotherapy with INH 150 mg for 14 days.
- The ranking of agents by EBA revealed by this study differed from that arising from sterilizing activity as defined in studies of regimens and associated relapse rates. This has cast doubt on the ultimate value of EBA studies, as it is rapid sterilizing activity that is now considered the most valuable attribute of an anti-tuberculosis agent.

Following this study a series of EBA evaluations were undertaken that make a considerable contribution to our understanding of the actions of anti-tuberculosis agents and, in particular, the relationship between dose and bactericidal efficacy and, in some instances, pharmacokinetics. These studies also elicited discussion about the role of EBA studies in the evaluation of new drugs and related methodological issues.

Isoniazid

In all EBA studies thus far, INH at a 300 mg dose consistently had the highest EBA 0–2 of any agent (Table 1). The bactericidal action of INH is dose related, reaches a plateau at a dose of approximately 300 mg and can be detected at a dose as low as 18 mg.[16] The N-acetyltransferase-2 genotype (NAT2) also influences the EBA of INH and faster INH acetylators consistently have a lower EBA. Nonetheless at a 300 mg dose, commonly used in clinical practice, the EBA of homozygous fast acetylators of INH is higher than that of any other anti-tuberculosis agent.[24]

The rifamycins

As with INH, EBA studies confirm a dose-related increase in the EBA of RMP.[10,13,15,26] Furthermore, the original study of Jindani et al and a recently published study show that a 20 mg/kg dose (approximately 1000 mg) causes a further linear increase in the EBA 0–2 of RMP.[10,27] It remains uncertain whether this will be reflected in increased sterilizing activity and treatment shortening. Studies evaluating the EBA 0–2 of a RMP dose of 600 mg are summarized in Table 2 and show, with one exception, an EBA ranging from 0.174 to 0.293.

Table 1 The early bactericidal activity (EBA) 0–2 days of isoniazid (INH) 300 mg

		EBA of INH 300 mg		
Authors	N	0–2 days	0–5 days	0–14 days
Jindani et al (1980)[10]	4	0.722 (SD 0.10)		0.192
Chan et al (1992)[13]	14	0.431 (SD 0.37)		
Kennedy et al (1993)[14]	5		0.25 (0.19–0.40)*	
Sirgel et al (1993)[15]	11	0.495 (SD 0.19)		
Donald et al (1997)[16]	12	0.554 (SD 0.21)		
Sirgel et al (1997)[17]	11	0.539 (SD 0.21)		
	10	0.656 (SD 0.30)		
Hafner et al (1997)[18]	16		0.21 (SE 0.03)	
Mitchison and Sturm (1997)[12]	12	0.457 (SD 0.47)		
Chambers et al (1998)[19]	11	0.60 (SD 0.30)		
Sirgel et al (2000)[20]				
Cape Town	8	0.636 (SD 0.17)		
Hong Kong	10	0.371 (SD 0.39)		
Dietze et al (2001)[21]	15			0.189
Gosling et al (2003)[22]	14	0.77 (SD 0.37)		
Pletz et al (2004)[23]	9		0.209 (CI: 0.01–0.40)	
Donald et al (2004)[24]	21	0.553 (SE 0.05)		
Johnson et al (2006)[25]	10	0.67 (SD 0.17)		

*Range

Table 2 The early bactericidal activity (EBA) of rifampicin 600 mg

		EBA of rifampicin 600 mg		
Authors	N	0–2 days	0–5 days	2–14 days
Jindani et al (1980)[10]	8	0.174 (SD 0.23)		0.113 (SD 0.096)
Chan et al (1992)[13]	11	0.293 (SD 0.30)		
Sirgel et al (1993)[15]	10	0.204 (SD 0.04)		
Mitchison & Sturm (1997)[12]	14	0.299 (SD 0.36)		
Sirgel et al (2000)[20]				
Cape Town	10	0.174 (SD 0.16)		
Hong Kong	8	0.631 (SD 0.48)		
Gosling et al (2003)[22]	12	0.28 (SD 0.21)		
Sirgel et al (2005)[26]	14	0.221 (SD 0.25)	0.28 (SD 0.21)	

Three other rifamycins have been evaluated; in two studies the EBA 0–2 of rifabutin (RFB) was determined.[13,15] In both a dose related response was found, but at the lowest doses, 75 mg and 150 mg, there was no activity, and at doses of 300 mg and 600 mg a response considerably less than that of equivalent RMP doses. Despite these findings, when RFB 300 mg replaced RMP 600 mg in a clinical trial, neither treatment failure nor the relapse rates were affected.[15] This emphasizes again the divergence between EBA and sterilizing activity.

