REVIEW

Bacillus anthracis and antibacterial agents

A. Bryskier

Aventis Pharma SA, Infectious Disease Group, Romainville, France

Anthrax is one of the oldest threats to humankind, and remains endemic in animals in many parts of the world. Human cases are infrequent, and some result from biological warfare. This review summarizes the current knowledge on the antibacterial activity of available antibiotics. For potential use in the most severe cases of anthrax, antibacterials need to exhibit potent in vitro activity, intracellular bioactivity, and suitable locations in lymph nodes. In animal models, it has been shown that doxycycline and fluoroquinolones are the most active compounds. There is a lack of data for animal models for macrolides and ketolides, some of them exhibiting good in vitro activity. However, systemic anthrax (inhalation or gastrointestinal) is mainly due to anthrax toxin, and therapy directed against intoxication is needed as basic treatment.

Keywords Anthrax, fluoroquinolones, doxycycline, β-lactams, macrolides, ketolides, antibiotics

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INTRODUCTION

Anthrax is one of the oldest documented infectious diseases and is believed to be the fifth Egyptian plague at the time of Moses, described in the book of Genesis (Exodus 9); clinical cases were also clearly reported by the ancient Romans and Hindus. In 25 BC, Virgil, in the third Georgic Period, described the illness [1]. Anthrax was probably the disease behind the 'Black Bane' which swept through Europe in the Middle Ages, causing a large number of human and animal deaths. A panzootic of anthrax that killed approximately one-half of the sheep in Europe in the mid-1800s resulted in intensive research by early microbiologists. Rayer, in 1850 [2], showed that administration of anthrax-contaminated blood containing 'small bodies' from a sick animal could induce anthrax in healthy sheep. This observation was confirmed in 1855, by Pollender [3], who was unable to prove the involvement of these organisms in anthrax. Davaine demonstrated in 1868 that the 'bacteridia' is the causative agent of anthrax [4]. In 1877, *Bacillus anthracis* was isolated in pure culture from the vitreous humour of a bovine eye and was proved to be the anthrax etiologic agent by Robert Koch [5–7]. Louis Pasteur in 1881 [8] and William Greenfield in 1880 [9] were pioneers in anthrax vaccination [10].

B. anthracis is a Gram-positive spore-forming bacillus; it is the etiologic agent of anthrax.

Anthrax commonly occurs in both wild and domestic mammals (e.g. sheep, cattle, horses, pigs, goats, camels, antelopes, bison, elephants, hippotami, kudu, and other herbivores) [11]. The results of studies of agricultural outbreaks have suggested that conditions for multiplication are favorable when the soil has a pH above 6.0 and is rich in organic matter [12].

Humans can develop anthrax infection following exposure to the organism through infected animals or tissue from infected animals, or by direct exposure to *B. anthracis* [13,14]. Sporadic outbreaks have occurred as a result of both agricultural and military disruptions. Anthrax is endemic in rural India [15], Pakistan, Sudan and Egypt, as well as in many parts of Asia.

In the last 25 years, only a few major outbreaks have occurred. The first was in Zimbabwe in 1979–80, during the Rhodesian civil war, when failure of veterinary vaccination programs led to a human epidemic, causing 6500 anthrax cases and 200 fatalities [16]. The second outbreak was in Paraguay in 1980 [17]. In France, some human cases were

Corresponding author and reprint requests: A. Bryskier, Aventis Pharma SA, Infectious Disease Group, 102 route de Noisy, 93230 Romainville, France
Tel: +33 1 49 91 51 21
Fax: +33 1 49 91 50 20
E-mail: andre.bryskier@aventis.com

reported in 1997, as well as two animal anthrax outbreaks in two areas [18]. An accident in the former Soviet Union in the research center at Sverdlosk (Ekateringburg) led to the death of 66 adults due to inhaled anthrax [19]. Sixteen anthrax cases in humans were recorded in the UK between 1980 and 2000; all were cutaneous cases, and associated with workers who handled bone meal, animal carcasses and skin [20]. Twenty-five human cases of anthrax occurred between 1978 and 1981 in Switzerland in textile workers handling infected goat hair from Pakistan [21]. In 1992, a family outbreak of anthrax was reported in northern Italy [22]. In 1971, outbreaks were reported in sub-Saharan Africa—(Chad) [23] and The Gambia—due to the use of communal loofahs when bathing [24]. In Thailand, a gastrointestinal anthrax outbreak was reported after ingestion of contaminated water-buffalo meat [25].

