CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

19-537/S038

19-847/S024

19-857/S027

19-858/S021

20-780/S008

MICROBIOLOGY REVIEW

MICROBIOLOGY REVIEW

DIVISION OF SPECIAL PATHOGENS AND IMMUNOLOGIC DRUG PRODUCTS (HFD-590)

NDA #: 19-537/S-038

REVIEWER:	Peter A. Dionne
CORRESPONDENCE DATE:	29-FEB-00
CDER DATE:	01-MAR-00
REVIEW ASSIGN DATE:	04-MAR-00
REVIEW COMPLETE DATE:	20-MAR-00

SPONSOR:
Bayer Pharmaceutical Division
Bayer Corporation
400 Morgan Lane
West Haven, CT 06516-4175

CONTACT PERSON:
Andrew S. Verderame
Associate Director Regulatory Affairs
Phone Number: (203) 812-5172

SUBMISSION REVIEWED: Supplement for the addition of Anthrax (post exposure prophylaxis) as an approved indication

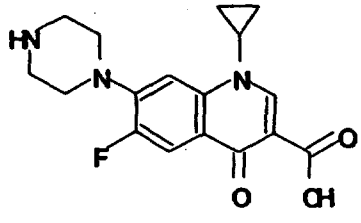
DRUG CATEGORY: Antimicrobial: Fluoroquinolone

INDICATIONS: Acute Sinusitis, Lower Respiratory Tract Infections, UTI, Acute Uncomplicated Cystitis in females, Chronic Bacterial Prostatitis, Complicated Intra-Abdominal Infections, Skin and Skin Structure Infections, Bone and Joint Infections, Infectious Diarrhea, Typhoid Fever, Uncomplicated cervical and urethral gonorrhea; Now Requesting Anthrax (post-exposure prophylaxis)

DOSAGE FORM: Tablets 100, 250, 500, and 750 mg/tablet

DRUG PRODUCT NAME

PROPRIETARY: CIPRO® Tablets
NONPROPRIETARY/USAN: Ciprofloxacin hydrochloride tablets
CHEMICAL NAME: 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid, monohydrochloride monohydrate salt
STRUCTURAL FORMULA:



Molecular Formula: $C_{17}H_{18}FN_3O_3 \cdot HCl \cdot H_2O$
Molecular Weight: 385.8

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APPEARS THIS WAY
ON ORIGINAL

SUPPORTING DOCUMENTS

NDA 20-780/S-008—Ciprofloxacin Oral Suspension
NDA 19-847/S-024—Ciprofloxacin Injection 1% solution vials
NDA 19-857/S-027—Ciprofloxacin Injection in 5% Dextrose
NDA 19-858/S-021—Ciprofloxacin Injection in 0.9% NaCl

INTRODUCTION

This submission contains information that Bayer has accumulated on the use of ciprofloxacin for anthrax (post-exposure prophylaxis). Bayer has relied entirely on published literature for the evidence to support this claim. No human clinical trial data have been included to support this claim. The sponsor has requested the addition of *Bacillus anthracis* to the *in vitro* activity listing in the microbiology subsection of the label. The sponsor has also added information on an animal study in rhesus monkeys exposed to inhaled *Bacillus anthracis* to the label in a section at the end of the label entitled "Other Information".

IN VITRO ACTIVITY AGAINST *BACILLUS ANTHRACIS*

The sponsor has submitted two references that give susceptibility data for ciprofloxacin against *Bacillus anthracis*. A literature search by this reviewer found the same two studies and no other references.

In the first study (1) 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 for 25 antimicrobial drugs. 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 in TABLE 1.

All isolates were sensitive to benzylpenicillin and other penicillins. The MIC values were ≤ 0.03 µg/mL. No isolates showed beta-lactamase activity. Cefazolin and cefoperazone showed good activity. A high rate of resistance was seen against cefuroxime, cefotaxime, ceftizoxime, ceftriaxone, and ceftazidime. All isolates were resistant to aztreonam. Ofloxacin and ciprofloxacin showed good activity with MICs of 0.03 to 0.06 µg/mL.

TABLE 1
Antimicrobial Susceptibility of 22 stains of *Bacillus anthracis* (Reference #1)

Drug	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
Amoxicillin		0.015	0.015
Amoxicillin/clavulanate		0.015	0.015
Piperacillin		0.25	0.5
Mezlocillin		0.06	0.06
Cefazolin		0.015	0.015
Cefuroxime		64	64
Cefotaxime		32	32
Ceftriaxone		32	32
Ceftazidime		16	32
Cefoperazone		128	128
Aztreonam		>128	>128
Clindamycin		1	1
TRM/SMZ		3.2/16	3.2/16
Chloramphenicol		2	2
Gentamicin		0.06	0.125
Streptomycin		2	4
Amikacin		0.03	0.06
Netilmicin		0.06	0.125
Tobramycin		0.25	1
Vancomycin		1	1
Ofloxacin		0.06	0.06
Ciprofloxacin		**0.03**	**0.06**

In the other reference (2) seventy strains were tested in the United Kingdom. These strains were isolated from diverse areas over a long period of time. Agar dilution susceptibility was tested to nine drugs. Mueller-Hinton agar was used and plates were incubated overnight at 37°C. The average inoculum size was 4.2×10^4 organisms/mL. Two strains were found to be resistant to penicillin and produced a beta-lactamase constitutively. Three other strains could be induced to produce beta-lactamase after growth in subinhibitory concentrations of flucloxacillin. Sixty-nine strains were resistant to cefuroxime (MIC 8-64 µg/mL) and two isolates showed intermediate resistance to streptomycin. All 70 isolates were sensitive to gentamicin, erythromycin, chloramphenicol, and tetracycline. Ciprofloxacin MICs were 0.03 to 0.06 µg/mL. Results are shown in TABLE 2.

TABLE 2
Antimicrobial Susceptibility of 70 stains of *Bacillus anthracis* (Reference #2)

Drug	Range (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Penicillin		0.06	0.125
Amoxicillin		0.06	0.125
Cefuroxime		32	64
Gentamicin		0.125	0.25
Streptomycin		1.0	1.0
Erythromycin		0.5	1.0
Tetracycline		0.125	0.125
Chloramphenicol		4.0	4.0
Ciprofloxacin		**0.06**	**0.06**

Most strains were sensitive to penicillin and amoxicillin with MIC of 0.03 µg/mL or less. Two strains were found to be resistant with MICs >0.25 µg/mL. The two strains, however, No. 32 and No. 70 originated from the same fatal case.

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.

Following the induction of beta-lactamase, the MICs were repeated for penicillin and amoxicillin using a larger inoculum of 1.3×10^7 organisms/mL. The MICs increased slightly for both antibiotics probably due to the larger inoculum size. Only strain 32 was highly resistant to both penicillin and amoxicillin while strain 70, which had previously been found to be resistant to penicillin had a MIC of 0.03 µg/mL to both drugs. Following induction strain 32 was still resistant and strains 52 and 76 now also had MICs of >64 µg/mL. All three strains were positive for beta-lactamase activity. Before induction, strains 52 and 76 had MICs of 0.06 and 0.03 µg/mL to penicillin and amoxicillin, respectively. Strain 23 had a MIC of 0.25 µg/mL to both drugs prior to induction and a MIC of 8.0 µg/mL following induction. Strain 59A had a MIC of 0.015 µg/mL to penicillin and 0.03 µg/mL to amoxicillin prior to induction and a MIC of 32.0 µg/mL and 16.0 µg/mL after induction. Both strains 23 and 59A were negative for beta-lactamase production. Strain 67 was once again positive for beta-lactamase production both before and after induction but was sensitive on MIC testing. This information is summarized in TABLE 3.

TABLE 3
MIC (µg/mL) following induction of beta-lactamase

Strain No.	23	32	52	59A	67	70	76
MIC to penicillin prior to induction	0.25	64	0.06	0.015	0.06	0.03	0.06
MIC to penicillin following induction	8.0	64	64	32.0	0.125	0.03	64
MIC to amoxicillin prior to induction	0.25	64	0.03	0.03	0.06	0.03	0.03
MIC to amoxicillin following induction	8.0	64	64	16.0	0.06	0.03	64
Beta-lactamase activity prior to induction	-ve	+ve	-ve	-ve	+ve	-ve	-ve
Beta-lactamase activity following induction	-ve	+ve	+ve	-ve	+ve	-ve	+ve

STUDIES IN ANIMAL MODELS

In a murine model, mice were injected subcutaneously with 10-1000 LD_{50} spores of *Bacillus anthracis* (3). The animals were treated orally with ciprofloxacin, pefloxacin, or lomefloxacin. Prophylaxis with ciprofloxacin protected 90-100% of mice against 10 LD_{50} after dosing with 2 to 3 milligrams and 50-90% of mice were protected against 1000 LD_{50}. Treatment with pefloxacin and lomefloxacin was comparable to that with ciprofloxacin.

A Rhesus monkey model was evaluated [redacted] After exposure to an inhaled dose of 8 LD_{50} spores of *Bacillus anthracis*, groups of 10 Rhesus monkeys were treated with penicillin, doxycycline, ciprofloxacin, doxycycline plus vaccine, vaccine alone, or saline. The MIC of ciprofloxacin for the *Bacillus anthracis* strain used was 0.08 µg/mL. The ciprofloxacin group was treated with 125 mg orally by orogastric tube every 12 hours beginning one day after exposure and continuing for 30 days. Controls were given saline intramuscularly every 12 hours beginning one day after exposure. Procaine penicillin G was given intramuscularly every 12 hours beginning one day after exposure. Doxycycline was given at a 30 mg dose every 12 hours. Vaccine was given on days 1 and 15 after exposure. At one hour post dose, the peak ciprofloxacin serum concentrations were 0.98 to 1.69 µg/mL, and trough concentrations were 0.12 to 0.19 µg/mL at 12 hours post dose.

Nine of the ten control animals exposed to an inhaled dose of anthrax died 3 to 8 days after challenge. The survivor appears to not have become infected, since blood cultures were negative and an antibody response never developed. Eight of ten animals treated with vaccination alone died. The time of death and clinical and autopsy findings did not differ from untreated controls. None of the antibiotic treated animals died during the 30 days of treatment. TABLE 4 shows the survival of various groups.

TABLE 4
Survival after postexposure treatment of inhalation anthrax

Treatment	Anthrax Deaths
Control Untreated	9/10
Vaccine Alone	8/10
Penicillin	3/10
Ciprofloxacin	1/9*
Doxycycline	1/10
Doxycycline + vaccine	0/9**

* One animal died 5 days after exposure from aspiration pneumonia, had no evidence of anthrax at autopsy, and was excluded from analysis.
** One animal died 5 days after discontinuing doxycycline with no evidence of anthrax on autopsy.

In the penicillin group, three of ten animals died of anthrax on days 9, 12, and 20 after penicillin was stopped.

One animal given ciprofloxacin died 5 days after exposure from aspiration pneumonia 24 hours after the inadvertent introduction of drug into the trachea. All other ciprofloxacin treated monkeys survived the 30 days of treatment with negative blood cultures. One animal died of anthrax 6 days after ciprofloxacin treatment was stopped. Another animal in the ciprofloxacin group died 73 days after antibiotic was discontinued. Autopsy revealed no evidence of anthrax. This animal died of urethral obstruction due to rubbery plugs in the urethra and bladder.