In the aftermath of trials showing emergence of rifamycin resistance when once weekly rifapentine (RFP) and INH was compared to twice or thrice weekly RMP and INH, a 5 day dose ranging study of RFP and RMP was undertaken.[26] Single RFP doses of 300, 600, 900 or 1,200 mg were compared to RMP in daily doses of 150, 300 and 600 mg. At the 300 mg and 600 mg doses, where a direct comparison between RMP and RFP was possible, the EBA for each drug (days 0–2, 2–5 and 0–5) was not significantly different, although those for RFP were higher. Whereas the values for RFP tended to level out at the highest doses indicating a possible maximum effect, there was no such indication for RMP.

Rifalazil (RFZ), a semisynthetic rifamycin with a long half-life of approximately 60 h, has exceptional bactericidal activity against *M. tuberculosis* in vitro and in animal models.[28] RFZ in doses of 10 mg/kg and 25 mg/kg combined with INH (5 mg/kg) was compared to INH (5mg/kg) alone and combined with RMP (10 mg/kg) daily over 14 days.[21] Although all the regimens decreased the sputum bacillary load measured by viable CFU counts, the mean 14 day decrease in the rifamycin arms was not significantly different from that of INH. The authors commented that short-term quantitative sputum cultures may not be the best indicator of rifamycin efficacy.

Table 3 The early bactericidal activity of fluoroquinolones (dose mg)

					Days 0–2					
Authors	n	CFX (1500)	n	OFX (800)*	n	MFX (400)	n	LFX (1000)	n	GFX (400)
Sirgel et al (1997)[17]	11	0.21 (SD 0.17)								
Mitchison & Sturm (1997)[12]			10	0.32 (SD 0.36)						
Chambers et al (1998)[19]			10	0.32 (SD 0.05)						
Sirgel et al (2000)[20]			11	0.38 (SD 0.31)						
Gosling et al (2003)[22]					8	0.53 (SD 0.31)				
Johnson et al (2006)[25]					9	0.33 (SD 0.39)	10	0.45 (SD 0.35)	10	0.35 (SD 0.27)
					Days 0–5[a], 0–7[b] or 2–7[c]					
Kennedy et al (1993)[14,b]	5	0.20 (range 0.09–0.40)								
Chambers et al (1998)[19,c]			10	0.16 (SD 0.02)						
Pletz et al (2004)[23,a]					8	0.27 (CI 0.00–0.55)				
Johnson et al (2006)[25,c]					9	0.17 (SD 0.10)	10	0.18 (SD 0.13)	10	0.17 (SD 0.13)

CFX=ciprofloxacin, OFX=ofloxacin, MFX=moxiflaxacin, LFX=levofloxacin, GFX=gatifloxacin.
*In the study of Chambers et al 1998 an OFX dose of 600 mg was used.

Fluoroquinolones

The EBA of fluoroquinolones has been examined in 7 studies summarized in Table 3. In nearly all studies, whether over 0–2, 0–5 or 0–7 days, the fluoroquinolones have shown bactericidal activity close to, or equal to, that of INH. The single exception was ciprofloxacin (CFX); in a dose-ranging study, a dose-related rise in EBA 0–2 was found, but even at the highest dose (1,500 mg), the EBA (0.205, SD 0.17) was significantly lower than that of INH (0.539, SD 0.21).[17] However, in an EBA 0–7 study, the activity of CFX (0.20, range 0.09–0.40) matched that of INH (0.20, range 0.19–0.40).[14] In a second arm of this study 11 patients received INH/RMP/PZA/EMB and 9 INH/RMP/CFX. The fall in CFU counts is shown in the paper in figure form; there was no difference in the early rate of fall, but after the first week the INH/RMP/CFX regimen lags and at 4 weeks only 3/9 INH/RMP/CFX patients , but 9/10 INH/RMP/PZA/EMB patients were culture-negative (Fisher exact test P=0.02). It is of interest that this study reveals the role of PZA during the first month of treatment, despite the presence of RMP in both regimens.