In the USA, the microorganism remains endemic in the soil of Texas, Oklahoma, and the lower Mississippi [26]. Since 1974, due to the risk of contamination, importation of goat skins have been banned in the USA from Haiti [20,27]. In the USA, from 1955 to 1987, there were 233 human cases of anthrax, and before the current crisis following the atrocities of 11 September 2001, the last human report was published in August 2001, in which one patient was exposed to *B. anthracis* during an epizootic anthrax among livestock in North Dakota [28]. Anthrax has been reported in deer in the USA and in wood buffalo in Canada [20].

Anthrax was used as a biological weapon for the first time by Moses. In the 20th century, Germany developed plans during World War I to contaminate sheep herds from Romania. In 1917–18, in Argentina, livestock destined for the Allied forces was infected with anthrax and *B. mallei* (glanders), resulting in the death of more than 200 mules [29]. Japan conducted biological warfare experiments in Manchuria from 1932 to 1945. In Unit 731, located near the town of Ping Fan, prisoners were infected with *B. anthracis* and other biological organisms [30]. The Japanese also deliberately contaminated water supplies and food with *B. anthracis*. In 1941–42, to be prepared for retaliation against German biological weapons, the British carried out bomb experiments with weaponized spores of *B. anthracis* on Gruinard Island near the coast of Scotland. Viable spores of anthrax persisted for 45 years after World War II, until 1986 when the island was decontaminated with formaldehyde and sea water [31]. From the 1950s to 1970, the USA experimented with biological weapons, including anthrax spores, until President Nixon terminated the program.

A Japanese terrorist group (Aum Shirikyo) dispersed anthrax aerosols in Tokyo subway stations on many occasions in 1995 without succeeding to induce anthrax, due to the fact that they used the avirulent (non-capsulated) strain 'Sterne' [20,32]. During the Gulf War, it was clearly demonstrated that Iraq possesses anthrax as a biological weapon.

At least three clinical pictures can be described: cutaneous infection, gastrointestinal infection, and inhaled (pulmonary disseminated infection). There have been no reports in the literature of direct human-to-human transmission. Most cases in industrialized countries are associated with exposure to animal products, especially goat hair imported from Turkey, Sudan and Pakistan, where anthrax remains common among domestic livestock.

ANTIBACTERIAL ACTIVITY OF ANTIBIOTICS

In vitro activity of antibacterial agents

In the different studies carried out to investigate the in vitro susceptibility of *B. anthracis* to various antibacterial agents, there has been variation in the methodologies used to determine MICs.

In the studies of Heine et al. [33,34], the microbroth dilution method in cation-adjusted Mueller–Hinton was used. The strains were added in microwells in the log growth phase at an inoculum of 5×10^4 CFU/mL. In the CDC study [35], strains were grown on TSA blood agar and an inoculum adjusted to 0.5 MacFarland was prepared in Mueller–Hinton broth. MICs were determined in microbroth cation-adjusted Mueller–Hinton. When comparing MICs from both studies, there is an up to two doubling dilution difference, especially for ciprofloxacin, chloramphenicol, and doxycycline, but not for penicillin G. In other studies, MICs were determined using an agar dilution method, with incubation at 37 °C overnight in ambient air [36,37].

It has been shown that, with β-lactam antibiotics, the percentage of spores in the inoculum did not influence MICs of benzylpenicillin, amoxicillin, and amoxicillin–clavulanate (Heine, personal communication). However, available antibacterials against *B. anthracis* are not active against spores.

B. anthracis KC-1 was tested for its susceptibility to all new investigational compounds (Tables 1

and 2) at Kyoto University (Japan). The KC-1 strain is highly susceptible to benzylpenicillin and ampicillin or amoxicillin, but less susceptible to N-acyl- or α-sulfopenicillins. Even if some oral cephems, such as cefaclor or cefadroxil, display high in vitro activity, all the cephems have low activity or are inactive against *B. anthracis*. Flomoxef, an oxa-1-cephamycin, displays interesting activity, with an MIC of 0.2 mg/L, in comparison with latamoxef, which has an MIC of 1.56 mg/L. Among carbapenems, panipenem seems to be highly active, as well as imipenem, with low MICs. Aminoglycosides have low activity. Vancomycin has low activity, but teicoplanin seems to exhibit good in vitro activity, with an MIC of 0.20 mg/L. In the macrolide field, clarithromycin exhibits the highest activity, and other compounds share similar antibacterial activity. Fluoroquinolones are highly active; however, lomefloxacin, fleroxacin, enoxacin and norfloxacin are less active. MICs were determined using brain–heart infusion agar with an inoculum size of 10^6 CFU/mL. An inoculum effect has been demonstrated with β-lactams when increasing the size of inoculum from 10^6 to 10^8 CFU/mL.