In the group treated with doxycycline alone, all animals survived during treatment and had negative blood cultures. One monkey died from anthrax 28 days after treatment was stopped.

None of the animals treated with doxycycline plus postexposure vaccine died of anthrax. One animal in this group died 6 days after discontinuance of doxycycline but had no evidence of anthrax on autopsy.

Animals that survived the aerosol challenge were examined for evidence of an immune response 131-142 days after exposure by measuring antibody to the protective antigen component of anthrax toxin. No surviving animals treated with antibiotic alone had an immune response. In contrast, the animals given doxycycline plus vaccine all developed a fourfold or greater rise in antibody. The two surviving animals given vaccine alone also developed an antibody response.

When rechallenged with a 50 LD_{50} spore suspension of anthrax only the group treated with doxycycline plus vaccine had significant number of survivors. In this group 9 of 9 animals remained free of disease one year after rechallenge. No significant protection was afforded by antibiotic treatment alone. In the penicillin group 0/7 survived. In the ciprofloxacin group only 1/7 survived and in the doxycycline group 0/9 survived.

The results of this experiment suggest that therapy for an unimmunized person exposed to an aerosol of anthrax spores should consist of long-term suppressive antibiotics. Vaccination may provide an additional degree of protection against relapse after antibiotic treatment and would protect against a subsequent exposure.

CONCLUSIONS

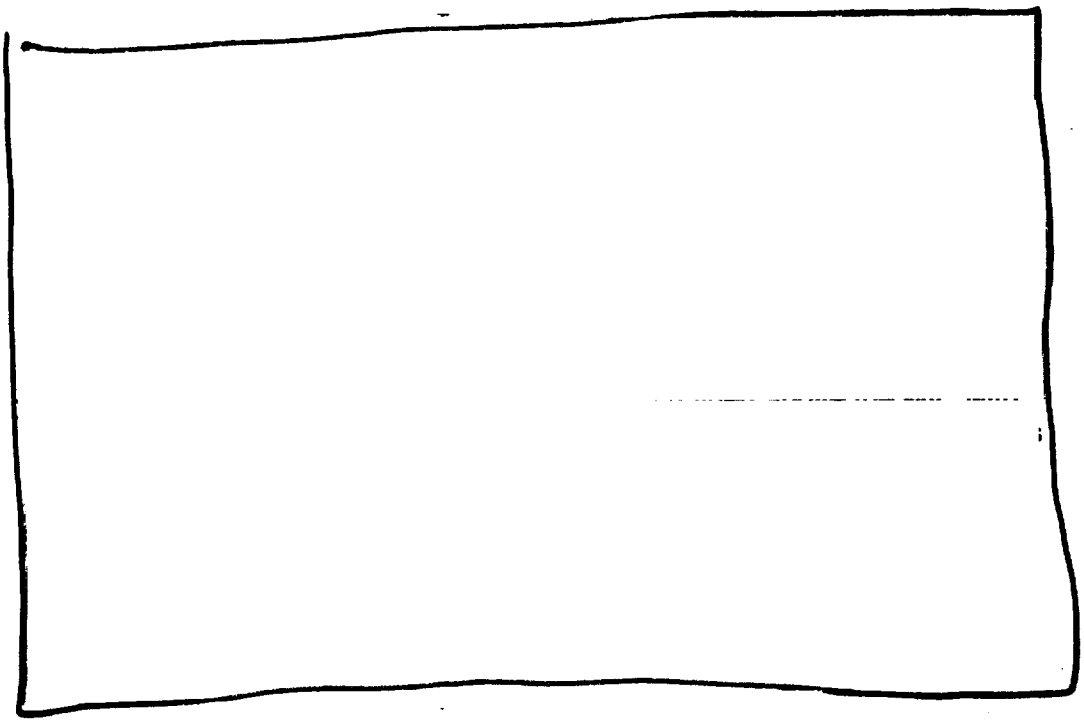
In this submission Bayer is requesting approval of an indication of Anthrax (post exposure prophylaxis) for ciprofloxacin. Bayer has submitted data from a study of inhalation anthrax in Rhesus monkeys and from published literature as evidence to support this claim.

The sponsor is requesting the addition of *Bacillus anthracis* to list #2 (*in vitro* activity only) in the Microbiology subsection of the label. Two studies on the *in vitro* susceptibility of *Bacillus anthracis* to ciprofloxacin have been submitted to support the addition of *Bacillus anthracis* to this list. A total of more than 90 isolates of *Bacillus anthracis* have been tested and the MIC_{90} value in both studies was 0.06 μg/mL, which is well below ciprofloxacin's susceptible breakpoint of 1 μg/mL. Both studies were performed over 5 years ago, but no newer data are available. Also no United States studies are available. There is no reason to believe that the susceptibility pattern for this species has changed recently or that testing of United States strains would give different results. From the microbiological standpoint the addition of *Bacillus anthracis* to the label seems justified.

Testing in an animal model also seems to justify the addition of *Bacillus anthracis* to the label. The sponsor has included information about a study in rhesus monkeys exposed to an inhaled dose of 8 LD_{50} spores of *Bacillus anthracis*. The statement correctly states that none of the nine ciprofloxacin treated animals died from anthrax within the 30 day treatment period. The information, however, does not state that one animal died of anthrax six days after treatment stopped. This section should be revised to include the dosage regime that was used for ciprofloxacin to give the stated serum concentrations and to include the fact that one of the nine animals died from anthrax after treatment.

RECOMMENDATIONS

From the microbiological viewpoint this supplement should be approved. The section at the end of the label entitled "Other Information" should be revised to read as follows:



The first two references in the label should be updated to the January 2000 reversions.

1. National Committee for Clinical Laboratory Standards, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Fifth Edition. Approved Standard NCCLS Document M7-A5, Vol. 20, No. 2, NCCLS, Wayne, PA, January 2000.
2. National Committee for Clinical Laboratory Standards, Performance Standards for Antimicrobial Disk Susceptibility Tests—Seventh Edition. Approved Standard NCCLS Document M2-A7, Vol. 20, No. 1, NCCLS, Wayne, PA, January 2000.

REFERENCES

1. Doganay M, Aydin N. Antimicrobial susceptibility of *Bacillus anthracis*. Scand J Infect Dis, 23:333=335, 1991.

2. Lightfoot NF, Scott RJD, Turnbull PCB. Antimicrobial susceptibility of *Bacillus anthracis*. Salisbury Med Bull 68, Suppl: 95-98, 1990.

3. Dyakov SI, Katsalukha VV, Lebedeva IK, et al. Comparative estimation of fluoroquinolone efficacy in experimental anthrax. Antibiot Chimioter 39:15-19, 1994.

4. Friedlander AM, Welkos SL, Pitt MLM, et al. Postexposure prophylaxis against experimental inhalation anthrax. J Infect Dis 167:1239-1242, 1993.

/S/

Peter A. Dionne
Microbiologist HFD-590

CONCURRENCES:

HFD-590/Div Dir /S/ Signature 4/7/00 Date
HFD-590/TLMicro /S/ Signature 3/23/2000 Date

CC:
HFD-590/Original NDA # 19-537/S-038
HFD-590/Division File
HFD-590/Micro/PDionne
HFD-590/MO/AMeyerhoff
HFD-520/Pharm/SHundley
HFD-590/Chem/DMatecka
HFD-590/CSO/VJensen

MICROBIOLOGY REVIEW

DIVISION OF SPECIAL PATHOGENS AND IMMUNOLOGIC DRUG PRODUCTS (HFD-590)

NDA #: 19-847/S-024

REVIEWER:	Peter A. Dionne
CORRESPONDENCE DATE:	29-FEB-00
CDER DATE:	01-MAR-00
REVIEW ASSIGN DATE:	04-MAR-00
REVIEW COMPLETE DATE:	20-MAR-00

SPONSOR:
Bayer Pharmaceutical Division
Bayer Corporation
400 Morgan Lane
West Haven, CT 06516-4175

CONTACT PERSON:
Andrew S. Verderame
Associate Director Regulatory Affairs
Phone Number: (203) 812-5172

SUBMISSION REVIEWED: Supplement for the addition of Anthrax (post exposure prophylaxis) as an approved indication.

DRUG CATEGORY: Antimicrobial: Fluoroquinolone

INDICATIONS: Acute Sinusitis, Lower Respiratory Tract Infections, UTI, Acute Uncomplicated Cystitis in females, Chronic Bacterial Prostatitis, Complicated Intra-Abdominal Infections, Skin and Skin Structure Infections, Bone and Joint Infections, Infectious Diarrhea, Typhoid Fever, Uncomplicated cervical and urethral gonorrhea; Now Requesting Anthrax (post-exposure prophylaxis)

DOSAGE FORM: IV solution—10mg/mL; 20 mL and 40 mL vials

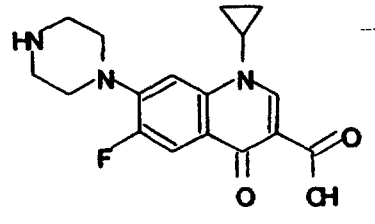
DRUG PRODUCT NAME

PROPRIETARY: CIPRO® I. V.

NONPROPRIETARY/USAN: Ciprofloxacin for intravenous infusion

CHEMICAL NAME: 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid.

STRUCTURAL FORMULA:



Molecular Formula: $C_{17}H_{18}FN_3O_3$

Molecular Weight: 331.4

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APPEARS THIS WAY
ON ORIGINAL

SUPPORTING DOCUMENTS

NDA 19-537/S-038—Ciprofloxacin Hydrochloride Tablets
NDA 20-780/S-008—Ciprofloxacin Oral Suspension
NDA 19-857/S-027—Ciprofloxacin Injection in 5% Dextrose
NDA 19-858/S-021—Ciprofloxacin Injection in 0.9% NaCl

INTRODUCTION

This submission contains information that Bayer has accumulated on the use of ciprofloxacin for anthrax (post-exposure prophylaxis). Bayer has relied entirely on published literature for the evidence to support this claim. No human clinical trial data have been included to support this claim. The sponsor has requested the addition of *Bacillus anthracis* to the *in vitro* activity listing in the microbiology subsection of the label. The sponsor has also added information on an animal study in rhesus monkeys exposed to inhaled *Bacillus anthracis* to the label in a section at the end of the label entitled "Other Information".

IN VITRO ACTIVITY AGAINST *BACILLUS ANTHRACIS*

The sponsor has submitted two references that give susceptibility data for ciprofloxacin against *Bacillus anthracis*. A literature search by this reviewer found the same two studies and no other references.

In the first study (1) 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 for 25 antimicrobial drugs. 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 in TABLE 1.

All isolates were sensitive to benzylpenicillin and other penicillins. The MIC values were ≤ 0.03 µg/mL. No isolates showed beta-lactamase activity. Cefazolin and cefoperazone showed good activity. A high rate of resistance was seen against cefuroxime, cefotaxime, ceftizoxime, ceftriaxone, and ceftazidime. All isolates were resistant to aztreonam. Ofloxacin and ciprofloxacin showed good activity with MICs of 0.03 to 0.06 µg/mL.