The first EBA study to evaluate ofloxacin (OFX) found a very promising EBA 0–2 of 0.32 (SD 0.36) amongst 10 African patients in Durban.[12] This was identical to the EBA 0–2 of 0.32 (SD 0.05) in a study enrolling 10 patients from California, USA and Ankara, Turkey.[19] A later study enrolling 11 patients from Cape Town reported EBAs 0–2 and 3–5 of 0.391 (SD 0.19) and 0.17 (SD 0.20) respectively.[20]

The EBA 0–5 of moxifloxacin (MFX) in comparison to those of INH 300 mg and RMP 600 mg was studied in 43 patients in Tanzania.[22] The results were presented as EBA 0–2 and as the time taken to reduce the viable count by 50% (vt_{50}). The EBA 0–2 of INH, MFX and RMP were 0.77 (SD 0.37), 0. 53 (SD 0.31) and 0.28 (SD 0.21), respectively; the vt_{50} was 0.46, 0.88 and 0.71 respectively. In a subsequent study the same researchers evaluated the combination of MFX and INH.[29] The mean EBA 0–2 for INH/MFX was 0.60 (95% CI 0.23–0.97; SEM 0.14), and this did not differ significantly from that for INH or MFX alone. Although no benefit was shown from combining INH and MXF there was no evidence of an antagonistic effect of MFX on INH. The vt_{50} for INH/MFX was 0.38 days and this, also, did not differ from that for INH and MFX independently.

In the most recent fluoroquinolone study the EBA 0–2 and 2–7 of levofloxacin (LFX), MFX and gatifloxacin (GFX) was ascertained.[25] The mean INH EBA 0–2 (0.67, SD 0.17) was greater than those of GFX (0.35, SD 0.27, P=0.01) and MFX (0.33, SD 0.39 P=0.02), but did not differ (P=0.14) from that of LFX (0.45, SD 0.35). Although no significant differences in the mean EBA 2–7 or in the

Table 4 The early bactericidal activity of aminoglycosides

Authors		Jindani et al (1980)[10]		Donald et al (2002)[30]		Donald et al (2001)[31]		Donald et al (2000)[32]
Dose (mg/kg)	n	Streptomycin	n	Streptomycin	n	Amikacin	n	Paromomycin
30			8	0.133 (SD 0.09)				
20	4	0.094						
15			9	0.043 (SD 0.10)	15	0.052 (SD 0.10)	15	0.092 (SD 0.14)
10					13	0.045 (SD 0.14)		
7.5			10	−0.133 (SD 0.16)	12	0.041 (SD 0.10)	7	0.066 (SD 0.22)

mean rates of fall of CFU were found between treatment with single agents, combination of the fluoroquinolone groups had greater activity than INH (P=0.036). It is to be hoped that these encouraging findings will be matched by fluoroquinolone sterilizing activity in long-term multidrug studies.

Aminoglycosides

The aminoglycosides are the oldest drug class with a consistent place in tuberculosis treatment; SM is still listed as an essential drug by the World Health Organization. Three aminoglycosides SM, amikacin (AM) and paromomycin (PM) have been the subject of five studies and in three a range of doses was evaluated (Table 4). In all of these a relatively low EBA of <0.1 was found at doses usually used in clinical practice, although the EBA 2–14 of SM rose to 0.128.[10] Low-clearance liposomal encapsulated AM (30 mg/kg) was studied for 3 days, but found to be without effect.[33]

The third aminoglycoside evaluated was PM, also known as aminosidine, a broad spectrum aminoglycoside structurally related to neomycin and kanamycin. PM has been used to treat visceral leishmaniasis and there is anecdotal evidence of its use in drug-resistant tuberculosis.[34] Two doses of PM, 7.5 and 15 mg/kg, were evaluated, and an EBA 0–2 of 0.066 (SD 0.216) and 0.092 (SD 0.140) respectively found. Thus, despite being in vitro amongst the most bactericidal agents available[35] all the aminoglycosides have a relatively low EBA.