In other studies, more isolates have been tested, collected from different sources. In a study published in 1991, 22 isolates collected from cutaneous anthrax cases were shown to be highly susceptible to penicillin G, ampicillin, ofloxacin and ciprofloxacin (Table 3) [36]. In a second study, 70 isolates were tested, demonstrating good activity of benzyl-

Table 1 In vitro activity of β-lactam and aminoglycoside antibiotics against *Bacillus anthracis*

Antibiotics	MIC (mg/L)
Benzylpenicillin	0.015
Amoxicillin	0.025
Ampicillin	0.025
Piperacillin	0.78
Sulbenicillin	0.78
Cefaclor	0.78
Cefadroxil	0.78
Cephalexin	1.56
Cefuroxime	25
Cefixime	>100
Cefetamet	100
Cefteram	6.25
Ceftibuten	>100
Cefpodoxime	6.25
Cefdinir	1.56
Cefditoren	6.25
Cefazolin	0.09
Cefoperazone	1.56
Cefpimizole	12.5
Cefotaxime	6.25
Ceftriaxone	12.5
Ceftizoxime	25
Cefmenoxime	12.5
Cefotiam	3.13
Ceftazidine	50
Cefepime	12.5
Cefpirome	12.5
Cefsulodin	50
Cefodizime	25
Latamoxef	1.56
Flomoxef	0.20
Cefminox	3.13
Imipenem	0.012
Panipenem	≤0.006
Ritipenem	0.10
Aztreonam	>100
Carumonam	>100
Gentamicin	1.56
Netilmicin	1.56
Amikacin	6.25
Dibekacin	3.12
Arbekacin (souche TMS-1)	3.12

Adapted from Nishino et al. *Chemotherapy* (Tokyo). Numerous supplements on new antibacterials.

Table 2 In vitro activity of fluoroquinolones, macrolides and other antibacterials against *Bacillus anthracis* KC-1

Antibiotics	MIC (mg/L)
Erythromycin A	0.20
Roxithromycin	0.39
Azithromycin	0.39
Clarithromycin	0.10
Josamycin	0.20
Midecamycin	0.39
Miokamycin	0.78
Rokitamycin	0.20
Leucomycin	0.39
Dalfopristin–quinupristin	0.20
Teicoplanin	0.20
Vancomycin	1.56
Minocycline	0.10
Nalidixic acid	6.25
Pipemidic acid	1.56
Miloxacin	3.13
Norfloxacin	0.39
Ofloxacin	0.10
d-ofloxacin	0.10
Levofloxacin	0.05
Sparfloxacin	0.05
Tosufloxacin	0.01
Pazufloxacin	0.05
Balofloxacin	0.05
Lomefloxacin	0.20
Enoxacin	0.39
Fleroxacin	0.39
Grepafloxacin	0.025

Adapted from Nishino et al. *Chemotherapy* (Tokyo). Numerous supplements on new antibacterials.

penicillin and amoxicillin; however, two isolates were highly resistant to penicillin G (MIC 64 mg/L), tetracycline and ciprofloxacin (Table 3) [37].

In vitro, *B. anthracis* is susceptible to rifampicin (MIC 0.5 mg/L); however, in an in vivo murine model, oral rifampicin caused only an increase in mean lifespan and had no significant effect on survival rate [38]. Recently, the antibacterial susceptibilities of the isolates related to the recent infections in the USA, as well as other data, were released from the CDC [33–35,39] (Table 3).

A working group on MIC harmonization for *B. anthracis* was set up at the NCCLS subcommittee meeting, held in January 2002 in Tampa (Florida),

