CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

19-537/S038

19-847/S024

19-857/S027

19-858/S021

20-780/S008

MEDICAL REVIEW

MEDICAL OFFICER'S REVIEW OF SUPPLEMENTAL NDAs

NDA 19-537/S-038
NDA 19-847/S-024
NDA 19-857/S-027
NDA 19-858/S-021
NDA 20-780/S-008
CIPRO®

Applicant
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Drug Identification

Generic name:	ciprofloxacin
Trade name:	Cipro®
Pharmacologic category:	antimicrobial-fluoroquinolone
Dosage formulations:	tablet, suspension, solution
Routes of administration:	oral, intravenous

Regulatory materials reviewed
NDA 19-537/SE1-038 volumes 1-3 (these materials were also submitted to NDAs 19-847, 19-857, 19-858, 20-780)

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I. INTRODUCTION

Background

Anthrax is a zoonotic infection that has been recognized as a human disease since antiquity. Cutaneous, gastrointestinal, and inhalational forms of infection with *Bacillus anthracis* have been traditionally associated with agricultural or industrial exposures. Today, human anthrax is rare in the United States though it remains an endemic disease in other areas of the world. Most recently, attention has turned to *Bacillus anthracis* as a possible agent of biological warfare or bioterrorism.

The Bayer Corporation, responding to an expressed public health need, has submitted an application for the addition of the indication of post-exposure prophylaxis of disease caused by inhaled *B. anthracis* to the label of Cipro® (ciprofloxacin). This is the first antimicrobial drug application submitted to the Food and Drug Administration (FDA) for an indication resulting from the intentional use of a biological agent.

In February 1999, the first National Symposium on Medical and Public Health Response to Bioterrorism was held in Arlington, Virginia. This meeting recognized the lead role of the US Department of Health and Human Services in responding to the needs of a US population exposed to a biological agent. Disease surveillance, medical preparedness, and availability of appropriate pharmaceuticals and vaccines were noted as important elements of the public health response. As a member agency of the US Public Health Service, the responsibilities of the Food and Drug Administration include facilitation of the development of products that can be used to prevent, treat, or diagnose conditions caused by the exposure to an intentionally deployed biological agent.

In June 1999, the Centers for Disease Control and Prevention convened a panel of experts to identify the biological agents considered to be of greatest potential concern. The result was three categories of agents. The organisms in Category A were thought to be of greatest concern, warranting increased surveillance and the availability of appropriate therapy or prophylaxis for diseases caused by them (see Table 1).

Table 1.Biological agents-category A (US CDC, June 1999)

Organism	Disease
Variola major	Smallpox
Bacillus anthracis	Anthrax
Yersinia pestis	Plague
Clostridium botulinum toxin	Botulism
Francisella tularensis	Tularemia
Filoviruses/Arenaviruses	Hemorrhagic fever

Anthrax

Anthrax has been regarded as a possible agent of biowarfare or bioterrorism for almost a century. The inhalational form of the disease, which affects the mediastinal lymph nodes, other organs of the reticuloendothelial system, and the central nervous system, is considered the most likely clinical entity resulting from the intentional use of an aerosolized preparation of the spores of *B. anthracis.* Historically, penicillin or a tetracycline has been the drug of choice to treat inhalational anthrax, but survival is poor once clinical manifestations are present. Mortality in inhalational anthrax is 80-100% even in patients infected with penicillin-susceptible *B. anthracis* who receive appropriate treatment [Gold H. Treatment of anthrax, Fed Proc, Conference on anthrax, 26: 1563-1568; 1967 and Knudson, GB. Treatment of anthrax in man: history and current concepts, Milit Med, 151: 71-77; 1986]. The possibility of the use of bioengineered penicillin- and tetracycline-resistant strains in an intentional attack has been raised.

Among the Category A biological agents, inhalational anthrax is somewhat unique in that there was an outbreak of this infection in 1979 among the human population of Sverdlovsk, former USSR. This outbreak, considered the result of an accident at a military microbiology facility, is one of the few opportunities for systematic study of human disease resulting from any category A agent. [Abramova et al, Pathology of inhalational anthrax in 42 cases from the Sverdlovsk outbreak of 1979, Proc Natl Acad Sci USA 90: 2291-94 March 1991 and Meselson et al, The Sverdlovsk anthrax outbreak of 1979, Science 266: 1202-08; Nov 18, 94].

Regulatory status of drugs for anthrax

US government agencies seeking to make policy regarding appropriate drugs for treatment or prevention of disease in civilian or military populations must consider the regulatory status of those products. Government agencies considering large-scale shipment across state lines would usually be expected to use agents for which there is FDA-approved labeling for the clinical indication of interest. Though products are frequently used 'off-label' in the practice of medicine, large-scale use of a product off-label is more appropriately administered under the IND regulations. If there are sufficient data to support approval of an indication for a drug for which there is substantial clinical experience and a well-characterized safety profile, the preferred approach for such drugs is to approve labeling for the indication. Consideration of the data to support such an indication warrants careful weighing of risks and benefits.

The study of agents for the management of inhalational anthrax raises a number of issues. Inhalational anthrax is an extremely rare disease. Due to its high mortality, it cannot ethically be studied in human subjects under circumstances of intentional exposure. There are drugs with currently approved labeling by FDA for disease associated with *B. anthracis.* Labels for penicillin, tetracycline, doxycycline, and minocycline products list *B. anthracis* among the organisms susceptible to these agents. None of these agents is indicated specifically for post-exposure prophylaxis for disease caused by inhaled *B. anthracis.* When consideration is given to the development of a large-scale use program following a possible exposure, resistance, safety, and compliance issues are raised. As noted above, there are reports of bioengineered strains of *B. anthracis* resistant to

traditionally active agents. In addition, rates of penicillin allergy and gastrointestinal intolerance of the tetracyclines warrant the availability of a choice of agents in the event of a need for drug administration following exposure of a large population to aerosolized *B. anthracis.*

Cipro®

Cipro® in tablet form was approved for human use in the US in 1987; the intravenous solutions were approved in 1990. Dosing is specific to the approved indication, and ranges from 100-750 mg po q 12 hours for the tablet. For the intravenous formulations, the approved doses range from 200-400 mg iv q 12 hours. The proposed regimen for oral ciprofloxacin for inhalational anthrax (post-exposure) is given below:

Patient population	Oral dose	Intravenous dose
Adult	500 mg q 12 h x 60 days	400 mg q 12 h
Pediatric	15 mg/kg q 12 h x 60 days	15 mg/kg q 12 h

Cipro® has been used by 250 million patients worldwide and approximately 100 million patients in the US. It is approved for 13 indications, including several that are relevant to the proposed use that is the subject of this review. Cipro® is approved for deep tissue infections including lower respiratory tract and complicated intra-abdominal infections; it is approved for a long-term use indication, bone and joint infections for ≥ 4-6 weeks; it is approved for another infection of the reticuloendothelial system, typhoid fever.

II. EXPERIMENTAL DATA

The discussion below begins with the microbiology of *B. anthracis*. It is followed by a review of the pharmacology of ciprofloxacin in the Rhesus monkey (macaque) with focus on differential tissue distribution. Rhesus pharmacology is then compared with that of various human populations, and it is demonstrated that ciprofloxacin serum concentrations are reached or exceeded in humans receiving ciprofloxacin in the doses recommended for post-exposure inhalational anthrax. These human and Rhesus serum concentrations are shown to consistently exceed the MIC_{90} of ciprofloxacin for *B. anthracis*. The serum levels achieved in the Rhesus monkey will then be shown to correlate with improved survival in animals that received ciprofloxacin compared with animals that received no antimicrobial following exposure to aerosolized spores of *B. anthracis*. This animal model is then discussed in the context of previous animal models of inhalational anthrax and epidemiologic studies of human experience.

MICROBIOLOGY - *B. anthracis*

The reader is also referred to the microbiology review of Mr Peter Dionne.

Life cycle

Bacillus anthracis is a large gram-positive spore-forming rod. In clinical specimens, the organism appears as an encapsulated rod. In culture, it grows in a filamentous, unencapsulated form. Under certain conditions, it undergoes spore formation that permits the organism to survive under extreme conditions in the environment. Endospores are resistant to toxic chemicals including antimicrobial agents because their outer layers are highly impermeable. They are also heat resistant, and can survive heat exposures sufficient to denature proteins and nucleic acids. The water content of spores is extremely low. In the dehydrated state, proteins and nucleic acids become far more resistant to thermal denaturation.