TABLE 1
Antimicrobial Susceptibility of 22 stains of *Bacillus anthracis* (Reference #1)

Drug	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
Amoxicillin		0.015	0.015
Amoxicillin/clavulanate		0.015	0.015
Piperacillin		0.25	0.5
Mezlocillin		0.06	0.06
Cefazolin		0.015	0.015
Cefuroxime		64	64
Cefotaxime		32	32
Ceftriaxone		32	32
Ceftazidime		16	32
Cefoperazone		128	128
Aztreonam		>128	>128
Clindamycin		1	1
TRM/SMZ		3.2/16	3.2/16
Chloramphenicol		2	2
Gentamicin		0.06	0.125
Streptomycin		2	4
Amikacin		0.03	0.06
Netilmicin		0.06	0.125
Tobramycin		0.25	1
Vancomycin		1	1
Ofloxacin		0.06	0.06
Ciprofloxacin		**0.03**	**0.06**

In the other reference (2) seventy strains were tested in the United Kingdom. These strains were isolated from diverse areas over a long period of time. Agar dilution susceptibility was tested to nine drugs. Mueller-Hinton agar was used and plates were incubated overnight at 37°C. The average inoculum size was 4.2×10^4 organisms/mL. Two strains were found to be resistant to penicillin and produced a beta-lactamase constitutively. Three other strains could be induced to produce beta-lactamase after growth in subinhibitory concentrations of flucloxacillin. Sixty-nine strains were resistant to cefuroxime (MIC 8-64 µg/mL) and two isolates showed intermediate resistance to streptomycin. All 70 isolates were sensitive to gentamicin, erythromycin, chloramphenicol, and tetracycline. Ciprofloxacin MICs were 0.03 to 0.06 µg/mL. Results are shown in TABLE 2.

TABLE 2
Antimicrobial Susceptibility of 70 stains of *Bacillus anthracis* (Reference #2)

Drug	Range (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Penicillin		0.06	0.125
Amoxicillin		0.06	0.125
Cefuroxime		32	64
Gentamicin		0.125	0.25
Streptomycin		1.0	1.0
Erythromycin		0.5	1.0
Tetracycline		0.125	0.125
Chloramphenicol		4.0	4.0
Ciprofloxacin		**0.06**	**0.06**

Most strains were sensitive to penicillin and amoxicillin with MIC of 0.03 µg/mL or less. Two strains were found to be resistant with MICs >0.25 µg/mL. The two strains, however, No. 32 and No. 70 originated from the same fatal case.

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.

Following the induction of beta-lactamase, the MICs were repeated for penicillin and amoxicillin using a larger inoculum of 1.3×10^7 organisms/mL. The MICs increased slightly for both antibiotics probably due to the larger inoculum size. Only strain 32 was highly resistant to both penicillin and amoxicillin while strain 70, which had previously been found to be resistant to penicillin had a MIC of 0.03 µg/mL to both drugs. Following induction strain 32 was still resistant and strains 52 and 76 now also had MICs of >64 µg/mL. All three strains were positive for beta-lactamase activity. Before induction, strains 52 and 76 had MICs of 0.06 and 0.03 µg/mL to penicillin and amoxicillin, respectively. Strain 23 had a MIC of 0.25 µg/mL to both drugs prior to induction and a MIC of 8.0 µg/mL following induction. Strain 59A had a MIC of 0.015 µg/mL to penicillin and 0.03 µg/mL to amoxicillin prior to induction and a MIC of 32.0 µg/mL and 16.0 µg/mL after induction. Both strains 23 and 59A were negative for beta-lactamase production. Strain 67 was once again positive for beta-lactamase production both before and after induction but was sensitive on MIC testing. This information is summarized in TABLE 3.

TABLE 3
MIC (μg/mL) following induction of beta-lactamase

Strain No.	23	32	52	59A	67	70	76
MIC to penicillin prior to induction	0.25	64	0.06	0.015	0.06	0.03	0.06
MIC to penicillin following induction	8.0	64	64	32.0	0.125	0.03	64
MIC to amoxicillin prior to induction	0.25	64	0.03	0.03	0.06	0.03	0.03
MIC to amoxicillin following induction	8.0	64	64	16.0	0.06	0.03	64
Beta-lactamase activity prior to induction	-ve	+ve	-ve	-ve	+ve	-ve	-ve
Beta-lactamase activity following induction	-ve	+ve	+ve	-ve	+ve	-ve	+ve

STUDIES IN ANIMAL MODELS

In a murine model, mice were injected subcutaneously with 10-1000 LD_{50} spores of *Bacillus anthracis* (3). The animals were treated orally with ciprofloxacin, pefloxacin, or lomefloxacin. Prophylaxis with ciprofloxacin protected 90-100% of mice against 10 LD_{50} after dosing with 2 to 3 milligrams and 50-90% of mice were protected against 1000 LD_{50}. Treatment with pefloxacin and lomefloxacin was comparable to that with ciprofloxacin.

A Rhesus monkey model was evaluated at [redacted]. After exposure to an inhaled dose of 8 LD_{50} spores of *Bacillus anthracis*, groups of 10 Rhesus monkeys were treated with penicillin, doxycycline, ciprofloxacin, doxycycline plus vaccine, vaccine alone, or saline. The MIC of ciprofloxacin for the *Bacillus anthracis* strain used was 0.08 μg/mL. The ciprofloxacin group was treated with 125 mg orally by orogastric tube every 12 hours beginning one day after exposure and continuing for 30 days. Controls were given saline intramuscularly every 12 hours beginning one day after exposure. Procaine penicillin G was given intramuscularly every 12 hours beginning one day after exposure. Doxycycline was given at a 30 mg dose every 12 hours. Vaccine was given on days 1 and 15 after exposure. At one hour post dose, the peak ciprofloxacin serum concentrations were 0.98 to 1.69 μg/mL, and trough concentrations were 0.12 to 0.19 μg/mL at 12 hours post dose.

Nine of the ten control animals exposed to an inhaled dose of anthrax died 3 to 8 days after challenge. The survivor appears to not have become infected, since blood cultures were negative and an antibody response never developed. Eight of ten animals treated with vaccination alone died. The time of death and clinical and autopsy findings did not differ from untreated controls. None of the antibiotic treated animals died during the 30 days of treatment. TABLE 4 shows the survival of various groups.

TABLE 4
Survival after postexposure treatment of inhalation anthrax

Treatment	Anthrax Deaths
Control Untreated	9/10
Vaccine Alone	8/10
Penicillin	3/10
Ciprofloxacin	1/9*
Doxycycline	1/10
Doxycycline + vaccine	0/9**

* One animal died 5 days after exposure from aspiration pneumonia, had no evidence of anthrax at autopsy, and was excluded from analysis.
** One animal died 5 days after discontinuing doxycycline with no evidence of anthrax on autopsy.

In the penicillin group, three of ten animals died of anthrax on days 9, 12, and 20 after penicillin was stopped.

One animal given ciprofloxacin died 5 days after exposure from aspiration pneumonia 24 hours after the inadvertent introduction of drug into the trachea. All other ciprofloxacin treated monkeys survived the 30 days of treatment with negative blood cultures. One animal died of anthrax 6 days after ciprofloxacin treatment was stopped. Another animal in the ciprofloxacin group died 73 days after antibiotic was discontinued. Autopsy revealed no evidence of anthrax. This animal died of urethral obstruction due to rubbery plugs in the urethra and bladder.

In the group treated with doxycycline alone, all animals survived during treatment and had negative blood cultures. One monkey died from anthrax 28 days after treatment was stopped.

None of the animals treated with doxycycline plus postexposure vaccine died of anthrax. One animal in this group died 6 days after discontinuance of doxycycline but had no evidence of anthrax on autopsy.

Animals that survived the aerosol challenge were examined for evidence of an immune response 131-142 days after exposure by measuring antibody to the protective antigen component of anthrax toxin. No surviving animals treated with antibiotic alone had an immune response. In contrast, the animals given doxycycline plus vaccine all developed a fourfold or greater rise in antibody. The two surviving animals given vaccine alone also developed an antibody response.

When rechallenged with a 50 LD_{50} spore suspension of anthrax only the group treated with doxycycline plus vaccine had significant number of survivors. In this group 9 of 9 animals remained free of disease one year after rechallenge. No significant protection was afforded by antibiotic treatment alone. In the penicillin group 0/7 survived. In the ciprofloxacin group only 1/7 survived and in the doxycycline group 0/9 survived.

The results of this experiment suggest that therapy for an unimmunized person exposed to an aerosol of anthrax spores should consist of long-term suppressive antibiotics. Vaccination may provide an additional degree of protection against relapse after antibiotic treatment and would protect against a subsequent exposure.

CONCLUSIONS

In this submission Bayer is requesting approval of an indication of Anthrax (post exposure prophylaxis) for ciprofloxacin. Bayer has submitted data from a study of inhalation anthrax in Rhesus monkeys and from published literature as evidence to support this claim.

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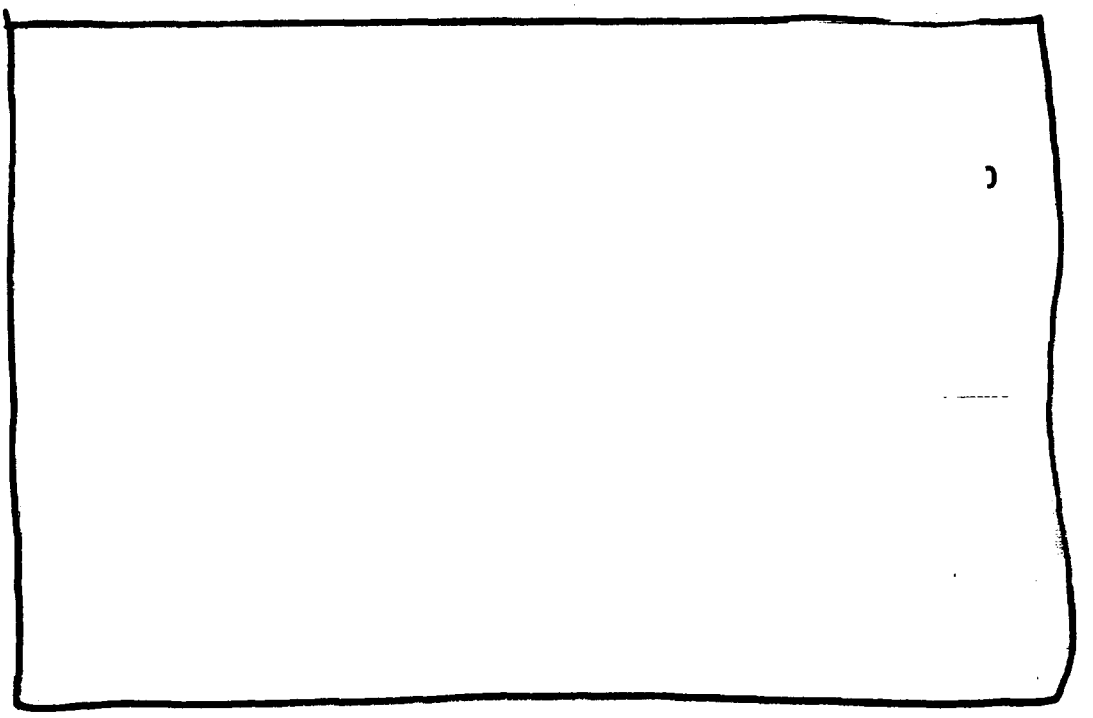
Testing in an animal model also seems to justify the addition of *Bacillus anthracis* to the label. The sponsor has included information about a study in rhesus monkeys exposed to an inhaled dose of 8 LD_{50} spores of *Bacillus anthracis*. The statement correctly states that none of the nine ciprofloxacin treated animals died from anthrax within the 30 day treatment period. The information, however, does not state that one animal died of anthrax six days after treatment stopped. This section should be revised to include the dosage regime that was used for ciprofloxacin to give the stated serum concentrations and to include the fact that one of the nine animals died from anthrax after treatment.