Table 3 In vitro susceptibility of *Bacillus anthracis*

		MIC (mg/L)			
Compounds	***N***	**50**	**90**	**Range**	**References**
Ciprofloxacin	65	0.06	0.06	0.03–0.12	[35]
	28	0.03	0.03	0.01–0.125	[39]
	96	0.06	0.06	0.03–0.06	[37]
	22	0.06	0.06	0.03–0.06	[37]
	18	0.25	2.0	0.06–0.2	[34]
Levofloxacin	1				
	96	0.125	0.25	0.03–2.0	[37]
	18	0.25	1.0	0.06–2.0	[34]
Gatifloxacin	1	–	–	0.025	[44]
	20	0.12	0.12	0.12	[35]
	96	0.12	0.12	0.12	
Trovafloxacin	1	–	–	1.6	[44]
Pefloxacin	96	0.125	0.5	0.03–1.0	[37]
Nalidixic acid	96	4.0	8.0	0.03–32	[37]
Ofloxacin	96	0.25	0.25	0.03–1.0	[37]
	22	0.06	0.06	0.03–0.06	[36]
	18	1.0	2.0	0.5–8.0	[34]
Sparfloxacin	18	0.5	0.5	0.12–2.0	[33]
Clarithromycin	28	0.125	0.125	0.06–0.125	[39]
	18	0.5	1.0	0.25–2.0	[33]
Erythromycin A	28	1.0	2.0	0.5–8.0	[39]
	65	1.0	1.0	0.5–1.0	[35]
	12	0.5	0.5	0.5	Data on file, 1997
	70	0.5	1.0	0.25–1.0	[37]
	96	1.0	1.0	1.0–4.0	[37]
Azithromycin	12	1.0	1.0	0.5–2.0	Data on file, 1997
	18	8.0	8.0	2.0–8.0	[33]
Telithromycin	96	0.03	0.25	0.03–1.0	Data on file, 2001*
	12	0.25	0.25	0.03–0.25	Data on file, 1997
ABT 773	28	0.03	0.03	0.01–0.125	[39]
Clindamycin	64	≤0.5	1.0	≤0.5–1.0	[35]
	18	0.25	0.5	0.12–1.0	[33]
	96	0.12	0.25	0.25–1.0	[37]
Roxithromycin	12	0.5	0.5	0.25–1.0	Data on file, 1997
Quinupristin–dalfopristin	18	1.0	1.0	0.12–0.5	[33]
Linezolid	18	2.0	4.0	1.0–8.0	[34]
Penicillin G	65	≤0.06	≤0.06	≤0.06–128	[35]
	70	0.06	0.125	0.01–64	[37]
	18	64	>64	2.0 to >64	[33]
	96	0.12	8.0	0.12–16	[37]
Ceftriaxone	74	16	32	4.0–32	[35]
	18	16	64	16 to >64	[33]
Ampicillin	22	0.03	0.03	0.01–0.03	[36]
	18	64	>64	4.0 to >64	[33]
Ampicillin–sulbactam	22	0.01	0.01	0.01–0.03	[36]
Amoxicillin	70	0.06	0.125	0.01–64	[37]
	22	0.01	0.01	0.01–0.03	[36]
	18	64	>64	8.0 to >64	[33]

Table 3 continued

Compounds	*N*	MIC (mg/L) 50	90	Range	References
	96	0.12	4.0	0.12–16	[37]
Amoxicillin–clavulanic acid	22	0.01	0.01	0.01	[36]
	18	1.0	2.0	0.5–16	[33]
Piperacillin	22	0.25	0.5	0.125–0.5	[36]
	18	64	>64	16 to >64	[33]
	96	1.0	1.0	0.25–32	[37]
Mezlacillin	22	0.06	0.06	0.01–0.06	[36]
Cefazolin	22	0.01	0.01	0.01–0.03	[36]
	0.5	1.0	1.0	0.5–8.0	[33]
Cephalothin	96	0.5	16	0.12–32	[37]
Cefuroxime	22	64	64	16–64	[36]
	18	64	>64	16 to >64	[33]
	70	32	64	1.0–64	[37]
Imipenem	18	≤0.03	0.12	≤0.03 to >64	[33]
	96	0.12	0.12	0.12–2.0	[37]
Meropenem	18	0.06	0.12	≤0.03–012	[33]
Rifampicin	65	≤0.25	0.5	≤0.25–0.5	[35]
	18	0.5	0.5	≤0.03–1.0	[33]
	96	0.12	0.12	0.12–2.0	[37]
GAR-936	18	0.12	0.5	<0.03–0.5	[34]
Doxycycline	18	0.06	0.12	<0.03–0.25	[34]
	96	0.12	0.12	0.12–0.25	[37]
Tetracycline	65	0.03	0.06	0.03–0.06	[35]
	70	0.125	0.125	0.06–1.0	[37]
Chloramphenicol	74	4.0	4.0	2.0–8.0	[35]
	22	2.0	2.0	1.0–2.0	[36]
	18	16	16	8.0–64	[33]
	70	4.0	4.0	2.0–4.0	[37]
	96	2.0	2.0	1.0–4.0	[37]
Vancomycin	74	2.0	2.0	0.5–2.0	[35]
	22	1.0	1.0	0.25–1.0	[36]
	18	2.0	2.0	1.0–4.0	[34]
Teicoplanin	96	0.25	0.5	0.12–2.0	[37]
Daptomycin	18	2.0	2.0	1.0–4.0	[33]
Novobiocin	18	2.0	2.0	1.0–4.0	[33]
Clofazimine	18	16	32	8.0–64	[33]
Co-trimoxazole	22	3.2/16	3.2/16	1.6/8–3.2/16	[36]
	18	>64	>64	2.0 to >64	[33]
	96	>4/76	>4/76	>4/76	[37]
Sulfamethoxazole	18	>64	>64	>64	[33]
Trimethoprim	18	>64	>64	64	[33]
Oritavancin	18	0.25	0.5	<0.03–1.0	[35]
Cefotaxime	22	32	32	8.0–32	[36]
	18	32	>64	16 to >64	[33]
Ceftriaxone	96	32	32	4.0–64	[37]
65	16	32	32	4.0–32	[35]
Ceftizoxime	22	32	32	16–64	[36]
Cefotetan	18	16	16	8.0 to >64	[33]
Ceftazidime	22	128	128	128–256	[36]
	18	>64	>64	>64	[33]
Cefoperazone	22	2.0	4.0	0.5–4.0	[36]
Cefoxitin	96	8	32	1.0–64	[37]
Aztreonam	22	>128	>128	>128	[36]
	18	>64	>64	>64	[33]
Gentamicin	22	0.06	0.125	0.03–0.25	[36]
	18	2.0	2.0	1.0–4.0	[33]
	70	0.12	0.12	0.06–0.5	[37]
	96	0.15	0.5	0.12–5.0	[37]