Under certain favorable conditions, such as heat activation or the presence of certain chemicals, spores will start to germinate. If the necessary nutrients for growth are present, germination is followed by the conversion of the spore cell into a vegetative cell. The vegetative state of *B. anthracis* possesses two virulence factors. The presence of a polypeptide capsule confers resistance to phagocytosis; it is associated with the presence of a plasmid, pXO_2. The other virulence factor is the production of toxin, composed of three proteins that combine in pairs to produce two different effects. Protective antigen (PA) is necessary in combination with edema factor (EF) or lethal factor (LF) for the two latter toxins to cause tissue pathology. None of these three proteins has toxic effects by itself. Genes for all three are encoded on a plasmid, pXO_1 [Debord T, Vidal D. Le charbon pulmonaire, Rev Pneumol Clin, 54(6): 377-81; 1998 and Hanna P. Anthrax pathogenesis and host response, Curr Top Microbiol Immunol, 225: 13-35; 1998].

Current hypotheses regarding the pathophysiology of inhalational anthrax suggest that inhaled spores are deposited on the pulmonary alveolar epithelium where they are phagocytosed by pulmonary macrophages, transported to local lymph nodes, and

converted to the vegetative state. The reader is referred to sections below for a more detailed discussion of pathophysiology.

***B. anthracis - in vitro* susceptibility data**

Traditionally *B. anthracis* is susceptible to drugs of the penicillin and tetracycline classes. In one of the two studies submitted [Doganay M, Aydin N. Antimicrobial susceptibility of *Bacillus anthracis*. Scand J Infect Dis, 23:333-335, 1991], twenty-two strains of *Bacillus anthracis* were isolated from cutaneous anthrax lesions in Turkey between 1981 and 1988. Minimum inhibitory concentrations (MICs) were determined by agar dilution. Mueller-Hinton agar was used and the inoculum was between 3×10^4 and 5×10^5 cfu/mL. Incubation was overnight at 37°C. Beta-lactamase production was also determined for each isolate. The results are shown below.

Table 2. Antimicrobial Susceptibility of 22 strains of *Bacillus anthracis*

Drug	MIC Range (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Benzylpenicillin		0.015	0.015
Ampicillin		0.03	0.03
Ampicillin/sulbactam		0.015	0.015
Ofloxacin		0.06	0.06
Ciprofloxacin		**0.06**	**0.06**

In the second study [Lightfoot NF, Scott RJD, Turnbull PCB. Antimicrobial susceptibility of *Bacillus anthracis*. Salisbury Med Bull 68, Suppl: 95-98, 1990], seventy strains were tested in the United Kingdom. These strains were isolated from diverse areas over a long period of time. Agar dilution susceptibility testing was performed. Mueller-Hinton agar was used, the inoculum was 4.2×10^4 cfu/mL and incubation was at 37°C. Two strains were resistant to penicillin and produced a beta-lactamase constituitively. Three other strains could be induced to produce beta-lactamase. The results of this study are shown below.

Table 3. Antimicrobial Susceptibility of 70 strains of *Bacillus anthracis* (2)

Drug	MIC Range (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Penicillin		0.06	0.125
Amoxicillin		0.06	0.125
Tetracycline		0.125	0.125
Ciprofloxacin		**0.06**	**0.06**

The MIC_{90} value in both studies was 0.06 µg/mL. All tested isolates had MICs of 0.06 µg/mL or less.

***B. anthracis* -penicillin resistance**

In the study performed by Doganay et al it appears that all 22 strains were susceptible to penicillin since all isolates had MICs ≤0.03 µg/mL. In the study performed by Lightfoot et al, which tested 70 strains, two strains were resistant with MICs of >0.25 µg/mL (Strains No. 32 and No. 70). Three strains showed strong beta-lactamase activity. These were strains No. 32 and No. 70, the penicillin-resistant strains, and No. 67, which was susceptible to penicillin and amoxicillin with MICs of 0.03 µg/mL. This is a resistant rate of 2.85% (2/70).

A search of the literature identified an epidemiologic study of interest [Patra G, Vaissaire J, Weber-Levy M, Le Doujet C, Mock M. Molecular characterization of *Bacillus* strains involved in outbreaks of anthrax in France in 1997, J Clin Micro, 36: 3412-14, 1998]. These authors found one penicillin-resistant strain among 11 strains isolated during outbreaks of anthrax in two different regions of France in 1997. This is about a 1% resistance rate but only a small number of strains were tested. This reference states that about 3% of naturally occurring anthrax strains overall are penicillin-resistant.

The rate of penicillin-resistance observed in *in vitro* studies of *B. anthracis* is consistent with observations from the published literature that state that about 3% of naturally occurring anthrax strains are resistant to penicillin [LaForce, FM. Anthrax, Clin Infect Dis, 19:1009-14; 1994 and Lalitha MK, Thomas MK. Penicillin resistance in *Bacillus anthracis*, Lancet, 349: 1522; 1997]. It has been reported that *B. anthracis* strains resistant to penicillin and tetracycline have been engineered by scientists in other countries [Inglesby TV, Henderson DA, et al. Anthrax as a biological weapon, JAMA, 281: 1735-45; 1999]. The possibility of penicillin and/or tetracycline resistance should be considered in the management of the patient exposed to aerosolized spores of *B. anthracis*.

CIPRO®- ANIMAL PHARMACOLOGY

The reader is also referred to the animal pharmacology/toxicology review of Dr Stephen Hundley and the biopharmaceutics and clinical pharmacology review of Dr Joette Meyer.

The data presented below summarize monkey pharmacology studies performed during the development of the oral and intravenous formulations of Cipro®:

Oral dosing-tissue levels in monkeys

Cipro® tissue levels following 13 weeks of 15 mg/kg oral dosing:

Liver	1.0 μg/g
Lung	0.1 to 0.3 μg/g
Lymph nodes	0.1 to 0.3 μg/g

Cipro® tissue levels following 13 weeks of 45 mg/kg oral dosing:

Liver	3.3 μg/g
Lung	0.3 to 0.9 μg/g
Lymph nodes	0.3 to 0.9 μg/g

Animals were sacrificed 24 hours after terminal dose (Week 13); no plasma data were reported from this procedure. Actual lung and lymph node values were not specified but fell within the listed ranges.

Intravenous dosing-tissue and blood levels in monkeys

Table 4. Tissue Cipro® Concentrations (24 Hours Post-Dosing at Week 26)

	Tissue Concentrations (μg/g or μg/ml)		
Tissues	5 mg/kg	10 mg/kg	20 mg/kg
Liver	0.11	0.34	1.0
Spleen	0.15	0.16	0.3
Lymph Nodes	0.13	0.42	1.1
Plasma*	-	-	0.1

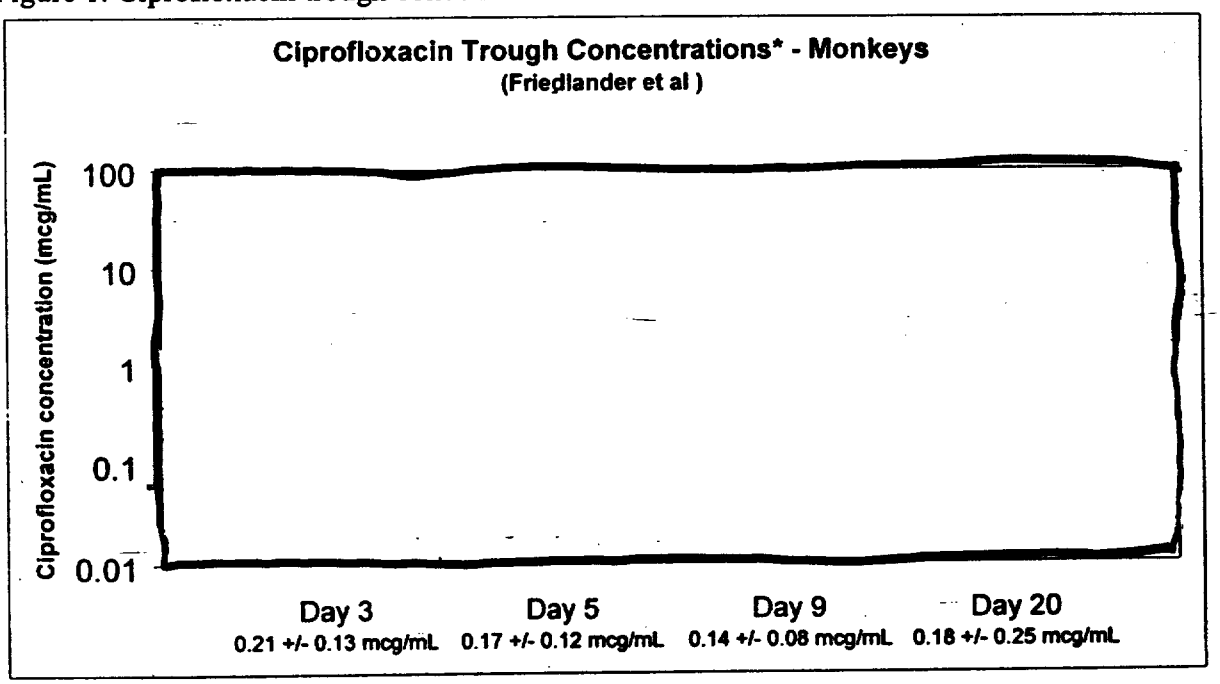
* In a separate segment of this study a 24-hour post-infusion plasma sample was analyzed following a 20 mg/kg dose; the ciprofloxacin concentration was approximately 0.1 μg/ml.