Other anti-tuberculosis agents

Other agents evaluated in EBA studies include EMB, PZA, PAS and amoxicillin/clavulanate.

EMB was evaluated in two studies. In the first arm of the study of Jindani et al doses of 15, 25 and 50 mg/kg, were studied over 5 days.[10] The EBA 0–2 of these doses in 3, 4 and 5 patients was 0.05, 0.37 and 0.38 respectively and these differences were not significant. However, the EBA 0–5 of these doses was 0.07, 0.12 and 0.42 respectively; these differences were significant (P=<0.05). In planning dose ranging studies for new drugs these results should be recalled as the optimal study duration to distinguish between the efficacy of different doses of an agent may vary considerably.[12] It is also relevant to draw attention to the very low EBA 0–2 of 0.05 of EMB 15 mg/kg[10] (the dose currently recommended by the World Health Organization). EMB (25 mg/kg) was also given over 14 days; the EBA 0–2 was now 0.25 and the EBA 2–14, 0.16. The inclusion of EMB in combined regimens led, however, to a decrease in mean EBA 2–14 from 0.13 to 0.10. In the second study[36] an EMB dose of approximately 25 mg/kg led to a moderate EBA 0–2 of 0.26 (95% CI 0.36 and 0.13).

PZA (dose 2 g) was evaluated in Nairobi and Cape Town.[10,36] The EBA of PZA is particularly interesting as PZA contributes significantly to sterilization of lesions and thus treatment shortening. The EBA 0–2 of PZA in Nairobi was 0.044 and 0.004 (SD 0.129) in Cape Town. Despite this negligible EBA 0–2 the Nairobi EBA 0–14 was 0.110. The individual PZA EBA 0–14 results appear in a figure and it is interesting that in 4 of the 9 patients evaluated there appears to be a fairly rapid fall in counts, while in the remaining 5 patients the counts increase or remain static, falling only at day 4 or later. The effects of SM and PZA combined (EBA 0–2, 0.118 and 2–14, 0.176) appear to be complementary.[10]

The EBA of para-aminosalicylic acid (PAS) has been evaluated once and then in only 4 patients.[10] Although considered a weak drug, mainly useful for preventing the development of resistance to companion drugs, a moderate EBA 0–2 of 0.259 was recorded for PAS, although the EBA 2–14 was low at 0.076.

Amoxicillin/clavulanate has been evaluated in two EBA studies. In the first, 10 pulmonary tuberculosis patients from California, USA and Ankara, Turkey received amoxicillin 1 g and clavulanate 250 mg three times daily for 7 days and an EBA of 0.39 (SD 0.32) was found, although the EBA 2–7 fell to 0.02 (SD 0.04).[19] In one of the few instances where the results of EBA studies for particular agents from different locations have differed, a later study from Cape Town enrolling 10 patients to receive amoxicillin 3g and clavulanate 750 mg in a single dose found an EBA 0–2 of 0.018 (SD 0.130) that did not differ from that found in patients receiving no drug (0.016, SD 0.069).[37] The reason for these conflicting findings must remain speculative, but it seems unlikely that the different dosing regimens used were responsible.

Relationship between pharmacokinetics and early bactericidal activity

One of the most valuable aspects of EBA studies is the opportunity to evaluate the pharmacokinetics of an

agent in the context of the dynamic interaction of agent and bacillus in the complex environment of pulmonary tuberculosis lesions.

The first occasion when the pharmacokinetics of an agent was studied simultaneously with EBA was during an evaluation of RMP and RFB.[13] The peak serum concentrations of RMP after the maximum dose of 600 mg (9.53 μg/mL) were more than ten times higher than those of RBU following the same dose (0.73 μg/mL), while the respective EBA's 0–2 were 0.293 and 0.049.