Table 3 continued

Compounds	*N*	MIC (mg/L) 50	90	Range	References
Streptomycin	22	2.0	4.0	1.0–4.0	[36]
	18	4.0	8.0	4.0–16	[33]
	70	1.0	1.0	0.5–4.0	[37]
	96	1.0	1.0	0.5–2.0	[37]
Amikacin	22	0.03	0.06	0.03–0.06	[36]
	18	2.0	2.0	1.0–2.0	[33]
Netilmicin	22	0.06	0.125	0.01–0.125	[36]
	18	2.0	4.0	2.0–8.0	[33]
Tobramycin	18	2.0	4.0	1.0–16	[33]
	22	0.25	1.0	0.25–1.0	[36]

*Partly replaced by Antibiotic susceptibilities of 96 isolates of Bacillus anthracis isolated in France between 1994 and 2000. Cavallo JD, Ramisse F, Girardet M, Vaissaire J, Mock M, Hernandez E. *Antimicrob Agents Chemother* 2002; 46: 2307–9.

to propose the most accurate method to determine in vitro activity against *B. anthracis*.

When comparing the microbroth dilution methods and E test strip methods for *B. anthracis*, it was demonstrated that MICs obtained with the E test were up to eight times lower than those obtained with the reference methods [34,35].

Resistant mutants

Resistance can be developed experimentally to most of the current antibacterial agents.

Rifampicin

In the population of various strains of *B. anthracis*, formation of spontaneous rifampicin-resistant mutants was detected at a rate of 10^{-8}. The level of rifampicin resistance in the mutants ranged from 16 to 512 mg/L. The clones of the rifampicin-resistant population of the virulent strain CH-7 were heterogeneous in their biological properties [40]. Rifampicin-resistant mutants were selected from UV-light-treated attenuated *B. anthracis* (strain Ames pXO1-pXO2), and spontaneous rifampicin-resistant mutants were also isolated on selective media. Mutations conferring rifampicin resistance are commonly due to mutations in the β-subunit of RNA polymerase, encoded by the *rpoB* gene. These mutations are located in four clusters in the N-terminal section. The majority of mutations occur in cluster I. Twelve amino acid positions are known to interact directly with rifampicin. Mutations were observed at four of these positions for *B. anthracis.* Of four amino acid mutations surrounding the rifampicin-binding pocket, two of these changes—position 450 (Ser→Cys) and 468 (Lys→Gln)—are unique for *B. anthracis.* There is a greater diversity among UV-generated rifampicin-resistant *B. anthracis* strains (positions 472, 468, 459, 467, 450, 453, 450, 454 and 467) than among spontaneously occurring mutants (positions 454, 467 and 472). The spontaneous rate of resistance was estimated at 1.57×10^{-9} mutations/generation by a Luria–Delbrück fluctuation test [41,42].

Fluoroquinolones

In a serial passage study, the potential for ofloxacin and doxycycline to select mutants of the vaccine strain *B. anthracis* Sterne was investigated. Repeated subcultures of *B. anthracis* Sterne increased the MIC of ofloxacin on the 13th passage from 0.20 mg/L to 0.80 mg/L. The MIC of 0.8 mg/L was stable for the next five passages. However, *B. anthracis* remains susceptible to ofloxacin according to the available breakpoints (not given for *B. anthracis*) [43] (Table 4).

B. anthracis Sterne was used to investigate the selection of resistant mutants after 21 sequential subcultures in subinhibitory concentrations of doxycycline, ciprofloxacin, trovafloxacin, and gatifloxacin. The number of passages required for selection of resistant mutants varied from nine (trovafloxacin) to 10 (ciprofloxacin and gatifloxacin). Currently, the mechanism of resistance to fluoroquinolones of *B. anthracis* is unknown.

After sequential passages with a single fluoroquinolone, each isolate was cross-resistant to other fluoroquinolones. In this study, MICs were determined using a macrodilution method in brain–heart infusion broth [44] (Table 4).