Although the studies cited above vary in dosing regimens, sampling times, and tissues sampled and therefore cannot be compared directly, a few salient points can be noted. Following 13 weeks of oral dosing, animals that received ciprofloxacin 15 mg/kg were found to have drug levels in the liver that were approximately 3-10x that in the lung or lymph node. Tissue levels resulting from 3x this dose were 3x as high as those observed in the 15 mg/kg group, and the differential tissue distribution observed in the 45 mg/kg group were similar to those seen in the lower dose group.

Differential tissue distribution was seen in animals in the repeat dose intravenous study, but this was only observed in those animals that received 20 mg/kg. Interestingly, both liver and lymph nodes were found to have drug levels that were ~3x that seen in the spleen. The intravenous studies also provide a means to compare plasma levels with drug levels found in the various organs of the reticuloendothelial system, the site of much of the early pathology noted following infection with inhaled *B. anthracis*. Following a 20 mg/kg dose, lymph node and liver drug levels were found to be ~10x that of plasma; spleen drug levels were similar to that of plasma.

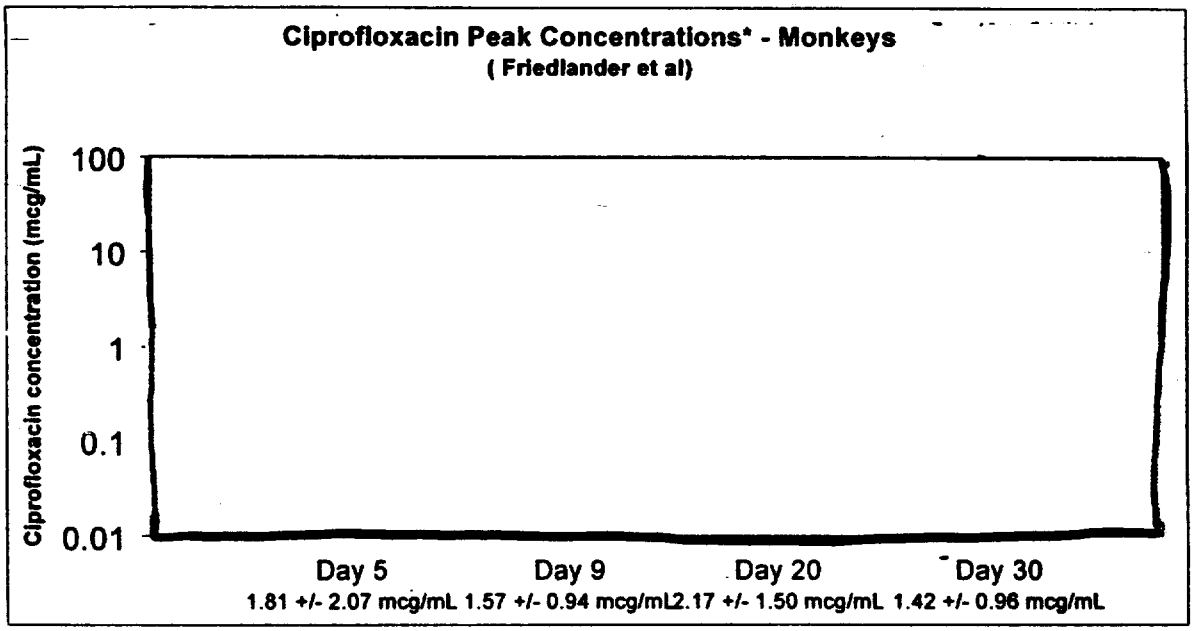
The Rhesus monkeys studied in the animal model of inhalational had a mean body weight of 7.7 kg (range 5.1 to 13.0 kg), and a mean body surface area of 0.46 m^2 (0.65 m^2). This represents a mean of 26% of the 1.73 m^2 body surface area of a 65-kg, 170-cm human. The animals exposed to aerosolized anthrax spores received a single ciprofloxacin 250 mg dose per nasogastric tube (pngt) 24 hours following exposure, followed twelve hours later by ciprofloxacin 125 mg pngt q 12 hours for 30 days. Figures 1 and 2 below present individual (mean ± SD) peak and trough blood levels of ciprofloxacin, respectively for these animals during the 30-day period of drug administration. The MIC_{90} of ciprofloxacin for *B. anthracis* is presented as a horizontal line.

Figure 1. Ciprofloxacin trough concentrations.



*Different symbols represent individual animal serum concentrations at a given time point.

Figure 2. Ciprofloxacin peak concentrations



*Different symbols represent individual animal serum concentrations at a given time point.

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CIPRO®-COMPARISON OF ANIMAL AND HUMAN PHARMACOLOGY

Studies of Cipro® pharmacokinetics in a number of human populations were undertaken during the course of the development of this drug. Serum concentration data were collected for both the oral and intravenous formulations in adult and pediatric populations. These included populations in which the doses of Cipro® administered were the same as those being recommended for the indication under review. Table 5 describes these populations, dosing regimens, serum concentrations, and the year of publication of these data, and compares them with the pharmacokinetic data from the Rhesus monkeys studied in the animal model of inhalational anthrax. Table 5 demonstrates that ciprofloxacin peak and trough serum concentrations achieved in the Rhesus monkey are reached or exceeded in human populations receiving the doses recommended for the post-exposure inhalational anthrax. Peak and trough concentrations reported in both monkey and human populations are shown to consistently exceed 0.06 μg/ml, the value of the MIC_{90} for *B. anthracis.*

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Table 5. Summary of Ciprofloxacin Pharmacokinetics

Population	Dose/Regimen	Route	N	$C_{max,ss}$ (µg/mL) ± SD	$C_{min,ss}$ (µg/mL) ± SD	Notes	Year of Publication
Monkeys	**250 mg x 1, then 125 mg po Q12h (*32 mg/kg x 1, then 16 mg/kg*)**	**PO**	**10**	**1.74 ± 1.41**	**0.17 ± 0.15**	**Loading dose of 2x used for 1st dose**	**1992**
Adults	**500 mg Q12h (*7.1 mg/kg*)**	**PO**	**12**	**2.89 ± 0.54**	**0.28 ± 0.13**	**At steady state**	**1984**
Adults	**400 mg Q12h (*5.6 mg/kg*)**	**IV**	**--**	**4.56**	**0.2**	**At steady state**	**NA**
Human Males	400 mg x 1 (*5.6 mg/kg*)	IV	11	3.11 ± 0.61*	--	*Single dose, not at steady state	1993
Obese Human Males	400 mg x 1 (*3.6 mg/kg*)	IV	17	2.66 ± 0.53*†	--	*Single dose, not at steady state	1993
Peds, CF	10 mg/kg Q8h	IV	18	5.0 ± 1.5	0.39 ± 0.18	Q8h dosing; different from proposed regimen	1997
Peds, CF	20 mg/kg Q12h	PO	18	3.7 ± 1.4	0.42 ± 0.21	Total dose 40 mg/kg; higher than proposed regimen	1997
Peds, CF	**10 mg/kg Q12h**	**IV**	**10**	**8.3**	[redacted]	**Similar to proposed regimen; levels obtained after 2nd IV dose (30 min infusion)**	**1996**
Peds, CF	**15 mg/kg Q12h**	**PO**	**8**	**3.5**	--	**Similar to proposed regimen; levels obtained after 1st oral dose and preceded by two IV doses of 15 mg/kg over 30 min**	**1996**

Data in **boldface** are for populations of animals or humans that received dosing regimens relevant to the dosing proposed for post-exposure inhalational anthrax.

CF- cystic fibrosis

†The C_{max} obtained after a single dose in obese human males is similar to that obtained after multiple dosing in adults of ideal body weight

MO COMMENT: It should be noted that the data from the monkey population in Table 5 reflects the use of a loading dose of ciprofloxacin (250 mg) that was twice the repeat dose administered to these animals during the 30-day period following exposure to aerosolized *B. anthracis*. Similarly, it is noteworthy that peak and trough drug concentrations achieved in the human populations that received doses similar to the proposed regimens reached or exceeded those seen in the experimental animals. A loading dose was not used in any of the human populations presented in Table 5.

MO COMMENT: Table 5 also demonstrates that the relationship between human and Rhesus serum concentrations and the MIC_{90} is preserved in an obese human population.

MO COMMENT: It should be noted that the pediatric data is derived from patients with cystic fibrosis (CF). The pharmacokinetics of ciprofloxacin in this patient population is known to be comparable to healthy subjects.

Figures 3 and 4 below provide a graphic depiction of the data presented in Table 5.