RECOMMENDATIONS

From the microbiological viewpoint this supplement should be approved. The section at the end of the label entitled "Other Information" should be revised to read as follows:



The first two references in the label should be updated to the January 2000 reversions.

1. National Committee for Clinical Laboratory Standards, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Fifth Edition. Approved Standard NCCLS Document M7-A5, Vol. 20, No. 2, NCCLS, Wayne, PA, January 2000.
2. National Committee for Clinical Laboratory Standards, Performance Standards for Antimicrobial Disk Susceptibility Tests—Seventh Edition. Approved Standard NCCLS Document M2-A7, Vol. 20, No. 1, NCCLS, Wayne, PA, January 2000.

REFERENCES

1. Doganay M, Aydin N. Antimicrobial susceptibility of *Bacillus anthracis*. Scand J Infect Dis, 23:333=335, 1991.

2. Lightfoot NF, Scott RJD, Turnbull PCB. Antimicrobial susceptibility of *Bacillus anthracis*. Salisbury Med Bull 68, Suppl: 95-98, 1990.

3. Dyakov SI, Katsalukha VV, Lebedeva IK, et al. Comparative estimation of fluoroquinolone efficacy in experimental anthrax. Antibiot Chimioter 39:15-19, 1994.

4. Friedlander AM, Welkos SL, Pitt MLM, et al. Postexposure prophylaxis against experimental inhalation anthrax. J Infect Dis 167:1239-1242, 1993.

/S/

Peter A. Dionne
Microbiologist HFD-590

CONCURRENCES:

HFD-590/Div Dir /S/ Signature 4/7/00 Date
HFD-590/TLMicro /S/ Signature 3/23/2000 Date

CC:
HFD-590/Original NDA # 19-847/S-024
HFD-590/Division File
HFD-590/Micro/PDionne
HFD-590/MO/AMeyerhoff
HFD-520/Pharm/SHundley
HFD-590/Chem/DMatecka
HFD-590/CSO/VJensen

MICROBIOLOGY REVIEW

DIVISION OF SPECIAL PATHOGENS AND IMMUNOLOGIC DRUG PRODUCTS (HFD-590)

NDA #: 19-857/S-027

REVIEWER:	Peter A. Dionne
CORRESPONDENCE DATE:	29-FEB-00
CDER DATE:	01-MAR-00
REVIEW ASSIGN DATE:	04-MAR-00
REVIEW COMPLETE DATE:	20-MAR-00

SPONSOR:
Bayer Pharmaceutical Division
Bayer Corporation
400 Morgan Lane
West Haven, CT 06516-4175

CONTACT PERSON:
Andrew S. Verderame
Associate Director Regulatory Affairs
Phone Number: (203) 812-5172

SUBMISSION REVIEWED: Supplement for the addition of Anthrax (post exposure prophylaxis) as an approved indication

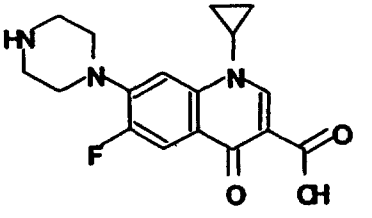
DRUG CATEGORY: Antimicrobial: Fluoroquinolone

INDICATIONS: Acute Sinusitis, Lower Respiratory Tract Infections, UTI, Acute Uncomplicated Cystitis in females, Chronic Bacterial Prostatitis, Complicated Intra-Abdominal Infections, Skin and Skin Structure Infections, Bone and Joint Infections, Infectious Diarrhea, Typhoid Fever, Uncomplicated cervical and urethral gonorrhea; Now Requesting Anthrax (post-exposure prophylaxis)

DOSAGE FORM: IV solution in 5% Dextrose—2 mg/mL; 100 mL and 200 mL in 5% Dextrose

DRUG PRODUCT NAME

PROPRIETARY: CIPRO® I. V.
NONPROPRIETARY/USAN: Ciprofloxacin for intravenous infusion
CHEMICAL NAME: 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid.
STRUCTURAL FORMULA:



Molecular Formula: $C_{17}H_{18}FN_3O_3$
Molecular Weight: 331.4

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Recommendations	9
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APPEARS THIS WAY
ON ORIGINAL

SUPPORTING DOCUMENTS

NDA 19-537/S-038—Ciprofloxacin Hydrochloride Tablets
NDA 20-780/S-008—Ciprofloxacin Oral Suspension
NDA 19-847/S-024—Ciprofloxacin Injection 1% solution vials
NDA 19-858/S-021—Ciprofloxacin Injection in 0.9% NaCl

INTRODUCTION

This submission contains information that Bayer has accumulated on the use of ciprofloxacin for anthrax (post-exposure prophylaxis). Bayer has relied entirely on published literature for the evidence to support this claim. No human clinical trial data have been included to support this claim. The sponsor has requested the addition of *Bacillus anthracis* to the *in vitro* activity listing in the microbiology subsection of the label. The sponsor has also added information on an animal study in rhesus monkeys exposed to inhaled *Bacillus anthracis* to the label in a section at the end of the label entitled "Other Information".

IN VITRO ACTIVITY AGAINST *BACILLUS ANTHRACIS*

The sponsor has submitted two references that give susceptibility data for ciprofloxacin against *Bacillus anthracis*. A literature search by this reviewer found the same two studies and no other references.

In the first study (1) 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 for 25 antimicrobial drugs. 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 in TABLE 1.

All isolates were sensitive to benzylpenicillin and other penicillins. The MIC values were ≤ 0.03 µg/mL. No isolates showed beta-lactamase activity. Cefazolin and cefoperazone showed good activity. A high rate of resistance was seen against cefuroxime, cefotaxime, ceftizoxime, ceftriaxone, and ceftazidime. All isolates were resistant to aztreonam. Ofloxacin and ciprofloxacin showed good activity with MICs of 0.03 to 0.06 µg/mL.

TABLE 1
Antimicrobial Susceptibility of 22 stains of *Bacillus anthracis* (Reference #1)

Drug	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
Amoxicillin		0.015	0.015
Amoxicillin/clavulanate		0.015	0.015
Piperacillin		0.25	0.5
Mezlocillin		0.06	0.06
Cefazolin		0.015 -	0.015
Cefuroxime		64	64
Cefotaxime		32	32
Ceftriaxone		32	32
Ceftazidime		16	32
Cefoperazone		128	128
Aztreonam		>128	>128
Clindamycin		1	1
TRM/SMZ		3.2/16	3.2/16
Chloramphenicol		2	2
Gentamicin		0.06	0.125
Streptomycin		2	4
Amikacin		0.03	0.06
Netilmicin		0.06	0.125
Tobramycin		0.25	1
Vancomycin		1	1
Ofloxacin		0.06	0.06
Ciprofloxacin		**0.03**	**0.06**

In the other reference (2) seventy strains were tested in the United Kingdom. These strains were isolated from diverse areas over a long period of time. Agar dilution susceptibility was tested to nine drugs. Mueller-Hinton agar was used and plates were incubated overnight at 37°C. The average inoculum size was 4.2×10^4 organisms/mL. Two strains were found to be resistant to penicillin and produced a beta-lactamase constitutively. Three other strains could be induced to produce beta-lactamase after growth in subinhibitory concentrations of flucloxacillin. Sixty-nine strains were resistant to cefuroxime (MIC 8-64 µg/mL) and two isolates showed intermediate resistance to streptomycin. All 70 isolates were sensitive to gentamicin, erythromycin, chloramphenicol, and tetracycline. Ciprofloxacin MICs were 0.03 to 0.06 µg/mL. Results are shown in TABLE 2.

TABLE 2
Antimicrobial Susceptibility of 70 stains of *Bacillus anthracis* (Reference #2)

Drug	Range (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Penicillin		0.06	0.125
Amoxicillin		0.06	0.125
Cefuroxime		32	64
Gentamicin		0.125	0.25
Streptomycin		1.0	1.0
Erythromycin		0.5	1.0
Tetracycline		0.125	0.125
Chloramphenicol		4.0	4.0
Ciprofloxacin		**0.06**	**0.06**

Most strains were sensitive to penicillin and amoxicillin with MIC of 0.03 µg/mL or less. Two strains were found to be resistant with MICs >0.25 µg/mL. The two strains, however, No. 32 and No. 70 originated from the same fatal case.

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.

Following the induction of beta-lactamase, the MICs were repeated for penicillin and amoxicillin using a larger inoculum of 1.3×10^7 organisms/mL. The MICs increased slightly for both antibiotics probably due to the larger inoculum size. Only strain 32 was highly resistant to both penicillin and amoxicillin while strain 70, which had previously been found to be resistant to penicillin had a MIC of 0.03 µg/mL to both drugs. Following induction strain 32 was still resistant and strains 52 and 76 now also had MICs of >64 µg/mL. All three strains were positive for beta-lactamase activity. Before induction, strains 52 and 76 had MICs of 0.06 and 0.03 µg/mL to penicillin and amoxicillin, respectively. Strain 23 had a MIC of 0.25 µg/mL to both drugs prior to induction and a MIC of 8.0 µg/mL following induction. Strain 59A had a MIC of 0.015 µg/mL to penicillin and 0.03 µg/mL to amoxicillin prior to induction and a MIC of 32.0 µg/mL and 16.0 µg/mL after induction. Both strains 23 and 59A were negative for beta-lactamase production. Strain 67 was once again positive for beta-lactamase production both before and after induction but was sensitive on MIC testing. This information is summarized in TABLE 3.

TABLE 3
MIC (μg/mL) following induction of beta-lactamase

Strain No.	23	32	52	59A	67	70	76
MIC to penicillin prior to induction	0.25	64	0.06	0.015	0.06	0.03	0.06
MIC to penicillin following induction	8.0	64	64	32.0	0.125	0.03	64
MIC to amoxicillin prior to induction	0.25	64	0.03	0.03	0.06	0.03	0.03
MIC to amoxicillin following induction	8.0	64	64	16.0	0.06	0.03	64
Beta-lactamase activity prior to induction	-ve	+ve	-ve	-ve	+ve	-ve	-ve
Beta-lactamase activity following induction	-ve	+ve	+ve	-ve	+ve	-ve	+ve

STUDIES IN ANIMAL MODELS

In a murine model, mice were injected subcutaneously with 10-1000 LD_{50} spores of *Bacillus anthracis* (3). The animals were treated orally with ciprofloxacin, pefloxacin, or lomefloxacin. Prophylaxis with ciprofloxacin protected 90-100% of mice against 10 LD_{50} after dosing with 2 to 3 milligrams and 50-90% of mice were protected against 1000 LD_{50}. Treatment with pefloxacin and lomefloxacin was comparable to that with ciprofloxacin.