Table 4 MICs after sequential passages [43,44]

Antibacterial agent	Number of subcultures	Initial MIC (mg/L)	MIC at 21 subcultures (mg/L)	Number of passages required to increase MIC 4-fold or greater
Ciprofloxacin	21	0.1	1.6	10
Trovafloxacin	21	1.6	12.5	9
Gatifloxacin	21	0.02	1.6	10
Ofloxacin	18	0.2	0.8	13
Doxycycline	21	0.02	0.1	14
Erythromycin A	15	6.25	6.25–50	4
Azithromycin	15	12.5	12.5–50	8
Clarithromycin	15	0.2	0.4–1.6	14

Doxycycline

In one study, no mutants were detected [43] after 18 passages, but in another study, after 14 passages, the initial MIC of 0.025 mg/L increased to 0.1 mg/L [44]. Strains resistant to doxycycline have been reported [45].

Macrolides

The number of passages required for selection of resistant mutants varied from four (erythromycin A) to 14 (clarithromycin). Mutants resistant to azithromycin were obtained after eight passages [44] (Table 4).

Resistance to antibacterial agents

A constitutive cephalosporinase is often produced by wild-type strains. Resistance to penicillins and doxycycline has been reported, and these strains are believed to have been engineered. *B. anthracis* CH-7 harbors the penicillinase gene in the repressed state [46]. There have been only a few reports on penicillin G resistance in *B. anthracis* [46–48]. The following antibiotics are naturally inactive against *B. anthracis*: sulfamethoxazole, trimethoprim, cefuroxime, cefotaxime and other 2-amino-5-thiazolyl cephems (such as ceftriaxone, ceftazidime, cefepime and cefpirome), and aztreonam.

Sensitivity to penicillin G has been used as a diagnostic tool to differentiate between *B. anthracis* and *B. cereus* isolates [49–51].

In *B. anthracis*, there are at least two β-lactamases which show more than 93% amino acid homology to the class A and B enzymes of *B. cereus* [52]. The main β-lactamase in *B. anthracis* seems to be a chromosomal metalloenzyme (class B).

In a study performed using the Microscan device, all *B. anthracis* isolates tested were susceptible to penicillin G. In the same study, the hydrolysis was quantitatively investigated. The hydrolysis speeds expressed in micromoles of β-lactam hydrolyzed per minute were 1.98×10^{-3}, 2.09×10^{-7}, 1.72×10^{-6}, 1.59×10^{-6}, 1.95×10^{-7}, 4.07×10^{-7}, $\leq 2.0 \times 10^{-7}$ and $\leq 1.0 \times 10^{-7}$ for penicillin G, cephaloridine, cefotaxime, cefuroxime, cefazolin, imipenem, cephalexin and cephradine, respectively, and the relative rates of hydrolysis versus penicillin G (100) were 1.1, 8.7, 8.0, 0.99, 2.1, ≤ 1.0 and ≤ 0.5 for cephaloridine, cefotaxime, cefuroxime, cefazolin, imipenem, cephalexin and cephradine, respectively [52]. In Kruger National park in South Africa, there is an area contaminated with *B. anthracis* spores. The in vitro susceptibilities of 44 *B. anthracis* isolates from this area were assessed against 16 antibacterial agents using a disk diffusion method in comparison with diameter zones obtained with *Staphylococcus aureus* NCTC 6571 on Mueller–Hinton agar according to Ericsson and Sherris [53]. Sensitivity to penicillin G, novobiocin and cefamandole was encountered in 84.1%, 86.4% and 68.18% of the isolates, respectively. Several isolates were moderately susceptible to penicillin G (15.9%), clindamycin (6.8%), fusidic acid (84%), novobiocin (13.6%), and cefamandole (31.8%) [54].

Animal models

The protective effects of ciprofloxacin, pefloxacin and lomefloxacin were investigated in animal anthrax induced with *B. anthracis* spores of three vaccinal strains. Protection was 50–80%, 40–70% and 40–70% for $10LD_{50}$, $100LD_{50}$ and $1000LD_{50}$, respectively [55]. A high therapeutic efficacy of minocycline was reported, irrespective of the contaminating dose and strains [45].

Female Dunkin–Hartley guinea pigs (500–600 g) were challenged for 7 min with aerosols of *B. anthracis* spores of Ames strain or Vollum strain to obtain a lung dose of 10^4-10^6 spores. Animals received either ciprofloxacin or doxycycline by the subcutaneous route for 21 days after bacterial exposure. Antibiotic levels were determined in non-infected animals.