1 page(s) have been removed because it contains trade secret and/or confidential information that is not disclosable.

Pharmacokinetics/Pharmacodynamics (PK/PD)

Fluoroquinolones demonstrate concentration-dependent killing. The goal of a dosing regimen for these drugs is to maximize the plasma concentrations. The peak concentration (Cmax)/MIC and/or area under the curve (AUC)/MIC ratios are considered PK/PD parameters that best correlate with drug efficacy. The relationship between these PK/PD parameters and drug efficacy has been demonstrated in animals models of infection as well as some clinical trials. Some recent data have demonstrated the usefulness of the AUC/MIC and Cmax/MIC ratio for infections treated with fluoroquinolones. Studies of PK/PD parameters suggest that Cmax/MIC values $\geq$10 correlate with clinical efficacy [Amsden GW, Ballow CH, et al. Pharmacokinetics and pharmacodynamics of anti-infective agents in Mandell, Bennett, and Dolin, *Prinicples and practice of infectious diseases*, Fifth edition, pp.257-9, Churchill-Livingstone, 2000].

There have been no prospective studies performed that link clinical outcome to drug exposure for infection with *B. anthracis*. However, in general, when there is a demonstrated relationship between plasma concentrations of drug and response, pharmacokinetic data may be used as one way to relate dose and possible outcome. A direct comparison of pharmacokinetic and pharmacodynamic parameters in an animal model of inhalational anthrax cannot be made. The optimal AUC/MIC or Cmax/MIC ratios for the treatment of infection due to *B. anthracis* not known. However, it is useful to compare the achievable blood levels in humans with the proposed dosing regimen and the blood levels in the Rhesus model. As shown in Figures 3 and 4, the pharmacokinetics of ciprofloxacin in monkeys and humans are similar. Ciprofloxacin peak concentrations achieved with repeat dosing regimens studied in macaques are ~33x MIC_{90} for *B. anthracis*. In humans, peak concentrations are ~50x MIC_{90} for *B. anthracis*.

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INHALATIONAL ANTHRAX- ANIMAL MODELS AND HUMAN EPIDEMIOLOGY

Animal models- early investigations

Since the 1940s, study of the interactions between host and pathogen in inhalational anthrax has been undertaken in a number of animal models. Epidemiologic studies have provided insight into the development of industrial disease in humans. The following discussion presents the findings of some of this earlier work in the context of the current application. This discussion will focus on those results from previous animal experiments that address questions that arise when considering the post-exposure administration of an antimicrobial to the human host exposed to aerosolized *B. anthracis.*

Inhalational anthrax was only described as a clinical entity in the mid-nineteenth century, when it was noted to be a serious public health problem among workers in the British textile industry. There were initially two theories on the pathogenesis of this infection. One was that the inhaled spores were phagocytosed by pulmonary macrophages and transported to the mediastinum where they germinated and produced toxin. The other theory was that the portal of entry was an erosion of the bronchial mucosa that then permitted the development of pneumonia by the vegetative stage of the organism. Henderson, Peacock, and Belton attempted to address this question in a study of penicillin prophylaxis in a Rhesus monkey model of inhalational anthrax. They hypothesized that if the first model of pathogenesis were correct, then penicillin might only be effective for as long as it was being administered and until the spores were completely removed from the lung. If the second were operative, then administration of an antimicrobial might effectively eradicate the organism and prevent disease entirely [Henderson et al, Observations on the prophylaxis of experimental pulmonary anthrax in the monkey, J Hyg 54: 28-36, 1956].

Penicillin administration that began 24 hours after exposure to aerosolized spores of *B. anthracis* and continued for five days was shown to only delay death in the animals exposed (Figure 5, Group A). When the duration of penicillin was extended to 10 or 20 days, a similar delay of death was observed, and the length of time by which death was delayed was generally proportional to the duration of antimicrobial administration (Figure 5, Groups B and C).

Survival curves

(Henderson et al 1956)

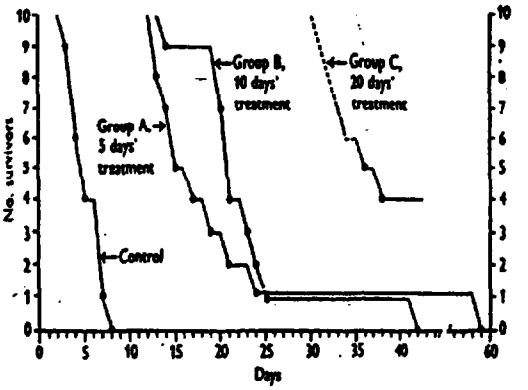

Figure 5. Survival curves for rhesus monkeys administered penicillin following exposure to aerosolized *B. anthracis* (from Henderson et al, 1956)

Passive immunization with hyperimmune horse serum after exposure to aerosolized spores again resulted in a similar delay of death and, like the penicillin groups, in survival curves that were largely parallel to those of the control (untreated) animals. The authors then investigated the efficacy of the combination of active immunization with soluble protective antigen and penicillin, both given 24 hours after exposure. Penicillin was administered for five days. Survival curves for this experiment showed that protection was conferred equally well by active immunization prior to exposure or by penicillin and active immunization after exposure. Animals who received a 5-day course of penicillin only demonstrated the same rapid drop in survival as was demonstrated in the earlier short-course penicillin experiments.

The authors then invoked the concept of spore retention when they questioned whether transitory modes of prophylaxis would be effective in the prevention of inhalational anthrax if spores could survive for such long periods. They noted that only a small proportion of spores was ultimately deposited in local lymph nodes; others were detected in the lung parenchyma 100 days after exposure. They also quantified the proportion of spores that were found in the lungs following exposure, and produced the following table:

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Table 6. Retention of *B. anthracis* spores in the lung following aerosol challenge (2-8 x 10^5 spores/L.) [from Henderson et al, 1956]

Time after exposure (days)	Estimated % of original retention
42	15-20
50	2
75	0.5-1
100	traces

Thus the idea of spore attrition was introduced. Small numbers of spores could be found as long as 100 days after exposure. The work of Henderson's group supported the idea of 'dormant infection,' and demonstrated a number of antimicrobial regimens that were too short to successfully protect the exposed macaque from inhalational anthrax. These experiments also invoked the concept of 'spore clearance,' suggesting that there existed a mode of exit from the lung for *B. anthracis* spores other than phagocytosis and subsequent development into the pathogenic vegetative state.

Other early studies of inhalational anthrax in the guinea pig by Ross permitted direct observation of spores deposited on pulmonary alveolar epithelium, and provided insight into possible mechanisms of 'spore attrition.' Ross noted that the number of spores that reached regional lymph nodes was substantially less than the number deposited on the alveolar epithelium. Using staining techniques that differentiated heat-stable and heat-labile spores, maturing spores, and bacilli, she demonstrated that some inhaled spores that were deposited on the alveolar epithelium were picked up by pulmonary macrophages, transported to regional lymph nodes, developed into vegetative organisms in the reticuloendothelial system (RES) and thus produced toxin and systemic disease. Other phagocytosed spores were shown to pass into the bronchioles and presumably leave the lung by the airways, and still others may have reached a stage of maturation in the macrophage that permitted spore lysis and destruction within the phagocytic cell. While only a rough quantification of inhaled spores was possible with Ross' experiments, she provided histologic evidence that not all inhaled spores develop into vegetative organisms that produce systemic disease [Ross, The pathogenesis of anthrax following the administration of spores by the respiratory route, J Path Bact 73: 495-494, 1957].

Animal model –study under review

The reader is also referred to the microbiology review of Mr Peter Dionne, the animal pharmacology /toxicology reviews of Dr Stephen Hundley and Dr Terry Peters, and the biostatistics review of Dr Karen Higgins.

This animal study of ciprofloxacin in post-exposure inhalational anthrax was performed in a Rhesus monkey model. It was planned and conducted by investigators at the US Army Medical Research Institute of Infectious Diseases (USAMRIID) in 1990. It was

intended that the results would permit the development of a practical scheme for treating susceptible humans exposed to *B. anthracis* in field conditions. To that end, it used a similar route of exposure of aerosolized spores of *B. anthracis* and weight-adjusted dosing regimens as would be anticipated in humans.

> **MO COMMENT**: The discussion below follows the medical officer's review of the study protocol and primary source data describing vital signs, clinical findings, microbiology, pathology, and mortality of experimental animals. Two papers summarizing this experiment [Friedlander et al, Postexposure prophylaxis against experimental inhalational anthrax, J Infect Dis 167: 1239-42, 1993 and Kelly et al, Serum concentrations of penicillin, doxycycline, and ciprofloxacin during prolonged therapy in rhesus monkeys, J Infect Dis 166:1184-7, 1992] were submitted by the sponsor and reviewed. The medical officer also conducted a MEDLINE search and reviewed the published literature to address several questions raised by these data (see REFERENCES).