During a five day study of daily RMP and a single dose of RFP, serum concentrations of RMP were determined on treatment days one and five from 0–12 h and RFP concentrations after the single RFP dose for up to 96 h. When it was assumed that only 14% of the measured RMP serum concentration was not protein bound and 2% of RFP, the ratios of the area under the curve (AUC) to minimal inhibitory concentration (MIC) for the drugs were similar and found to "associate well" with the EBA 0–5 in the dosage groups evaluated.[26] Protein binding may be an important factor limiting the bactericidal activity of anti-tuberculosis drugs. Pharmacokinetic studies were also undertaken during a more recent study of high-dose RMP (20 mg/kg)[27] and, although the mean peak RMP serum concentration on the first treatment day, before enzyme induction (13.0 μg/mL, SD 4.5), was similar to that after a 600 mg dose in the previous study of Sirgel et al (14.0, SD 4.7), also before enzyme induction,[26] the AUC was significantly higher (171 μg.h/mL vs 100 μg.h/mL). This suggests that the AUC is more closely associated with RMP bactericidal activity than peak serum concentrations.

Hafner et al determined the INH C_{max}, T_{max} and the AUC_{0-12},[18] however, in covariate analysis, none of these parameters had a significant effect on the INH EBA 0–5. INH serum concentrations at 3 h after dosing were determined during a dose-ranging study of INH.[16] The serum concentrations varied in proportion to dose and at the lowest effective dose of 18.7 mg were 0.12 μg/mL where the MIC of *M tuberculosis* was approximately 0.1 μg/mL. During a study of the influence of NAT2 genotype on EBA the serum concentrations of INH were determined 2, 3, 4 and 5 hours after dosing and a correlation shown between EBA and the AUC_{0-5}.[24] It was also shown that a maximum EBA was reached at a 2 h INH serum concentration of ~2.5 μg/mL.

In a further exploration of these findings the optimal EBA of INH was defined as that which was 90% of the mean maximum EBA 0–2 or the EBA_{90}; this was 0.512.[38] Kinetic factors associated with the INH EBA_{90} were a 2 h INH concentration of 2.19 (SEM 0.68) μg/mL and an $AUC_{0-\infty}$ of 10.52 (SEM 3.69) μg/mL per hour. Defining the EBA_{90} as 90% of the maximum EBA arising either, as in the case of INH, by a flattening of the dose-response curve or, as with SM, by an unacceptable incidence of toxicity at higher doses, and relating it to accompanying pharmacokinetics, introduces a further element of objectivity to the study of anti-tuberculosis agents.

In the case of amikacin (AM) serum concentrations were determined at 1, 2, 3 and 4 h after dosing, at the same time as the EBA was determined; despite concentrations well above MIC at the maximum dose of 15 mg/kg, the EBA remained low.[31] Achieving serum concentrations well in excess of MIC for an agent is no guarantee that the agent will have a high EBA. Other factors such as tissue penetration, lesional pH and presence of other inhibitors or excessive protein-binding may limit drug action.

The serum concentrations of RFZ were determined in a 14 day study of the combination of INH and RFZ. Approximate dose proportionality was found with C_{max} values of 13.5 (SD 4.6) and 26.4 (SD 11.0) μg/mL following 10 and 25 mg/kg doses.[21]

A pharmacokinetic study accompanied an EBA study of the fluoroquinolones LFX, GFX and MFX and INH.[25] The C_{max} and AUC_{0-24} were determined and a relationship sought between EBA and MIC of the drugs. When treatment groups were compared, the AUC_{0-24}/MIC paralleled the EBA 0–2 with INH having the highest value followed by LFX, MFX and GFX. AUC_{0-24}/MIC was correlated with EBA 0–2 for the GFX patients only, but not for subjects in the other arms of the study. No pharmacokinetic correlation was found for EBA 2–7.