A Rhesus monkey model was evaluated at [redacted] After exposure to an inhaled dose of 8 LD_{50} spores of *Bacillus anthracis*, groups of 10 Rhesus monkeys were treated with penicillin, doxycycline, ciprofloxacin, doxycycline plus vaccine, vaccine alone, or saline. The MIC of ciprofloxacin for the *Bacillus anthracis* strain used was 0.08 μg/mL. The ciprofloxacin group was treated with 125 mg orally by orogastric tube every 12 hours beginning one day after exposure and continuing for 30 days. Controls were given saline intramuscularly every 12 hours beginning one day after exposure. Procaine penicillin G was given intramuscularly every 12 hours beginning one day after exposure. Doxycycline was given at a 30 mg dose every 12 hours. Vaccine was given on days 1 and 15 after exposure. At one hour post dose, the peak ciprofloxacin serum concentrations were 0.98 to 1.69 μg/mL, and trough concentrations were 0.12 to 0.19 μg/mL at 12 hours post dose.

Nine of the ten control animals exposed to an inhaled dose of anthrax died 3 to 8 days after challenge. The survivor appears to not have become infected, since blood cultures were negative and an antibody response never developed. Eight of ten animals treated with vaccination alone died. The time of death and clinical and autopsy findings did not differ from untreated controls. None of the antibiotic treated animals died during the 30 days of treatment. TABLE 4 shows the survival of various groups.

TABLE 4
Survival after postexposure treatment of inhalation anthrax

Treatment	Anthrax Deaths
Control Untreated	9/10
Vaccine Alone	8/10
Penicillin	3/10
Ciprofloxacin	1/9*
Doxycycline	1/10
Doxycycline + vaccine	0/9**

* One animal died 5 days after exposure from aspiration pneumonia, had no evidence of anthrax at autopsy, and was excluded from analysis.
** One animal died 5 days after discontinuing doxycycline with no evidence of anthrax on autopsy.

In the penicillin group, three of ten animals died of anthrax on days 9, 12, and 20 after penicillin was stopped.

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In the group treated with doxycycline alone, all animals survived during treatment and had negative blood cultures. One monkey died from anthrax 28 days after treatment was stopped.

None of the animals treated with doxycycline plus postexposure vaccine died of anthrax. One animal in this group died 6 days after discontinuance of doxycycline but had no evidence of anthrax on autopsy.

Animals that survived the aerosol challenge were examined for evidence of an immune response 131-142 days after exposure by measuring antibody to the protective antigen component of anthrax toxin. No surviving animals treated with antibiotic alone had an immune response. In contrast, the animals given doxycycline plus vaccine all developed a fourfold or greater rise in antibody. The two surviving animals given vaccine alone also developed an antibody response.

When rechallenged with a 50 LD_{50} spore suspension of anthrax only the group treated with doxycycline plus vaccine had significant number of survivors. In this group 9 of 9 animals remained free of disease one year after rechallenge. No significant protection was afforded by antibiotic treatment alone. In the penicillin group 0/7 survived. In the ciprofloxacin group only 1/7 survived and in the doxycycline group 0/9 survived.

The results of this experiment suggest that therapy for an unimmunized person exposed to an aerosol of anthrax spores should consist of long-term suppressive antibiotics. Vaccination may provide an additional degree of protection against relapse after antibiotic treatment and would protect against a subsequent exposure.

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RECOMMENDATIONS

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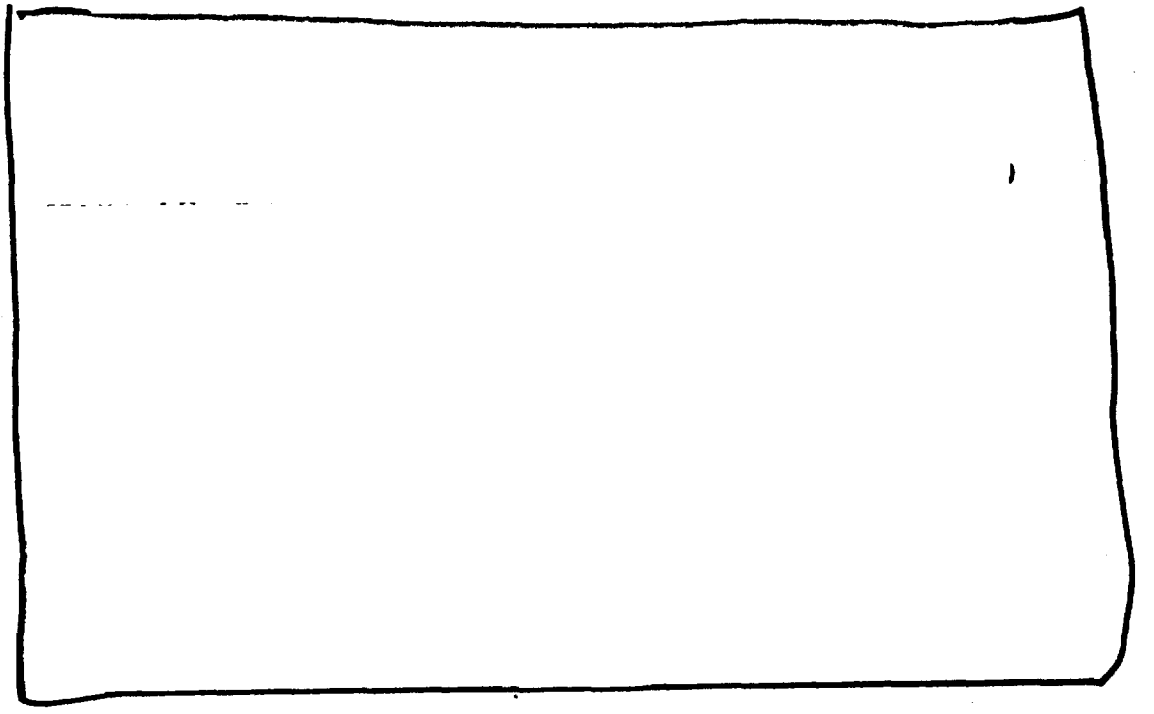

The first two references in the label should be updated to the January 2000 reversions.

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/S/

Peter A. Dionne
Microbiologist HFD-590

CONCURRENCES:

HFD-590/Div Dir /S/ /S/ Signature 4/7/00 Date
HFD-590/TLMicro _____ Signature 3/23/2000 Date

CC:
HFD-590/Original NDA # 19-857/S-027
HFD-590/Division File
HFD-590/Micro/PDionne
HFD-590/MO/AMeyerhoff
HFD-520/Pharm/SHundley
HFD-590/Chem/DMatecka
HFD-590/CSO/VJensen

MICROBIOLOGY REVIEW

DIVISION OF SPECIAL PATHOGENS AND IMMUNOLOGIC DRUG PRODUCTS (HFD-590)

NDA #: 19-858/S-021

REVIEWER:	Peter A. Dionne
CORRESPONDENCE DATE:	29-FEB-00
CDER DATE:	01-MAR-00
REVIEW ASSIGN DATE:	04-MAR-00
REVIEW COMPLETE DATE:	20-MAR-00

SPONSOR: Bayer Pharmaceutical Division
Bayer Corporation
400 Morgan Lane
West Haven, CT 06516-4175

CONTACT PERSON: Andrew S. Verderame
Associate Director Regulatory Affairs
Phone Number: (203) 812-5172

SUBMISSION REVIEWED: Supplement for the addition of Anthrax (post exposure prophylaxis) as an approved indication

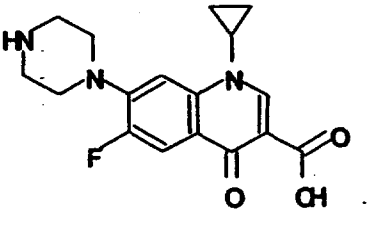
DRUG CATEGORY: Antimicrobial: Fluoroquinolone

INDICATIONS: Acute Sinusitis, Lower Respiratory Tract Infections, UTI, Acute Uncomplicated Cystitis in females, Chronic Bacterial Prostatitis, Complicated Intra-Abdominal Infections, Skin and Skin Structure Infections, Bone and Joint Infections, Infectious Diarrhea, Typhoid Fever, Uncomplicated cervical and urethral gonorrhea; Now Requesting Anthrax (post-exposure prophylaxis)

DOSAGE FORM: IV solution in 0.9% NaCl—2 mg/mL; 100 mL and 200 mL in 0.9% NaCl

DRUG PRODUCT NAME

PROPRIETARY: CIPRO® I. V.
NONPROPRIETARY/USAN: Ciprofloxacin for intravenous infusion
CHEMICAL NAME: 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid.
STRUCTURAL FORMULA:



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Molecular Weight: 331.4

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APPEARS THIS WAY
ON ORIGINAL

SUPPORTING DOCUMENTS

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Ofloxacin		0.06	0.06
Ciprofloxacin		0.03	0.06

In the other reference (2) seventy strains were tested in the United Kingdom. These strains were isolated from diverse areas over a long period of time. Agar dilution susceptibility was tested to nine drugs. Mueller-Hinton agar was used and plates were incubated overnight at 37°C. The average inoculum size was 4.2×10^4 organisms/mL. Two strains were found to be resistant to penicillin and produced a beta-lactamase constitutively. Three other strains could be induced to produce beta-lactamase after growth in subinhibitory concentrations of flucloxacillin. Sixty-nine strains were resistant to cefuroxime (MIC 8-64 µg/mL) and two isolates showed intermediate resistance to streptomycin. All 70 isolates were sensitive to gentamicin, erythromycin, chloramphenicol, and tetracycline. Ciprofloxacin MICs were 0.03 to 0.06 µg/mL. Results are shown in TABLE 2.

TABLE 2
Antimicrobial Susceptibility of 70 stains of *Bacillus anthracis* (Reference #2)

Drug	Range (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Penicillin		0.06	0.125
Amoxicillin		0.06	0.125
Cefuroxime		32	64
Gentamicin		0.125	0.25
Streptomycin		1.0	1.0
Erythromycin		0.5	1.0
Tetracycline		0.125	0.125
Chloramphenicol		4.0	4.0
Ciprofloxacin		0.06	0.06

Most strains were sensitive to penicillin and amoxicillin with MIC of 0.03 µg/mL or less. Two strains were found to be resistant with MICs >0.25 µg/mL. The two strains, however, No. 32 and No. 70 originated from the same fatal case.

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.

Following the induction of beta-lactamase, the MICs were repeated for penicillin and amoxicillin using a larger inoculum of 1.3×10^7 organisms/mL. The MICs increased slightly for both antibiotics probably due to the larger inoculum size. Only strain 32 was highly resistant to both penicillin and amoxicillin while strain 70, which had previously been found to be resistant to penicillin had a MIC of 0.03 µg/mL to both drugs. Following induction strain 32 was still resistant and strains 52 and 76 now also had MICs of >64 µg/mL. All three strains were positive for beta-lactamase activity. Before induction, strains 52 and 76 had MICs of 0.06 and 0.03 µg/mL to penicillin and amoxicillin, respectively. Strain 23 had a MIC of 0.25 µg/mL to both drugs prior to induction and a MIC of 8.0 µg/mL following induction. Strain 59A had a MIC of 0.015 µg/mL to penicillin and 0.03 µg/mL to amoxicillin prior to induction and a MIC of 32.0 µg/mL and 16.0 µg/mL after induction. Both strains 23 and 59A were negative for beta-lactamase production. Strain 67 was once again positive for beta-lactamase production both before and after induction but was sensitive on MIC testing. This information is summarized in TABLE 3.