Study title
Efficacy of antibiotic treatment and vaccination in protection of Rhesus monkeys following aerosol infection with *Bacillus anthracis*

Study objective: To determine the efficacy of each of 3 antibiotics, penicillin, doxycycline, and ciprofloxacin, alone or in combination with the human anthrax vaccine (MDPH), in protecting monkeys against lethal aerosol challenge with *B. anthracis.*

> **MO COMMENT**: The objective stated above is presented verbatim from the protocol, which went on to state that the results of this experiment should permit development of a practical scheme for treating susceptible humans exposed to *B. anthracis* in field conditions. The discussion below will focus on that part of the experiment that evaluates the role of antibiotics alone following exposure to aerosolized *B. anthracis.*

The protocol stated that aerosol infection with *Bacillus anthracis* has been studied previously in the rhesus monkey and primates appear to be the most appropriate known animal model for anthrax in man.

> **MO COMMENT**: Following a search of the medical literature, the MO reviewed animal studies of inhalational anthrax conducted in a number of species including the rhesus monkey, chimpanzee, sheep, and guinea pig [Albrink WS, Goodlow RJ. Experimental inhalation anthrax in the chimpanzee, Am J Path, 35: 1055-65; 1959. Fritz DL, Jaax NK, et al. Pathology of experimental inhalation anthrax in the Rhesus monkey, Lab Invest, 73(5):691-702; 1995. Gleiser CA, Berdjis CC, et al. Pathology of experimental respiratory anthrax in *Macaca mulatta,* Brit J Exp Path, 44: 416-26; 1963. Ivins BE, Pitt MLM, et al. Comparative efficacy of experimental anthrax vaccine candidates against inhalation anthrax in rhesus macaques, Vaccine, 16: 1141-48; 1998 and Jones MN, Beedham RJ, et al. Antibiotic prophylaxis for inhalational anthrax, Salisbury Med Bull, SS87: 127-8]. Also reviewed were studies of human cases of inhalational anthrax following

sporadic industrial exposures in the US and in the Sverdlovsk outbreak of 1979 [Abramova FA, Grinberg LM, et al. Pathology of inhalational anthrax in 42 cases from the Sverdlovsk outbreak of 1979, Proc Natl Acad Sci USA, 90: 2291-94; March 1991 and Brachman PS, Plotkin SA. et al. An epidemic of inhalation anthrax: the first in the twentieth century, Am J Hyg, 72:6-23; 1960 and Vessal K, et al. Radiological changes in inhalational anthrax: a report of radiological and pathological correlation in two cases, Clin Radiol, 26: 471-4; 1975]. The MO concurs that the pathology observed in primates exposed to aerosolized *B. anthracis* is comparable to that seen in humans with inhalational anthrax, and that there exists a body of literature using the rhesus monkey to study this infection.

Study design
This was a three part study:

1) Part One used two rhesus monkeys to verify that lethal infection could be established with the [redacted] planned for use. Microbiology, hematology, chemistry, antigen/antibody, and pathology studies were performed following exposure and at necropsy.

2) Part Two used six additional monkeys to collect pharmacokinetic data during a 30-day dosing period with each of three antimicrobials. None of these animals was exposed to aerosolized *B. anthracis*. Two animals received penicillin G intramuscularly, two received doxycycline by nasogastric tube, and two received ciprofloxacin by nasogastric tube. Blood samples were collected for determination of serum concentrations of antibiotic and of serum bactericidal levels against the Vollum B1 strain of *B. anthracis*.

3) Part Three used 60 additional monkeys that were exposed by aerosol route to the dose of spores determined in Part One. There were six groups of ten animals each. One group received vaccine only on days 1 and 14 post-exposure. Three groups received a 30-day regimen of antibiotic that was started at 24 hours post-exposure. The antibiotics administered were penicillin, doxycycline, and ciprofloxacin at the therapeutic doses determined in Part Two. One group received both doxycycline and vaccine, and a control group of ten monkeys received saline intramuscularly. Following this exposure, animals were observed for a total of 90 days.

   The published study report [Friedlander et al, 1993] stated that in the first challenge, the 60 monkeys (mean weight 7.7 kg) were exposed to an inhaled dose of $4.0 \pm 1.6 \times 10^5$ *B. anthracis* Vollum 1B spores, corresponding to ~8 LD_{50}.

MO COMMENT: Review of the line listings showed that the mean inhaled dose for the 20 animals in the ciprofloxacin and control groups was ~11 LD_{50}.

Serial blood specimens were drawn on all animals in part three for blood culture (antibiotic binding resin used in those animals receiving drug), [redacted] stain for bacilli, drug levels (when appropriate), routine hematology, chemistry, antigen/antibody, and coagulation studies.

At necropsy, blood was taken for [redacted] stain and culture and routine studies as listed above, and cerebrospinal fluid (CSF) was taken for stain, culture, and cell count. For animals that had a positive preterminal blood culture, a limited necropsy was performed. Specimens from six organs were taken for histology, culture, and immunocytologic analysis. These included brain, intrathoracic lymph node, spleen, lung, liver, and kidney. For animals with a negative pre-terminal blood culture, the spleen was cultured immediately and examined histologically to confirm anthrax. These animals had a complete necropsy performed. It was planned that two of the surviving monkeys in each group would be humanely euthanized and necropsied at the end of the experiment to determine the presence of viable spores.

MO COMMENT: The submission did not include primary source data from the determination of viable spores in two surviving animals in each group.

Endpoints

1) Part One- the point at which both monkeys became critically ill and were euthanized or died

2) Part Two- the determination of serum pharmacokinetics and toxicity for each antibiotic, approximately 60 days

3) Part Three- approximately 90 days following spore exposure.

MO COMMENT: The publication describing Part Three of this protocol [Friedlander et al, Postexposure prophylaxis against experimental inhalational anthrax, J Infect Dis 167: 1239-42, 1993] reported results as mortality rates or survival curves post-exposure.

MO COMMENT: The MO considered mortality at day 90 post-exposure to be the primary efficacy endpoint for Part Three. Mortality at day 120 (just prior to rechallenge) was the endpoint used in an additional analysis. The reader is referred to the RESULTS section below for further discussion of these analyses.

Evaluability criteria

The protocol did not include an explicit statement of evaluability criteria. The publication describing Part Three of this protocol [Friedlander et al,1993] stated the following in the *Materials and Methods* section:

> 'A diagnosis of anthrax was confirmed in all animals by isolating *B. anthracis* from the blood. In some cases, organs were cultured quantitatively. In all deaths in which antemortem blood cultures were negative, cultures were done of blood, spleen, lung, liver, intrathoracic lymph nodes, and brain.'

> **MO COMMENT**: The MO used the investigators' definition of anthrax, isolation of *B. anthracis* from the blood, to determine whether an individual animal had anthrax. For those animals with negative blood cultures, the results of quantitative culture of other organs, histopathologic examination of necropsy specimens, and/or immunocytologic analysis were used to determine whether or not an animal had anthrax.

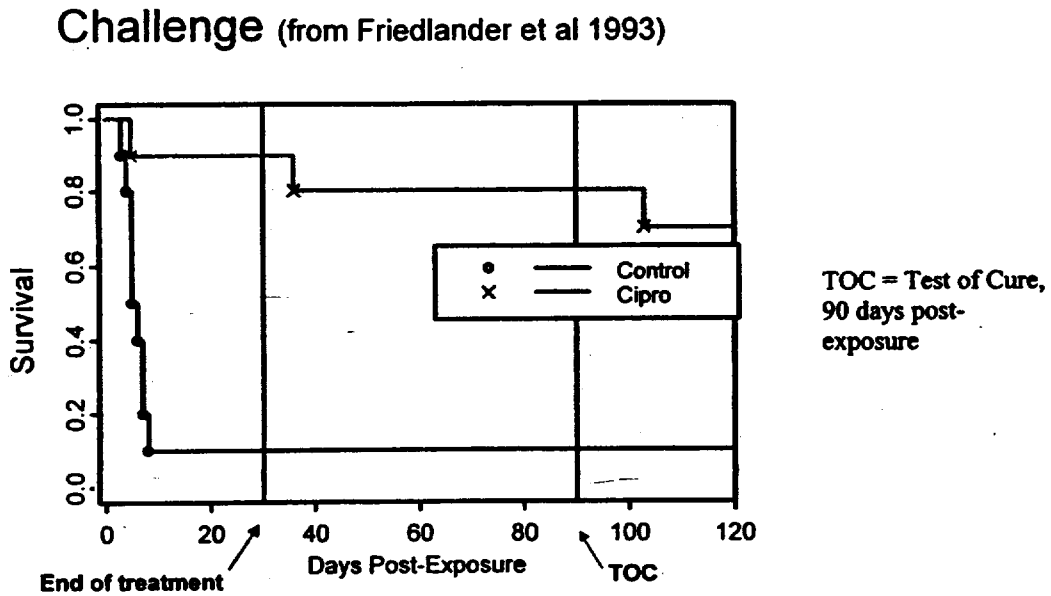
RESULTS

This section will discuss only the results from Part Three of the experiment because that is the part that evaluated antimicrobial efficacy. The MO analysis of results is divided into four parts, 1) Mortality, 2) Microbiology, 3) Pathology, and 4) Analysis of Failures.