Doxycycline and ciprofloxacin protected animals from infection following inhalation of up to 10^6 spores of both strains of *B. anthracis*, so long as administration of the antibiotics was continued. After antibiotic administration had been discontinued, some animals died, more quickly in the ciprofloxacin group than in the doxycycline group. *B. anthracis* was isolated from the lung tissue of these guinea pigs [56].

The in vivo efficacies of penicillin G, ofloxacin, trovafloxacin and gatifloxacin were investigated after *B. anthracis* challenge by the intratracheal route 4 days after ^{60}Co irradiation of female B6D2AF 1/J mice (16–20 g). The endpoint was the survival rate 30 days after challenge. The antibacterial therapy was started 6, 24 and 48 h after bacterial exposure, and continued for 7 or 21 days. It was demonstrated that non-lethal irradiation associated with a *B. anthracis* Sterne spore challenge increased the translocation of intestinal microflora. Antibacterial treatment must start within 24 h and be completed at 21 days to significantly reduce the mortality rate after exposure to *B. anthracis* Sterne following non-lethal irradiation. The survival rates after therapy with trovafloxacin, gatifloxacin, penicillin G+ ofloxacin, penicillin G and ofloxacin were 90%, 79%, 55%, 25% and 21%, respectively [57].

RATIONALE FOR LONG-TERM ANTIBIOTIC TREATMENT

In inhalation anthrax, the mortality rate is high. In the Sverdlovsk (Russia) outbreak, it was reported that 66 of 79 patients died, although the reliability of the diagnosis in the surviving patients is questionable. However, it seems that patients whose onset of disease was 30 or more days after inhalation had a higher rate of recovery in comparison with those who had early onset of the disease. In the case of fatalities, the interval between onset of symptoms and death averaged 3 days. In monkeys, there is a similar course of the disease, even after a latency of 58 days.

After inhalation of anthrax spores, the spore-bearing particles of 1–5 μm were deposited in alveolar spaces. The spores are engulfed by alveolar macrophages, and many of them are able to survive within their phagocytes, even if most of them are lyzed. Surviving spores are transported via the lymphatic system to mediastinal lymph nodes, where germination may occur up to 60 days later [58,59]. In the Sverdlovsk outbreak, clinical onset occurred from 2 to 43 days after exposure. In experimental monkeys, fatal disease occurred up to 98 days after exposure. Viable spores have been shown in the mediastinal lymph nodes of monkeys 100 days after exposure.

LIMITS OF ANTIBIOTIC THERAPY

Main characteristics needed for an antibacterial agent to be efficacious against *B. anthracis*

Owing to the pathophysiology of inhaled anthrax, the following studies are needed to determine potential clinical efficacy and possible use of the antibiotic for prevention after exposure to anthrax spores:

1. In vitro activities, determined by a mean of MIC values for a sufficient number of isolates collected from human and animal sources.
2. Mutant selection needs to be investigated.
3. Bactericidal activity has to be determined.
4. Animal infections (rhesus monkeys, guinea pigs, rabbits, and mice) must be assessed with determinations of pharmacokinetic parameters [60–62].
5. Determination of plasma levels in humans must be assessed, as well as respiratory tissue levels (bronchial mucosa, alveolar macrophages, epithelial lining fluid).
6. Intracellular concentration and efflux in macrophages must be assessed as well as antibacterial localization within the cell, *B. anthracis* being mainly located in the phagolysosome; intracellular bioactivity against this pathogen could be investigated. Although *B. anthracis* is an extracellular pathogen, it appears to require an intracellular step to initiate infection.
7. *B. anthracis* spores and germination occur in the lymph nodes [58,59]; determination of antibacterial levels at this site is therefore an important parameter. However, when the capacity of the lymph node is overwhelmed, the infection spreads to successive nodes, and the bacilli then enter the bloodstream and multiply.

Only a few families of antibacterial agents are able to concentrate in phagocytes: macrolides, ketolides, fluoroquinolones, cyclines, ansamycins (rifampicin and derivatives), streptogramins, clindamycin, and teicoplanin [63]. To combat *B. anthracis*, antibacterials need to be mainly concentrated in the phagolysosome. Data on lymph node concentrations are scarce. Data are available for ofloxacin [64], levofloxacin [65], pefloxacin [66], ciprofloxacin [67], fleroxacin [68], azithromycin [69], and doxycycline [70]. However, these studies have been carried out mainly in mesenteric lymph nodes for the treatment of typhoid fever.

B. anthracis

B. anthracis virulence depends on the bacterial capsule and the toxin complex, which is composed of three entities, a protective antigen, an edema factor, and a lethal factor. The three components of the anthrax toxin need to be associated for it to exert its effect [71].

Once germination occurs, disease follows rapidly. Replicating organisms release toxins, leading to hemorrhage, edema, and necrosis. In experimental animals, once toxin production has reached a critical threshold, death occurs even if sterility of the bloodstream is achieved with antibiotics.