Methodological issues

Patients enrolled in EBA studies should be newly diagnosed and not have received any previous anti-tuberculosis treatment; a high bacillary burden, as reflected by severe sputum microscopy smear-positivity will allow better opportunity to detect differences between individual drugs and regimens and different doses of the same drug. Hospitalization of patients for the duration of the study greatly facilitates supervision of dosing, better sputum collection and observation for possible toxicity, this being particularly important when a new investigational agent is being evaluated. The great majority of studies reviewed in this paper enrolled between approximately 10–15 patients in each group evaluated. This relatively small number of patients has proved sufficient to demonstrate differences between individual drugs and doses and means the exposure of only a small number of individuals to the potential, although limited danger, of delaying full anti-tuberculosis chemotherapy. It must be emphasized that only pulmonary tuberculosis patients with uncomplicated disease should be admitted to EBA studies; those with complications such as disseminated forms of disease or tuberculosis meningitis must receive immediate multi-drug therapy.

The use of monotherapy in patients with sputum microscopy smear-positive disease might be considered liable to elicit resistance to the investigational drug, however in the great majority of studies reviewed in this paper, sensitivity testing both before and after the EBA study demonstrated the acquisition of drug resistance in only one instance, a patient who received INH 150 mg monotherapy for 14 days.[10]

Other issues related to EBA methodology and data analysis that have elicited discussion include whether sputum should be collected overnight or whether a morning specimen is sufficient, whether sputum specimens should be decontaminated prior to culture, whether the use of a "no drug" control group is needed

and what statistical approach should be used to analyze results and the optimal length of time over which a study should be conducted.

During the first comprehensive study of Jindani et al sputum was collected overnight for 12 hours to ensure representative sampling of cavity contents.[12] The potential influence of sputum collection over different periods was examined by Hafner et al.[18] During this study of the EBA 0–5 of 300 mg INH, sputum was collected from 8:00 pm to 6:00 am the next morning (10 h) and from 6:00 am to 8:00 am (2 h). The sputum volume of the 2 h collection was 6.1 mL (SE 3.7 mL) and that of the 10 h collection 18.6 mL (SE 11.4 mL); the composite 10 h and 2 h collection (12 h) had a mean volume of 24.6 mL (SE 14.6 mL). The EBA estimated from the 10 or 12 h sputum collections had a 2-fold smaller SE than estimates from the 2 h collections. In the light of these findings it would seem wise to require that patients produce at least 10 mL of sputum overnight for inclusion in EBA studies.

In many earlier EBA studies untreated or "no drug" patients were included and their results compared to those of the treated groups. The inclusion of untreated patients poses ethical problems and adds considerably to the costs of any study. In a review of sources of variation in EBA studies the weighted mean of five "no drug" groups studied in Cape Town was 0.00036 and the suggestion made that it would be appropriate to test the means of treated groups against zero.[39] An alternative approach is to measure EBA as the fall in CFU between the mean of 2-baseline-days and the relevant treatment days[18], and this approach was adopted in several recent studies.[21,22,25]

On reviewing EBA studies, it is evident that bacterial and fungal contamination has been a frustrating problem. Two approaches to this are a decontamination procedure for sputum specimens or the use of selective media. In several studies a relationship was shown between the pretreatment CFU count and the fall in CFU.[16,19] In a dose ranging INH study multiple regression of log INH dose and pretreatment count found a significant effect for the pretreatment count ($P<0.01$) indicating a rise of 0.094 (CI 0.029–0.1580) in EBA for every 10-fold increase in pretreatment count.[16] In the study of Chambers et al (1998) CFU counts were done both with and without decontamination and those not decontaminated found to be ~0.5 to 1.0 $\log_{10}$ higher.[19] As EBA detects the action of an agent against actively metabolizing bacilli and it is precisely these that are killed during NaOH decontamination it would seem preferable to use selective media to carry out cultures for EBA studies.

It is during the first two days of treatment that the most significant differences are seen in the EBA of different drugs.[10] This led to the practice of studying agents for the first two treatment days only. Nonetheless, it is also evident, first, that the dose related response of certain agents, such as EMB, might become apparent only during longer study[12] and, second, that the activity of a drug such as PZA would not be detected if only the first two days of treatment are studied.[40,41] In the case of PZA, the least bactericidal of our current "essential drugs", its activity did not become apparent until approximately four days after starting treatment.[10]