Mortality

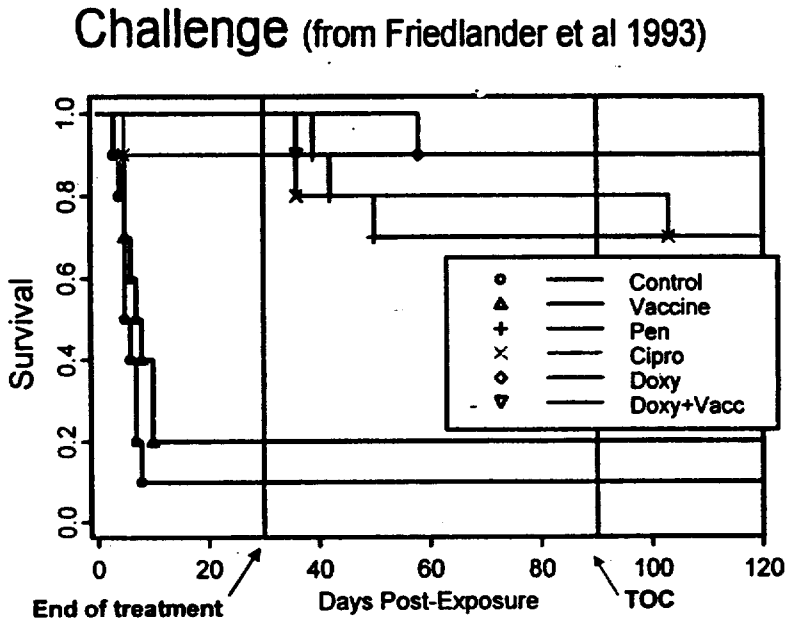
Inspection of the data presented in Figure 6 demonstrates markedly different survival curves for the animals that received post-exposure ciprofloxacin for 30 days compared with those animals that received saline control.

Figure 6. Survival curves for Rhesus monkeys administered ciprofloxacin or saline control following exposure to aerosolized *B. anthracis* (from Friedlander et al, 1993).



Inspection of the survival curves in Figure 7 shows the results for all 6 groups of monkeys. The animals in the control (•) and post-exposure vaccine (▲) groups demonstrated a rapid drop in survival and high mortality, while those that received 30 days of antimicrobial or antimicrobial plus vaccine demonstrated markedly improved survival. These data suggest that a number of different regimens studied in this series of experiments afford comparable protection following the first challenge.

Figure 7. Survival curves for rhesus monkeys administered antimicrobial, vaccine, antimicrobial plus vaccine, or saline control following exposure to aerosolized *B. anthracis* [from Friedlander et al, 1993].



Tables 7 and 8 below present statistical analyses of mortality rates in animals receiving ciprofloxacin compared to animals in the control group. While the small sample sizes result in wide confidence intervals, mortality rates for the ciprofloxacin cohort are similar to mortality rates for penicillin, doxycycline, and doxycycline plus vaccine cohorts for both the evaluable and intent to treat (ITT) study populations.

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Table 7. Evaluable Population Analysis: cause of death proven to be due to anthrax

Treatment	Anthrax deaths	P vs. control‡	95%¹ CI of treatment - control	95%¹ CI of ciprofloxacin - comparator
Control untreated	9/10			
Vaccine alone	8/10	> 0.1	(-54.1%, 37.2%)	(-96.8%, -23.9%)
Penicillin	3/10	0.0198	(-88.7%, -12.3%)	(-67.5%, 24.0%)
Ciprofloxacin	1/9*	0.0011	(-97.5%, -35.0%)	
Doxycycline	1/10	0.0011	(-97.6%, -36.2%)	(-42.9%, 50.5%)
Doxycycline + vaccine	0/9†	0.0001	(-99.8%, -51.6%)	(-37.4%, 56.3%)

*One animal (T292) died 5 days after exposure following the accidental instillation of drug into the trachea, had no evidence of anthrax at autopsy, and was excluded from the evaluable population for this analysis. Another animal (B7388) died 73 days after antibiotic treatment. Therefore, though this animal was evaluable, this death was not thought to be anthrax related and was not included in this analysis.

†One animal (H538) died 6 days after discontinuing doxycycline with no evidence of anthrax on autopsy. Cause of death remains unknown: the animal was excluded from this statistical analysis.

‡ P-value was calculated using a two-tailed Fisher's exact test.

¹ 95% confidence interval was calculated using an exact method.

Table 8. ITT Analysis: including all causes of death as failure

Treatment	All deaths	P vs. control‡	95%¹ CI of treatment - control	95%¹ CI of ciprofloxacin – comparator
Control untreated	9/10			
Vaccine alone	8/10	> 0.1	(-54.1%, 37.2%)	(-82.9%, -1.4%)
Penicillin	3/10	0.0198	(-88.7%, -12.3%)	(-45.9%, 45.9%)
Ciprofloxacin	3/10	0.0198	(-88.7%, -12.3%)	
Doxycycline	1/10	0.0011	(-97.6%, -36.2%)	(-28.3%, 62.0%)
Doxycycline + vaccine	1/10	0.0011	(-97.6%, -36.2%)	(-28.3%, 62.0%)

‡ P-value was calculated using a two-tailed Fisher's exact test.

¹ 95% confidence interval was calculated using an exact method.

Microbiology

The following discussion distinguishes between microbiology and pathology studies. Results of cultures of blood or other organs were considered by the MO to be microbiologic data, while results of staining and microscopic examination of tissue specimens were considered by the MO to be pathologic data. In order to determine the cause of death, the MO first reviewed the results of quantitative blood cultures in all 60 animals following the first challenge with aerosolized *B. anthracis*. All but three animals that died had *B. anthracis* bacteremia at the time of death. The three animals that did not have *B. anthracis* bacteremia at death included two in the group that received ciprofloxacin (#s T292 and B7388) and one in the group that received doxycycline plus vaccine (#H538).

Pathology

The necropsy reports for all 60 animals were then reviewed in order to meet two objectives:

1) To characterize and quantify the histologic findings in the animals in the control group such that these could serve as a standard against which to assess the histologic findings of the animals that received a 30-day post-exposure antimicrobial regimen and were found to have *B. anthracis* bacteremia at or before death
2) To better characterize the cause of death in those animals with negative blood cultures at the time of death

These two parts of the analysis of necropsy reports are presented separately below:

Pathology findings in the control animals

From a review of the published literature, the MO determined histologic findings that are considered characteristic of inhalational anthrax in the primate [Gleiser CA, Berdjis CC, et al. Pathology of experimental respiratory anthrax in *Macaca mulatta,* Brit J Exp Path, 44: 416-26; 1963 and Albrink WS, Goodlow RJ. Experimental inhalation anthrax in the chimpanzee, Am J Path, 35: 1055-65; 1959 and Gochenour WS, Gleiser CA, et al. Observations on penicillin prophylaxis of experimental inhalational anthrax in the monkey, J Hyg, Camb, 60: 29-33; 1962]. These were hemorrhage, edema, and necrosis. Table 9 presents a summary of these findings in the blood, intrathoracic lymph nodes, spleen, lung, liver, brain, and kidney.

MO COMMENT: These organs were included in this table because, as described in the protocol, it was planned that they be examined in all animals that died. A review of the literature shows that these are organs consistently found to have pathologic changes in primates with inhalational anthrax.

Table 9. Pathology of 9 control animals following first challenge

ORGAN	No. of evaluable specimens	% + stain for bacilli	No.* of evaluable specimens	% hemorrhage	% edema	% necrosis
Blood	9	66.7 (6/9)	NA	NA	NA	NA
Intrathoracic LN	9	55.6 (5/9)	6	66.7 (4/6)	50.0 (3/6)	-
Spleen	9	66.7 (6/9)	8	50.0 (4/8)	12.5 (1/8)	-
Lung	9	33.3 (3/9)	8	50.0 (4/8)	37.5 (3/8)	12.5 (1/8)
Liver	9	44.4 (4/9)	7	-	-	28.6 (2/7)
Brain/Meninges	9	66.7 (6/9)	7	85.7 (6/7)	-	-
Kidney	9	44.4 (4/9)	2	50.0 (1/2)	50.0 (1/2)	-

NA-Not Applicable

*A specimen was considered unevaluable for a given pathologic finding if there were no report for that finding

Nine of ten animals in the control group died. As noted above in the Microbiology section, all nine of these animals had positive blood cultures for *B. anthracis*. Table 9 shows that 67% of these animals were found to have bacillemia on direct microscopic examination of the peripheral blood and spleen. The most common pathologic finding in animals that died of anthrax was hemorrhage, seen most commonly in the brain and intrathoracic lymph nodes, and noted in half of the evaluable specimens of spleen, lung, and kidney. Hemorrhage and/or edema were seen in all organs examined except the liver.