TABLE 3
MIC (μg/mL) following induction of beta-lactamase

Strain No.	23	32	52	59A	67	70	76
MIC to penicillin prior to induction	0.25	64	0.06	0.015	0.06	0.03	0.06
MIC to penicillin following induction	8.0	64	64	32.0	0.125	0.03	64
MIC to amoxicillin prior to induction	0.25	64	0.03	0.03	0.06	0.03	0.03
MIC to amoxicillin following induction	8.0	64	64	16.0	0.06	0.03	64
Beta-lactamase activity prior to induction	-ve	+ve	-ve	-ve	+ve	-ve	-ve
Beta-lactamase activity following induction	-ve	+ve	+ve	-ve	+ve	-ve	+ve

STUDIES IN ANIMAL MODELS

In a murine model, mice were injected subcutaneously with 10-1000 LD_{50} spores of *Bacillus anthracis* (3). The animals were treated orally with ciprofloxacin, pefloxacin, or lomefloxacin. Prophylaxis with ciprofloxacin protected 90-100% of mice against 10 LD_{50} after dosing with 2 to 3 milligrams and 50-90% of mice were protected against 1000 LD_{50}. Treatment with pefloxacin and lomefloxacin was comparable to that with ciprofloxacin.

A Rhesus monkey model was evaluated at [redacted] After exposure to an inhaled dose of 8 LD_{50} spores of *Bacillus anthracis*, groups of 10 Rhesus monkeys were treated with penicillin, doxycycline, ciprofloxacin, doxycycline plus vaccine, vaccine alone, or saline. The MIC of ciprofloxacin for the *Bacillus anthracis* strain used was 0.08 μg/mL. The ciprofloxacin group was treated with 125 mg orally by orogastric tube every 12 hours beginning one day after exposure and continuing for 30 days. Controls were given saline intramuscularly every 12 hours beginning one day after exposure. Procaine penicillin G was given intramuscularly every 12 hours beginning one day after exposure. Doxycycline was given at a 30 mg dose every 12 hours. Vaccine was given on days 1 and 15 after exposure. At one hour post dose, the peak ciprofloxacin serum concentrations were 0.98 to 1.69 μg/mL, and trough concentrations were 0.12 to 0.19 μg/mL at 12 hours post dose.

Nine of the ten control animals exposed to an inhaled dose of anthrax died 3 to 8 days after challenge. The survivor appears to not have become infected, since blood cultures were negative and an antibody response never developed. Eight of ten animals treated with vaccination alone died. The time of death and clinical and autopsy findings did not differ from untreated controls. None of the antibiotic treated animals died during the 30 days of treatment. TABLE 4 shows the survival of various groups.

TABLE 4
Survival after postexposure treatment of inhalation anthrax

Treatment	Anthrax Deaths
Control Untreated	9/10
Vaccine Alone	8/10
Penicillin	3/10
Ciprofloxacin	1/9*
Doxycycline	1/10
Doxycycline + vaccine	0/9**

* One animal died 5 days after exposure from aspiration pneumonia, had no evidence of anthrax at autopsy, and was excluded from analysis.
** One animal died 5 days after discontinuing doxycycline with no evidence of anthrax on autopsy.

In the penicillin group, three of ten animals died of anthrax on days 9, 12, and 20 after penicillin was stopped.

One animal given ciprofloxacin died 5 days after exposure from aspiration pneumonia 24 hours after the inadvertent introduction of drug into the trachea. All other ciprofloxacin treated monkeys survived the 30 days of treatment with negative blood cultures. One animal died of anthrax 6 days after ciprofloxacin treatment was stopped. Another animal in the ciprofloxacin group died 73 days after antibiotic was discontinued. Autopsy revealed no evidence of anthrax. This animal died of urethral obstruction due to rubbery plugs in the urethra and bladder.

In the group treated with doxycycline alone, all animals survived during treatment and had negative blood cultures. One monkey died from anthrax 28 days after treatment was stopped.

None of the animals treated with doxycycline plus postexposure vaccine died of anthrax. One animal in this group died 6 days after discontinuance of doxycycline but had no evidence of anthrax on autopsy.

Animals that survived the aerosol challenge were examined for evidence of an immune response 131-142 days after exposure by measuring antibody to the protective antigen component of anthrax toxin. No surviving animals treated with antibiotic alone had an immune response. In contrast, the animals given doxycycline plus vaccine all developed a fourfold or greater rise in antibody. The two surviving animals given vaccine alone also developed an antibody response.

When rechallenged with a 50 LD_{50} spore suspension of anthrax only the group treated with doxycycline plus vaccine had significant number of survivors. In this group 9 of 9 animals remained free of disease one year after rechallenge. No significant protection was afforded by antibiotic treatment alone. In the penicillin group 0/7 survived. In the ciprofloxacin group only 1/7 survived and in the doxycycline group 0/9 survived.

The results of this experiment suggest that therapy for an unimmunized person exposed to an aerosol of anthrax spores should consist of long-term suppressive antibiotics. Vaccination may provide an additional degree of protection against relapse after antibiotic treatment and would protect against a subsequent exposure.

CONCLUSIONS

In this submission Bayer is requesting approval of an indication of Anthrax (post exposure prophylaxis) for ciprofloxacin. Bayer has submitted data from a study of inhalation anthrax in Rhesus monkeys and from published literature as evidence to support this claim.

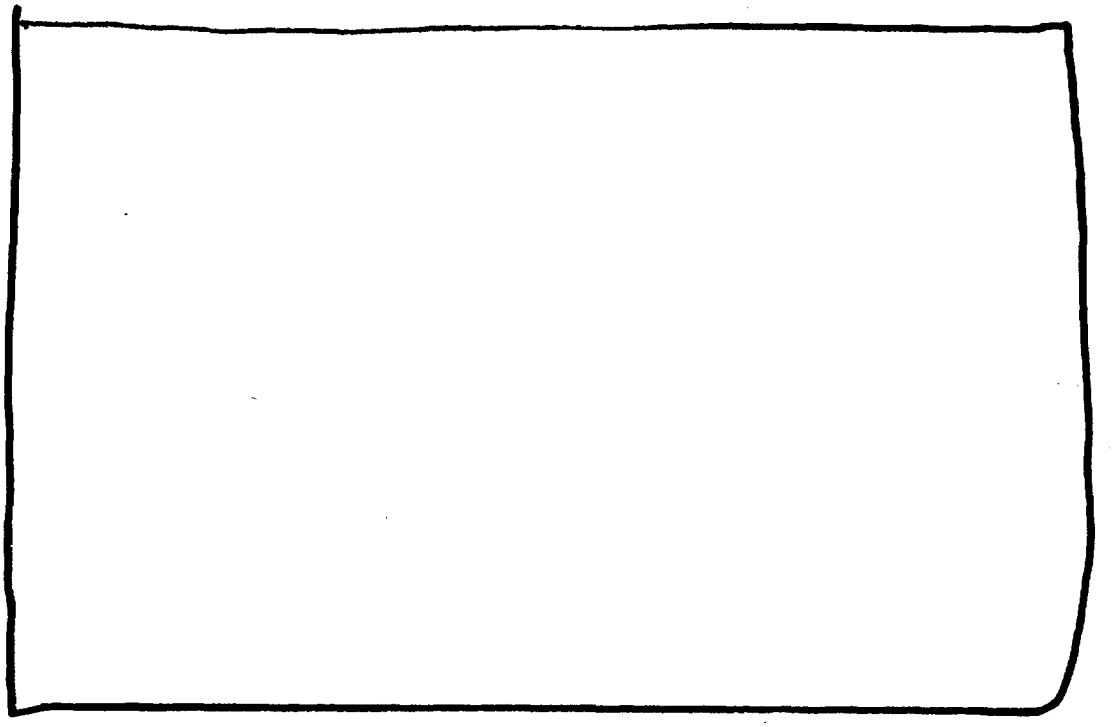
In this submission Bayer is requesting approval of an indication of Anthrax (post exposure prophylaxis) for ciprofloxacin. No clinical studies have been performed and Bayer has relied entirely on published literature for the evidence to support this claim.

The sponsor is requesting the addition of *Bacillus anthracis* to list #2 (*in vitro* activity only) in the Microbiology subsection of the label. Two studies on the *in vitro* susceptibility of *Bacillus anthracis* to ciprofloxacin have been submitted to support the addition of *Bacillus anthracis* to this list. A total of more than 90 isolates of *Bacillus anthracis* have been tested and the MIC_{90} value in both studies was 0.06 µg/mL, which is well below ciprofloxacin's susceptible breakpoint of 1 µg/mL. Both studies were performed over 5 years ago, but no newer data are available. Also no United States studies are available. There is no reason to believe that the susceptibility pattern for this species has changed recently or that testing of United States strains would give different results. From the microbiological standpoint the addition of *Bacillus anthracis* to the label seems justified.

Testing in an animal model also seems to justify the addition of *Bacillus anthracis* to the label. The sponsor has included information about a study in rhesus monkeys exposed to an inhaled dose of 8 LD_{50} spores of *Bacillus anthracis*. The statement correctly states that none of the nine ciprofloxacin treated animals died from anthrax within the 30 day treatment period. The information, however, does not state that one animal died of anthrax six days after treatment stopped. This section should be revised to include the dosage regime that was used for ciprofloxacin to give the stated serum concentrations and to include the fact that one of the nine animals died from anthrax after treatment.

RECOMMENDATIONS

From the microbiological viewpoint this supplement should be approved. The section at the end of the label entitled "Other Information" should be revised to read as follows:



The first two references in the label should be updated to the January 2000 reversions.

1. National Committee for Clinical Laboratory Standards, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Fifth Edition. Approved Standard NCCLS Document M7-A5, Vol. 20, No. 2, NCCLS, Wayne, PA, January 2000.
2. National Committee for Clinical Laboratory Standards, Performance Standards for Antimicrobial Disk Susceptibility Tests—Seventh Edition. Approved Standard NCCLS Document M2-A7, Vol. 20, No. 1, NCCLS, Wayne, PA, January 2000.

REFERENCES

1. Doganay M, Aydin N. Antimicrobial susceptibility of *Bacillus anthracis*. Scand J Infect Dis, 23:333=335, 1991.

2. Lightfoot NF, Scott RJD, Turnbull PCB. Antimicrobial susceptibility of *Bacillus anthracis*. Salisbury Med Bull 68, Suppl: 95-98, 1990.

3. Dyakov SI, Katsalukha VV, Lebedeva IK, et al. Comparative estimation of fluoroquinolone efficacy in experimental anthrax. Antibiot Chimioter 39:15-19, 1994.

4. Friedlander AM, Welkos SL, Pitt MLM, et al. Postexposure prophylaxis against experimental inhalation anthrax. J Infect Dis 167:1239-1242, 1993.