EBA studies over five or seven days or longer have several advantages. Of prime importance is detecting whether a new agent does kill mycobacteria in patients with pulmonary tuberculosis; the longer a study proceeds the better is the chance of detecting low bactericidal activity. Studies of at least 5–7-days will reveal the bactericidal activity of all currently available 'first-line' anti-tuberculosis agents. A second important advantage is the accumulation of more data. Determination of EBA is a clinical technique and it is impossible to control all relevant factors. In this process the sputum specimen is critical and uncertainties inherent in this procedure, such as irregular shedding of organisms by certain patients, have been highlighted.[12,42] In view of the tendency for CFU counts to vary, the availability of data collected over a longer period would also contribute to greater accuracy of EBA studies and allow the application of statistical techniques that accommodate anomalous results. In one design a reiterative method and an exponential decay model is proposed.[42] This also allows measurement of the time taken to reduce the viable count by 50% (vt_{50}) as a measure of drug action. Applying these methods recently led to a slightly different ranking of the agents studied[43] and the clinical relevance of the differences exposed by different statistical techniques needs exploration.

The earliest studies quantifying bacilli in sputum used counts of AFB either alone or in conjunction with viable CFU counts. The value of this double approach was assessed in two studies. In the first Hafner et al found that, although the baseline mycobacterial load was comparable by the two methodologies, the rate of decline of CFU counts was two-fold higher than that measured by quantitative microscopy.[18] In a multicenter study counts of the total number of AFB were used to increase the accuracy of EBA obtained by CFU counting.[39] No consistent pattern of AFB results was obtained, however, although there was a tendency for a fall in AFB counts during treatment. A method was then devised using total AFB counts to reduce the variability between patients within groups, but a significant gain in precision was found in only one center and was considered to be the result of patient factors increasing sputum dilution by saliva, pus and bronchial secretions.

The first extensive EBA study of Jindani et al was characterized by statistically significant differences between the various groups.[10] The next published study was undertaken in Hong Kong, but produced equivocal results as the error term between patients within groups was larger than anticipated.[13] This variation was shown not to be due to variation in CFU counting and led to discussion of possible differences between Chinese and African patients, the latter being considered to have more acute disease with connections between cavities, whereas Chinese patients had more chronic disease. In a later publication it was also pointed out that in earlier studies of identical drug regimens in East Africa and Hong Kong, sputum culture conversion to negative occurred more rapidly in Chinese patients, who had less cavitation and less extensive disease on chest radiography than African patients, but that Chinese patients were, nonetheless, more likely to relapse.[39] In

another analysis of patients studied in Cape Town inter-patient variation and not laboratory variation was identified as the most important source of variation in EBA results.[44]

Methodologies not addressed in this review, but which might aid the evaluation of anti-tuberculosis agents during the first weeks of therapy in conjunction with EBA studies, include time to detection of *Mycobacterium tuberculosis* growth in sputum culture,[45] the polymerase chain reaction,[46,47] the induction of antigen 85 complex of *M. tuberculosis* in sputum[48] and measurement of sputum *M. tuberculosis* messenger RNA.[49]

Conclusion

Since the introduction of the first chemotherapeutic agents for tuberculosis treatment more than 60 years ago the enumeration of mycobacteria in the sputum of pulmonary tuberculosis patients has contributed to our understanding of the actions of different agents. As illustrated by this review, the determination of EBA has also provided an objective, reproducible means of assessing the relationship between dose and the pharmacokinetics of an agent and its bactericidal effect and toxicity in relatively small numbers of patients. Applied to new anti-tuberculosis agents it can rapidly demonstrate that the agent has a detectable bactericidal effect in tuberculosis patients, offer guidance as to the most appropriate dose to take forward to further clinical studies and allow a preliminary evaluation of toxicity in patients.

Demonstration of the lowest effective dose will allow calculation of the therapeutic margin or ratio of the usual therapeutic dose, determined either by toxicity or by the drug reaching a maximum effect, and the lowest dose exercising a bactericidal effect. While it is attractive to hypothesize that the period days 2–7, or even 2–14, of an EBA study represents sterilizing activity there is little hard evidence to support this. A test that could with certainty detect killing of the subpopulation of dormant or intermittently metabolizing organisms that must be killed to sterilize tuberculosis lesions would considerably enhance the value of EBA studies.

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