> **MO COMMENT:** The finding of bacillemia on direct smear of the peripheral blood suggests a remarkably large organism load.

> **MO COMMENT:** The pathologic findings in the five animals that received a 30-day course of an antimicrobial following exposure and then died with *B. anthracis* cultured from the blood were reviewed and compared with the findings in the controls. All five of these animals (396D, C532, 088CC, L62, T308) had bacilli visualized on microscopic examination of at least one organ, the spleen. All five had hemorrhagic changes noted in at least two of the six organs listed in Table 9. The MO concurred that these animals had died of anthrax.

The pathologic findings in animals that received a 30-day course of an antimicrobial following exposure and then died with negative blood cultures were also reviewed individually. One of these animals (#H538) received doxycycline plus vaccine, and two (#s T292 and B7388) received ciprofloxacin. Pathologic findings in these three animals were not diagnostic of anthrax. The pathology report for one of the animals that received ciprofloxacin (#B7388) was initially difficult to interpret. This animal died on day 73 post-exposure (day 103 of study), and *B. anthracis* was not isolated from the blood at any time. No bacilli were visualized in any tissue preparations. Review of the necropsy findings did not reveal any hemorrhage, necrosis, or edema in the organs listed in Table 9. Examination of the organs of the urinary tract showed obstruction, fibrinous plugs in the urethra, and hemorrhagic urethritis. The conclusion to the necropsy report suggested that these changes were thought to be consistent with a diagnosis of systemic anthrax.

The MO requested clarification of this animal's diagnosis, and on July 26, 2000, received the following additional information: All microscopic slides from the necropsy of this animal were re-reviewed, and the findings in the pathology report were found to be accurate, but the conclusion that the findings were consistent with anthrax was found to be in error. This was corroborated by immunohistochemistry that showed no immunoreactivity against either *B. anthracis* polysaccharide antigen or capsular antigen in sections of the spleen. An amended report was submitted.

> **MO COMMENT**: The MO reviewed the amended report that presented the complete necropsy of animal B7388. The MO agreed that this animal did not have findings of anthrax. Thus, of the three animals in the ciprofloxacin group that died, only one (#T308) died of anthrax.

Analysis of failures

Table 7 shows that there were five animals that received post-exposure antimicrobial that died of anthrax following aerosol challenge. Analysis of the spore load received by these animals suggests that death was not associated with an unusually high inoculum of the infecting organism or the size of the animal. These data are presented in Table 8.

Table 8. Inoculum received by animals that died of anthrax

Animal # (cohort)	Inoculum (spore load as LD_{50})	Animal weight (kg)
T308 (ciprofloxacin)	8	8.8
C532 (penicillin)	13	8.8
088CC (penicillin)	16	9.2
L62 (penicillin)	17	7.0
396D (doxycycline)	10	9.0
Mean (survivors, n= 36)	11.9	7.6
Mean (all animals, n=60)	8	7.7

Underlying the consideration of a post-exposure regimen of ciprofloxacin is the question of the duration of drug administration. The early studies by Henderson and others demonstrated that regimens of 5, 10, and 20 days were too short; high mortality was only delayed. Review of the Friedlander data from the initial challenge phase of the survival curves suggests that a regimen of 30 days results in survival rates that begin to approximate the 'best case' results. However, among the animals that received ciprofloxacin was one that died of anthrax six days after the completion of drug administration. One of ten animals in this cohort was not sufficiently protected from inhalational anthrax following 30 days of ciprofloxacin. This suggests there were an adequate number of inhaled spores remaining to result in some proportion progressing to the vegetative phase, producing toxin, and causing disease after 30 days of antimicrobial. Data from the largest human outbreak in Sverdlovsk, 1979 show that one patient developed disease 43 days after the presumed exposure. Given the possibility that there

exist (s) some mechanism (s) of spore attrition over time, might there be a 'floor' to the spore load in the lung below which inhalational anthrax is unlikely to occur? If such a floor exists, is there a period of antimicrobial administration that will eradicate enough of the developing vegetative organisms such that the risk of disease is minimized? The discussion below addresses these questions.

Table 6 gave a rough approximation of spore load with the passage of time following exposure to aerosolized *B. anthracis* spores. It suggests that after 50 days post-exposure there are far fewer spores in the lungs than the original inhaled load. However it does not quantify the spore load, nor does it speculate about the number of spores that are sufficient for the development of clinical disease. Epidemiologic studies of mill workers with industrial exposure to anthrax spores provide some insight into this question. The aerosol infective dose for man is thought to be relatively high. Air sampling in animal-hair mills demonstrated that nonimmunized workers inhaled between 150-700 anthrax-contaminated particles with a diameter of 5 μ or less during a single eight hour shift, but clinical anthrax was rare in those mills. Other investigators recovered *B. anthracis* from the nose and pharynx of 14 of 101 healthy workers in two goat hair mills. Thus the possibility exists that there is a low organism load that is not associated with clinical disease [Carr EA, Rew RR. Recovery of *Bacillus anthracis* from the nose and throat of apparently healthy workers, J Infect Dis, 100: 169-171; 1957 and Dahlgren CM, Buchanan LM, et al. *Bacillus anthracis* aerosols in goat hair processing mills, Am J Hyg, 72: 24-31; 1960 and Knudson, Treatment of anthrax in man: history and current concepts, Military Med 151: 71-77, Feb 1986].

Human epidemiology

One might consider repeated low-level exposures in the workplace a different immunologic challenge than the exposure resulting from an intentional exposure to aerosolized anthrax spores. Perhaps the occupational exposures present enough antigen to elicit a protective immune response over time. Epidemiologic studies suggest that this is not the case. Brachman and Fekety compared the length of employment in goat-hair processing mills of a group of employees without a history of anthrax with the length of employment of those who did. They found that the likelihood of the development of anthrax was independent of the length of time of employment in the mill. This suggested that repeated low-level occupational exposures to anthrax spores do not confer protection against disease [Brachman and Fekety, Industrial anthrax, Annals NY Acad of Sci, 70:574-84, 1958].

The possibility exists that a relatively small load of inhaled anthrax spores can be carried asymptomatically by the unimmunized human host. The eradication of vegetative organisms that results from the administration of ciprofloxacin plus clearance route (s) for inhaled spores that do not result in toxin elaboration and disease raise the question of the appropriate duration of drug administration following exposure to aerosolized spores of *B. anthracis*.

The administration of an antimicrobial following exposure to an aerosol challenge is an effort to reduce risk of the development of clinical disease. Experimental and

epidemiologic data cited above suggest that a prophylactic regimen following exposure to aerosolized *B. anthracis* should be dosed for at least 45 days. At some period following that point, organism load in the lung passes a threshold below which disease is unlikely. The duration proposed for the administration of ciprofloxacin following exposure to aerosolized anthrax spores is 60 days.

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III. CIPROFLOXACIN-SAFETY PROFILE

Cipro® has been marketed in the US since 1987. Estimates of use in the US exceed [redacted] prescriptions; estimates of worldwide use are about [redacted] prescriptions. Consideration of a post-exposure regimen for inhalational anthrax warrants attention to certain aspects of the safety database, prolonged use regimens (≥ 60 days) and pediatric use.

A review was published which presented data from controlled clinical trials in the medical literature and the from manufacturer's US clinical data pool. This review provided information on a total of 1049 patients who received ciprofloxacin in prolonged use regimens. The mean duration of therapy in patients identified in the electronic literature search was 130 days, and the mean duration for patients in the manufacturer's database of US clinical trials was 80 days. The majority of patients received ciprofloxacin 500-750 mg bid. In both databases, adverse events occurred with similar frequency between the ciprofloxacin and comparator groups of the studies. In the clinical trial pool from the manufacturer, rates for all adverse events, regardless of attributability, were 31.6% in patients who received ciprofloxacin, and 34.8% in patients who-received a comparator agent. The most common adverse events were gastrointestinal, which were more frequent than neurologic or skin reactions. Pseudomembranous colitis was not observed, and no previously unidentified adverse events were noted [Segev et al, Safety of long term therapy with ciprofloxacin, Clin Infect Dis 28: 299-308, 1999].