/S/
Peter A. Dionne
Microbiologist HFD-590

CONCURRENCES:

HFD-590/Div Dir /S/ Signature 4/7/00 Date
HFD-590/TLMicro /S/ Signature 3/23/2000 Date

CC:
HFD-590/Original NDA # 19-858/S-021
HFD-590/Division File
HFD-590/Micro/PDionne
HFD-590/MO/AMeyerhoff
HFD-520/Pharm/SHundley
HFD-590/Chem/DMatecka
HFD-590/CSO/VJensen

MICROBIOLOGY REVIEW

DIVISION OF SPECIAL PATHOGENS AND IMMUNOLOGIC DRUG PRODUCTS (HFD-590)

NDA #: 20-780/S-008

REVIEWER:	Peter A. Dionne
CORRESPONDENCE DATE:	29-FEB-00
CDER DATE:	01-MAR-00
REVIEW ASSIGN DATE:	04-MAR-00
REVIEW COMPLETE DATE:	20-MAR-00

SPONSOR: Bayer Pharmaceutical Division
Bayer Corporation
400 Morgan Lane
West Haven, CT 06516-4175

CONTACT PERSON: Andrew S. Verderame
Associate Director Regulatory Affairs
Phone Number: (203) 812-5172

SUBMISSION REVIEWED: Supplement for the addition of Anthrax (post exposure prophylaxis) as an approved indication

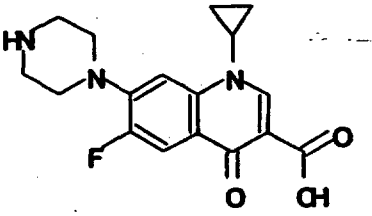
DRUG CATEGORY: Antimicrobial: Fluoroquinolone

INDICATIONS: Acute Sinusitis, Lower Respiratory Tract Infections, UTI, Acute Uncomplicated Cystitis in females, Chronic Bacterial Prostatitis, Complicated Intra-Abdominal Infections, Skin and Skin Structure Infections, Bone and Joint Infections, Infectious Diarrhea, Typhoid Fever, Uncomplicated cervical and urethral gonorrhea; Now Requesting Anthrax (post-exposure prophylaxis)

DOSAGE FORM: Oral Suspension-250 mg/5 mL and 500 mg/5 mL

DRUG PRODUCT NAME

PROPRIETARY: CIPRO® Oral Suspension
NONPROPRIETARY/USAN: Ciprofloxacin oral suspension
CHEMICAL NAME: 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid.
STRUCTURAL FORMULA:



Molecular Formula: $C_{17}H_{18}FN_3O_3$
Molecular Weight: 331.4

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APPEARS THIS WAY
ON ORIGINAL

SUPPORTING DOCUMENTS

NDA 19-537/S-038—Ciprofloxacin Hydrochloride Tablets
NDA 19-847/S-024—Ciprofloxacin Injection 1% solution vials
NDA 19-857/S-027—Ciprofloxacin Injection in 5% Dextrose
NDA 19-858/S-021—Ciprofloxacin Injection in 0.9% NaCl

INTRODUCTION

This submission contains information that Bayer has accumulated on the use of ciprofloxacin for anthrax (post-exposure prophylaxis). Bayer has relied entirely on published literature for the evidence to support this claim. No human clinical trial data have been included to support this claim. The sponsor has requested the addition of *Bacillus anthracis* to the *in vitro* activity listing in the microbiology subsection of the label. The sponsor has also added information on an animal study in rhesus monkeys exposed to inhaled *Bacillus anthracis* to the label in a section at the end of the label entitled "Other Information".

IN VITRO ACTIVITY AGAINST *BACILLUS ANTHRACIS*

The sponsor has submitted two references that give susceptibility data for ciprofloxacin against *Bacillus anthracis*. A literature search by this reviewer found the same two studies and no other references.

In the first study (1) 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 for 25 antimicrobial drugs. 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 in TABLE 1.

All isolates were sensitive to benzylpenicillin and other penicillins. The MIC values were ≤ 0.03 µg/mL. No isolates showed beta-lactamase activity. Cefazolin and cefoperazone showed good activity. A high rate of resistance was seen against cefuroxime, cefotaxime, ceftizoxime, ceftriaxone, and ceftazidime. All isolates were resistant to aztreonam. Ofloxacin and ciprofloxacin showed good activity with MICs of 0.03 to 0.06 µg/mL.

TABLE 1
Antimicrobial Susceptibility of 22 stains of *Bacillus anthracis* (Reference #1)

Drug	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
Amoxicillin		0.015	0.015
Amoxicillin/clavulanate		0.015	0.015
Piperacillin		0.25	0.5
Mezlocillin		0.06	0.06
Cefazolin		0.015	0.015
Cefuroxime		64	64
Cefotaxime		32	32
Ceftriaxone		32	32
Ceftazidime		16	32
Cefoperazone		128	128
Aztreonam		>128	>128
Clindamycin		1	1
TRM/SMZ		3.2/16	3.2/16
Chloramphenicol		2	2
Gentamicin		0.06	0.125
Streptomycin		2	4
Amikacin		0.03	0.06
Netilmicin		0.06	0.125
Tobramycin		0.25	1
Vancomycin		1	1
Ofloxacin		0.06	0.06
Ciprofloxacin		**0.03**	**0.06**

In the other reference (2) seventy strains were tested in the United Kingdom. These strains were isolated from diverse areas over a long period of time. Agar dilution susceptibility was tested to nine drugs. Mueller-Hinton agar was used and plates were incubated overnight at 37°C. The average inoculum size was 4.2×10^4 organisms/mL. Two strains were found to be resistant to penicillin and produced a beta-lactamase constitutively. Three other strains could be induced to produce beta-lactamase after growth in subinhibitory concentrations of flucloxacillin. Sixty-nine strains were resistant to cefuroxime (MIC 8-64 µg/mL) and two isolates showed intermediate resistance to streptomycin. All 70 isolates were sensitive to gentamicin, erythromycin, chloramphenicol, and tetracycline. Ciprofloxacin MICs were 0.03 to 0.06 µg/mL. Results are shown in TABLE 2.

TABLE 2
Antimicrobial Susceptibility of 70 stains of *Bacillus anthracis* (Reference #2)

Drug	Range (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Penicillin		0.06	0.125
Amoxicillin		0.06	0.125
Cefuroxime		32	64
Gentamicin		0.125	0.25
Streptomycin		1.0	1.0
Erythromycin		0.5	1.0
Tetracycline		0.125	0.125
Chloramphenicol		4.0	4.0
Ciprofloxacin		**0.06**	**0.06**

Most strains were sensitive to penicillin and amoxicillin with MIC of 0.03 µg/mL or less. Two strains were found to be resistant with MICs >0.25 µg/mL. The two strains, however, No. 32 and No. 70 originated from the same fatal case.

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.

Following the induction of beta-lactamase, the MICs were repeated for penicillin and amoxicillin using a larger inoculum of 1.3×10^7 organisms/mL. The MICs increased slightly for both antibiotics probably due to the larger inoculum size. Only strain 32 was highly resistant to both penicillin and amoxicillin while strain 70, which had previously been found to be resistant to penicillin had a MIC of 0.03 µg/mL to both drugs. Following induction strain 32 was still resistant and strains 52 and 76 now also had MICs of >64 µg/mL. All three strains were positive for beta-lactamase activity. Before induction, strains 52 and 76 had MICs of 0.06 and 0.03 µg/mL to penicillin and amoxicillin, respectively. Strain 23 had a MIC of 0.25 µg/mL to both drugs prior to induction and a MIC of 8.0 µg/mL following induction. Strain 59A had a MIC of 0.015 µg/mL to penicillin and 0.03 µg/mL to amoxicillin prior to induction and a MIC of 32.0 µg/mL and 16.0 µg/mL after induction. Both strains 23 and 59A were negative for beta-lactamase production. Strain 67 was once again positive for beta-lactamase production both before and after induction but was sensitive on MIC testing. This information is summarized in TABLE 3.

TABLE 3
MIC (μg/mL) following induction of beta-lactamase

Strain No.	23	32	52	59A	67	70	76
MIC to penicillin prior to induction	0.25	64	0.06	0.015	0.06	0.03	0.06
MIC to penicillin following induction	8.0	64	64	32.0	0.125	0.03	64
MIC to amoxicillin prior to induction	0.25	64	0.03	0.03	0.06	0.03	0.03
MIC to amoxicillin following induction	8.0	64	64	16.0	0.06	0.03	64
Beta-lactamase activity prior to induction	-ve	+ve	-ve	-ve	+ve	-ve	-ve
Beta-lactamase activity following induction	-ve	+ve	+ve	-ve	+ve	-ve	+ve

STUDIES IN ANIMAL MODELS

In a murine model, mice were injected subcutaneously with 10-1000 LD_{50} spores of *Bacillus anthracis* (3). The animals were treated orally with ciprofloxacin, pefloxacin, or lomefloxacin. Prophylaxis with ciprofloxacin protected 90-100% of mice against 10 LD_{50} after dosing with 2 to 3 milligrams and 50-90% of mice were protected against 1000 LD_{50}. Treatment with pefloxacin and lomefloxacin was comparable to that with ciprofloxacin.

A Rhesus monkey model was evaluated at [redacted]. After exposure to an inhaled dose of 8 LD_{50} spores of *Bacillus anthracis*, groups of 10 Rhesus monkeys were treated with penicillin, doxycycline, ciprofloxacin, doxycycline plus vaccine, vaccine alone, or saline. The MIC of ciprofloxacin for the *Bacillus anthracis* strain used was 0.08 μg/mL. The ciprofloxacin group was treated with 125 mg orally by orogastric tube every 12 hours beginning one day after exposure and continuing for 30 days. Controls were given saline intramuscularly every 12 hours beginning one day after exposure. Procaine penicillin G was given intramuscularly every 12 hours beginning one day after exposure. Doxycycline was given at a 30 mg dose every 12 hours. Vaccine was given on days 1 and 15 after exposure. At one hour post dose, the peak ciprofloxacin serum concentrations were 0.98 to 1.69 μg/mL, and trough concentrations were 0.12 to 0.19 μg/mL at 12 hours post dose.

Nine of the ten control animals exposed to an inhaled dose of anthrax died 3 to 8 days after challenge. The survivor appears to not have become infected, since blood cultures were negative and an antibody response never developed. Eight of ten animals treated with vaccination alone died. The time of death and clinical and autopsy findings did not differ from untreated controls. None of the antibiotic treated animals died during the 30 days of treatment. TABLE 4 shows the survival of various groups.

TABLE 4
Survival after postexposure treatment of inhalation anthrax

Treatment	Anthrax Deaths
Control Untreated	9/10
Vaccine Alone	8/10
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Doxycycline + vaccine	0/9**

* One animal died 5 days after exposure from aspiration pneumonia, had no evidence of anthrax at autopsy, and was excluded from analysis.
** One animal died 5 days after discontinuing doxycycline with no evidence of anthrax on autopsy.

In the penicillin group, three of ten animals died of anthrax on days 9, 12, and 20 after penicillin was stopped.

One animal given ciprofloxacin died 5 days after exposure from aspiration pneumonia 24 hours after the inadvertent introduction of drug into the trachea. All other ciprofloxacin treated monkeys survived the 30 days of treatment with negative blood cultures. One animal died of anthrax 6 days after ciprofloxacin treatment was stopped. Another animal in the ciprofloxacin group died 73 days after antibiotic was discontinued. Autopsy revealed no evidence of anthrax. This animal died of urethral obstruction due to rubbery plugs in the urethra and bladder.

In the group treated with doxycycline alone, all animals survived during treatment and had negative blood cultures. One monkey died from anthrax 28 days after treatment was stopped.

None of the animals treated with doxycycline plus postexposure vaccine died of anthrax. One animal in this group died 6 days after discontinuance of doxycycline but had no evidence of anthrax on autopsy.