No immune response has been demonstrated in experimental animals receiving antibiotic therapy during anthrax infection. Experimental studies demonstrated that treatment with penicillin G for 5–10 days, starting on day one after aerosol exposure of monkeys, was protective during the course of antibiotic treatment; however the monkeys died when the treatment was discontinued. Long-term protection was afforded only by combining penicillin G therapy with post-exposure immunization. Rhesus monkeys (*Macaca mulatta*) that survived the aerosol challenge were examined for evidence of an immune response 131–142 days after exposure, by measuring antibody to the protective component of anthrax toxin. No surviving animals treated with penicillin G, ciprofloxacin or doxycycline alone had an immune response. No protection was afforded against rechallenge of the surviving monkeys [72]. This suggests that even if the antibiotic-treated patients survive anthrax infection, the risk for recurrence remains for at least 60 days, due to the possibility of delayed germination of spores. Post-exposure vaccination in those patients may shorten the duration of antibiotic therapy to 30–45 days.

THERAPY FOR ANTHRAX (ANTIBIOTICS)

Cutaneous anthrax

It has been shown in cutaneous anthrax that negativation of blister fluid occurs 5 h after the first 2 million units of benzylpenicillin [73]. The standard recommendation is 2 million units every 6 h intravenously until the edema subsides, at which time oral penicillin (phenoxypenicillin or penicillin V) therapy can be used. The duration of treatment is at least 7–10 days.

Doxycycline, chloramphenicol, macrolides and fluoroquinolone are considered to be alternative therapies.

Antibiotic therapy does not stop the progress of anthrax lesions to an eschar phase, but does decrease systemic manifestations and local edema.

Inhalation anthrax

Aerosolized anthrax spores $>5\,\mu m$ in size are deposited in the upper airways (pharynx, larynx, and trachea), and effectively trapped or cleared by the mucociliary system. Spores between 2 and $5\,\mu m$ in size are able to reach the alveolar ducts and alveoli. These spores are engulfed by alveolar macrophages and transported to mediastinal and hilar lymph nodes. In the phagocytes, *B. anthracis* is located in the phagolysosome [71]. The minimum infectious inhaled dose in humans has not yet been determined. The minimum infectious dose in chimpanzees is 40 000–65 000 spores [74]. The alveolar macrophages represent the primary site of toxigenic *B. anthracis* germination during infection by inhalation [75]. Early antibiotic administration is essential, due to the rapid onset of the disease (started from 12 h). A delay in antibiotic treatment for patients with anthrax infection, even for several hours, may substantially lessen the chances of survival.

However, there are no clinical studies on the treatment of inhalation anthrax in humans. No data have been released from the Russian outbreak [76], and there is only limited clinical experience with the recent events [77]. In rhesus monkeys, a major change occurred within 3–8 days after inhalation of a lethal dose. Hemorrhages are found in mediastinal, mesenteric and tracheobronchial lymph nodes, and small intestinal serosa [78].

In studies of small numbers of monkeys infected with susceptible strains of *B. anthracis*, oral

doxycycline proved efficacious. Doxycycline is the preferred option in the cycline class being investigated for efficacy in animal models [79]. However, reports have been published of a *B. anthracis* vaccine strain that has been engineered to be resistant to tetracycline and benzylpenicillin. In rhesus monkeys, after exposure to aerolized spores of *B. anthracis*, administration of benzylpenicillin was shown only to delay death in the animals [80]. The delay was generally proportional to the duration of penicillin G administration and probably related to the low intracellular concentration of β-lactam antibiotics.

Engineering of fluoroquinolone-resistant *B. anthracis* may also be possible; however, there are no published reports on this.

Even though only ciprofloxacin was licensed by the FDA for this purpose in July 2000, a report from the CDC in 1998 [81] recommended vaccination and the use of oral fluoroquinolones such as ciprofloxacin, 500 mg bid, levofloxacin, 500 mg qd or ofloxacin, 400 mg bid, for post-exposure prophylaxis in adults.

CONCLUSION

Although *B. anthracis* seems to be very susceptible to penicillins, it is important to note that β-lactam antibiotics are not concentrated in phagocytes. In contrast, fluoroquinolones, macrolides, ketolides and cyclines are highly concentrated in the cell. Long-term tolerance needs to be considered to allow patient compliance. In the recent outbreak in the USA, 19% of the patients receiving prophylaxis complained of adverse events, and about 5% discontinued their medication [82]. Furthermore the selection of resistant mutants is the risk to be highlighted during long-term treatment. Antibacterial agents will significantly decrease the bacterial burden; however, for inhalation anthrax, the main factor remains the intoxication, against which antibacterials are ineffective [83].

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