At the Advisory Committee meeting of July 28, 2000, Bayer Corporation provided additional safety data regarding long-term use of Cipro®. Information from Bayer's database showed described three groups of patients: 1) 1051 patients, including 104 children, who received Cipro® for 60 days or longer in clinical trials sponsored by Bayer, 2) 12,799 patients who received Cipro® in any controlled study, and 3) 11,980 patients who received a comparator agent. Across the three populations adverse event rates overall were similar, 29%, 31%, and 33%, respectively. For those patients who received Cipro® for 60 days or longer, the most common adverse events were rash (3.9%), nausea (3.8%), abdominal pain (3.5%), diarrhea (2.5%), abnormal liver function tests (2.5%), vomiting (1.7%), pruritis (1.5%), headache (1.0%), and dizziness (0.5%). The rates for these events were generally similar when compared to groups (2) and (3) above, except that the patients who received ≥ 60 days of Cipro® had somewhat higher rates of abdominal pain and rash. Patients who received ≥ 60 days of Cipro® had somewhat lower rates of nausea, diarrhea, and headache.

The fluoroquinolone class of drugs has been associated with cartilage abnormalities in experimental juvenile animals, and the safety of drugs of this class has not been established in children. Compassionate use programs for pediatric patients and off-label uses by pediatricians have provided some safety data regarding ciprofloxacin in this population. These data were published in a review that reported safety information for oral and intravenous regimens in 1795 pediatric patients. This population included 517 children between 5 and 12 years, and 66 children less than 5 years of age. The median oral daily dose was 25 mg/kg and duration ranged from 1 to 303 days. Most patients (33%) were treated for 15-30 days. The most common intravenous dose was 6-10

mg/kg/day, and the duration ranged from 1 to 72 days. Treatment associated adverse events were noted in 10.9% of children receiving an oral regimen, and in 18.9% receiving an intravenous one. The most common adverse events were noted in the gastrointestinal system, including diarrhea, nausea, and vomiting. Headache and abdominal pain were also frequently reported. Arthralgia occurred in 1.5% of patients. More than 60% of these episodes were seen in children with cystic fibrosis, a disease which can be associated with arthropathy. The authors of the review under discussion here cited a previously published report of 1.3% arthralgia in pediatric patients receiving ciprofloxacin. Except for the arthralgia reported, the adverse event profile in pediatric patients appeared quite similar to that seen in adults [Hampel et al, Ciprofloxacin in pediatrics, Pediatr Infect Dis J, 16: 127-9, 1997].

At the Advisory Committee meeting of July 28, 2000, Bayer Corporation provided additional safety information regarding pediatric use of Cipro®. They reported that 3400 children have been treated in ciprofloxacin protocols. They provided comparative safety data on 167 who received Cipro® and 178 who received a comparator in controlled clinical trials. Occurring more commonly in children who received Cipro® were vomiting (10% v 4%), rash (7% v 4%), and nausea (7% v 3%). Arthralgia was less common in children in the Cipro® group (5% v 7%), and joint disorders were almost equally common between the two groups (5% v 6%). Abdominal pain, headache, diarrhea, and pruritis occurred with about equal frequency between the two groups. Dizziness was reported for 0% Cipro® group and 2% comparator group.

As noted above, Bayer also provided data on 104 children who received Cipro® for 60 days or longer; no serious adverse events were reported in this population. Data from these 104 patients was compared to data from all 2327 patients in the Cipro® global clinical trials pediatric database. Reported more commonly in children who received a prolonged course were arthralgia (5% v 1%), photosensitivity reaction (3% v <1%), and nervousness (2% v <1%). Arthritis was rare and about equally common in the two groups (0% v <1%). Nausea, liver function test abnormalities, diarrhea, and rash, occurred about equally commonly in the two groups and in ≤ 2% of either group.

Bayer also stated that in the US approximately [redacted] Cipro® prescriptions are written annually for patients younger than 10 years, [redacted] for patients between 10-14 years, and [redacted] for patients 15-17 years. Over the last 13 years, approximately 4.5 million pediatric patients have received ciprofloxacin. Planned pediatric studies include a comparative trial of Cipro® for complicated urinary tract infection, and a long-term observational safety study.

The development of fluoroquinolones for pediatric use has been an issue of discussion at meetings of the FDA Anti-Infective Drug Products Advisory Committee since 1989. Most recently, the issue was presented in November 1997, when the consensus was that pediatric development of fluoroquinolones was warranted for serious and life threatening infections. These included indications such as meningitis, fever and neutropenia, and complicated urinary tract infection [Anti-Infectives Advisory Committee minutes, 62nd meeting Nov 19, 1997].

MO COMMENT: Consideration should be given to the lethality of inhalational anthrax and the need for an agent to administer to an individual exposed to aerosolized *B. anthracis*. There is no evidence that this disease process is different in children than adults. Extrapolation regarding the course of disease suggests the response to therapy should be the same in children as in adults. The risk/benefit analysis of the use of ciprofloxacin in children for post-exposure inhalational anthrax suggests that the benefit afforded by ciprofloxacin administration in this setting outweighs the risk of arthropathy. The information about pediatric dosing that is already in the Cipro® label (Pediatric Use section) is different from the recommendations for dosing for post-exposure inhalational anthrax. With this information in mind, pediatric dosing guidelines for Cipro® for post-exposure inhalational anthrax should be provided.

IV. CIPROFLOXACIN-EXPERIENCE IN SELECTED HUMAN INFECTIONS

The efficacy profile of ciprofloxacin for the indication under discussion might be further characterized by information regarding ciprofloxacin treatment of human cases of anthrax as well as ciprofloxacin treatment of related infections. Data on ciprofloxacin treatment of human infections with *B. anthracis* and of mediastinitis due to a ciprofloxacin-susceptible organism are limited to case reports. A search of the medical literature provided one report of the successful treatment of a patient with *B. anthracis* sepsis following an extensive cutaneous infection. This patient received intravenous penicillin and ciprofloxacin as well as steroids for two weeks, then completed therapy with oral ciprofloxacin for an additional two weeks [Felek et al, A case of anthrax sepsis: non-fatal course, J Infect 38:201-2 May 1999]. Another report documents the successful use of imipenem, ciprofloxacin, and surgical debridement in a patient with postoperative *Nocardia mediastinitis* [Thaler et al, Mediastinitis due to *Nocardia asteroides* after cardiac transplantation, Intensive Care Med 18: 127-8, 1992].

Cipro has an approved indication for the treatment of lower respiratory tract infection due to *E. coli, K. pneumoniae, E. cloacae, P. mirabilis, P. aeruginosa, H. influenzae, H. parainfluenzae,* and (not a drug of first choice) *S. pneumoniae.* Cipro also has an indication for the treatment of an infection of the reticuloendothelial system, typhoid fever due to susceptible strains of *S. typhi* and *S. paratyphi.*

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V. ADVISORY COMMITTEE MEETING

This application was the subject of The Anti-Infective Drug Products Advisory Committee meeting on July 28, 2000. The questions asked of the Advisory Committee are presented below:

1) Do the data presented support the safety and efficacy of ciprofloxacin for post-exposure prophylaxis of inhalational anthrax?
2) If yes, is 60 days an appropriate duration of ciprofloxacin administration for this indication?

The committee voted 'yes' unanimously for both questions.

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VI. MEDICAL OFFICER'S CONCLUSION AND RECOMMENDATION

The data submitted by the applicant demonstrate that ciprofloxacin serum concentrations achievable in human populations reach or exceed those associated with improved survival in animals exposed to aerosol challenge with spores of *B. anthracis*. Serum concentrations in both human and animal populations consistently exceed the MIC_{90} of the causative organism, *B.anthracis*. Use of this [redacted] is consistent with accelerated approval regulations.

Review of animal models of inhalational anthrax and studies of human epidemiology of this rare and lethal disease suggest that a post-exposure 60-day regimen of ciprofloxacin can interrupt the development or progression of clinical disease in individuals exposed to aerosolized *B. anthracis*.

I recommend approval of ciprofloxacin for this indication.

/S/

Andrea Meyerhoff MD MSc DTMH
Medical Officer/DSPIDP

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Concurrence:
HFD-104/Dianne Murphy/Office Director /S/
HFD-590/Renata Albrecht/Acting Division Director /S/ 8/30/00
HFD-590/Rigoberto Roca/Medical Team Leader

CC:
Archival NDAs 19-537, 19-847, 19-857, 19-858, 20-780
HFD-590/Div. Files
HFD-590/V.Jensen/PM
HFD-590/Andrea Meyerhoff/Medical Reviewer
HFD-725/Karen Higgins/Statistical TL
HFD-590/Joette Meyer/Biopharm Reviewer
HFD-590/Peter Dionne/Microbiology
HFD-590/Stephen Hundley/Pharm-tox
HFD-520/Terry Peters/Pharm-tox Reviewer
HFD-590/Shukal Bala/Acting Microbiology TL
HFD-590/Funmi Ajayi/Biopharm TL
HFD-590/Ken Hastings/Pharm-tox TL
HFD-590/Rigo Roca/Medical TL
HFD-590/Renata Albrecht/Acting Div Dir
HFD-520/Gary Chikami/DivDir

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