Animals that survived the aerosol challenge were examined for evidence of an immune response 131-142 days after exposure by measuring antibody to the protective antigen component of anthrax toxin. No surviving animals treated with antibiotic alone had an immune response. In contrast, the animals given doxycycline plus vaccine all developed a fourfold or greater rise in antibody. The two surviving animals given vaccine alone also developed an antibody response.

When rechallenged with a 50 LD_{50} spore suspension of anthrax only the group treated with doxycycline plus vaccine had significant number of survivors. In this group 9 of 9 animals remained free of disease one year after rechallenge. No significant protection was afforded by antibiotic treatment alone. In the penicillin group 0/7 survived. In the ciprofloxacin group only 1/7 survived and in the doxycycline group 0/9 survived.

The results of this experiment suggest that therapy for an unimmunized person exposed to an aerosol of anthrax spores should consist of long-term suppressive antibiotics. Vaccination may provide an additional degree of protection against relapse after antibiotic treatment and would protect against a subsequent exposure.

CONCLUSIONS

In this submission Bayer is requesting approval of an indication of Anthrax (post exposure prophylaxis) for ciprofloxacin. Bayer has submitted data from a study of inhalation anthrax in Rhesus monkeys and from published literature as evidence to support this claim.

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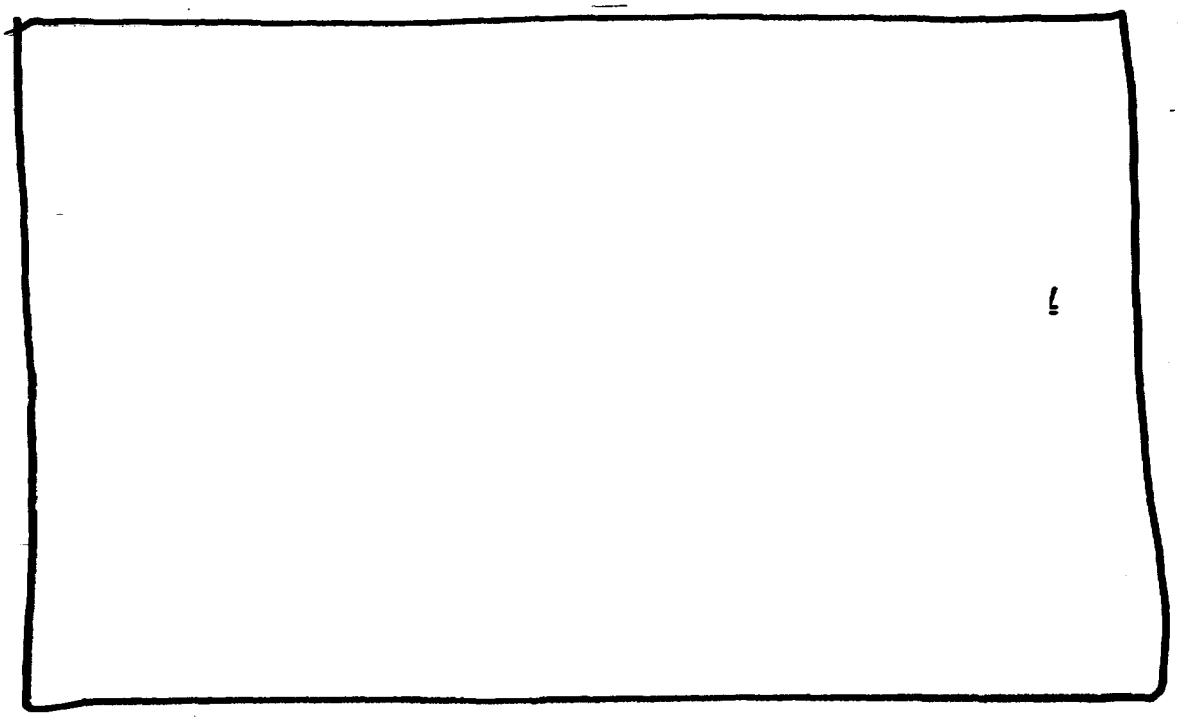
Testing in an animal model also seems to justify the addition of *Bacillus anthracis* to the label. The sponsor has included information about a study in rhesus monkeys exposed to an inhaled dose of 8 LD_{50} spores of *Bacillus anthracis*. The statement correctly states that none of the nine ciprofloxacin treated animals died from anthrax within the 30 day treatment period. The information, however, does not state that one animal died of anthrax six days after treatment stopped. This section should be revised to include the dosage regime that was used for ciprofloxacin to give the stated serum concentrations and to include the fact that one of the nine animals died from anthrax after treatment.

RECOMMENDATIONS

From the microbiological viewpoint this supplement should be approved. The section at the end of the label entitled "Other Information" should be revised to read as follows:



The first two references in the label should be updated to the January 2000 reversions.

1. National Committee for Clinical Laboratory Standards, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Fifth Edition. Approved Standard NCCLS Document M7-A5, Vol. 20, No. 2, NCCLS, Wayne, PA, January 2000.
2. National Committee for Clinical Laboratory Standards, Performance Standards for Antimicrobial Disk Susceptibility Tests—Seventh Edition. Approved Standard NCCLS Document M2-A7, Vol. 20, No. 1, NCCLS, Wayne, PA, January 2000.

REFERENCES

1. Doganay M, Aydin N. Antimicrobial susceptibility of *Bacillus anthracis*. Scand J Infect Dis, 23:333=335, 1991.

2. Lightfoot NF, Scott RJD, Turnbull PCB. Antimicrobial susceptibility of *Bacillus anthracis*. Salisbury Med Bull 68, Suppl: 95-98, 1990.

3. Dyakov SI, Katsalukha VV, Lebedeva IK, et al. Comparative estimation of fluoroquinolone efficacy in experimental anthrax. Antibiot Chimioter 39:15-19, 1994.

4. Friedlander AM, Welkos SL, Pitt MLM, et al. Postexposure prophylaxis against experimental inhalation anthrax. J Infect Dis 167:1239-1242, 1993.

/S/

Peter A. Dionne
Microbiologist HFD-590

CONCURRENCES:

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HFD-590/Div Dir /S/ Signature 4/7/00 Date
HFD-590/TLMicro Signature 3/23/2000 Date

CC:
HFD-590/Original NDA # 20-780/S-008
HFD-590/Division File
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HFD-590/CSO/VJensen

MICROBIOLOGY REVIEW

DIVISION OF SPECIAL PATHOGENS AND IMMUNOLOGIC DRUG PRODUCTS
(HFD-590)

Addendum to Microbiology Review
Review of Blood Cultures from Friedlander Study

NDAs #: 19-537/S-038	**REVIEWER:**	Peter A. Dionne
19-847/S-024	**CORRESPONDENCE DATE:**	29-FEB-00
19-837/S-027	**CDER DATE:**	01-MAR-00
19-838/S-021	**REVIEW ASSIGN DATE:**	04-MAR-00
20-780/S-008	**REVIEW COMPLETE DATE:**	24-AUG-00

SPONSOR: Bayer Pharmaceutical Division
Bayer Corporation
400 Morgan Lane
West Haven, CT 06516-4175

CONTACT PERSON: Andrew S. Verderame
Associate Director Regulatory Affairs
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SUBMISSION REVIEWED: Supplement for the addition of Anthrax (post exposure prophylaxis) as an approved indication

DRUG CATEGORY: Antimicrobial: Fluoroquinolone

INDICATIONS: Acute Sinusitis, Lower Respiratory Tract Infections, UTI, Acute Uncomplicated Cystitis in females, Chronic Bacterial Prostatitis, Complicated Intra-Abdominal Infections, Skin and Skin Structure Infections, Bone and Joint Infections, Infectious Diarrhea, Typhoid Fever, Uncomplicated cervical and urethral gonorrhea; Now Requesting Anthrax (post-exposure prophylaxis)

DOSAGE FORM: IV solution in 0.9% NaCl—2 mg/mL; 100 mL and 200 mL in 0.9% NaCl

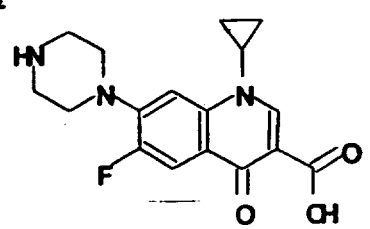
DRUG PRODUCT NAME

PROPRIETARY: CIPRO® I. V. CIPRO® Oral

NONPROPRIETARY/USAN: Ciprofloxacin for intravenous infusion and oral

CHEMICAL NAME: 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid.

STRUCTURAL FORMULA:



Molecular Formula: $C_{17}H_{18}FN_3O_3$ **Molecular Weight:** 331.4

BACKGROUND:

In a study performed by Friedlander et al., *Bacillus anthracis* Vollum 1B spores were prepared and Rhesus monkeys were exposed in a head-only chamber to an aerosol generated with a nebulizer. The median diameter of the particles generated was 1.2 μm. Animals were exposed to an inhaled dose of around 5.5×10^5 spores (~11 LD_{50}). Animals were randomly distributed into six groups of ten animals. One group was administered saline and used as the control group. Another group received human anthrax vaccine. One group received penicillin, another ciprofloxacin, and a third group was given doxycycline. The final group received doxycycline and vaccine. All groups were dosed for 30 days. Survivors from the first experiment were rechallenged with an inhaled dose of about 30 LD_{50} on Day 131 to 142.

Daily blood cultures were obtained from the saline controls and the group of animals given only vaccine, until death or for 14 days. In the antimicrobial treated groups, blood was cultured every other day until 80% of the controls died, and then twice weekly until Day 30, then every other day until about Day 60, and then once a week until rechallenge. Blood from animals not given antimicrobials was cultured in 10-fold dilutions in triplicate on trypticase soy agar. Blood from antimicrobial treated animals was cultured undiluted and at a 1:100 dilution on trypticase soy agar.

The results of blood cultures from this study are presented in TABLE 1.

TABLE 1
Blood Cultures from Friedlander Inhalation Anthrax Study

Group: Control

Monkey #	Survival	Bacteremia	Level (Log cfu/mL)	Day of Death
837T	Dead	neg day 0,1,2	6.514 day 3	
			8.918 day 3—necropsy	3
47G	Dead	neg day 0,1,2	3.05 day 3	
			3.898 day 4	
			8.124 day 5	5
3JH	Dead	neg day 1,2	2.166 day 3	
			1 day 4	
			1.79 day 5	
		neg day 6 and 7	2.301 day 8--terminal	7
D274	Dead	neg day 0,1,2,3,4	3.294 day 5	
			1.753 day 6	
			2.778 day 7	
			3.54 day 8	
			3.6 day 8—terminal	8
128N	Dead	neg day 0,1,2	1.477 day 3	
			8.882 day 4	
			8.185 day 4--terminal	4
981C	Dead	neg day 0,1,2,3	5.185 day 4	
			8.85 day 5—terminal	5
84A44	Dead	neg day 0,1,2,3	1.845 day 4	
			4.084 day 5	
			4.55 day 5--terminal	5
A027	Dead	neg day 0,1,2,3	2.821 day 4	
			1.946 day 5	
			5.817 day 6	6
3LP	Dead	neg day 0,1,2,3,4	4.214 day 5	
			3.322 day 6	
			8.191 day 7--terminal	7
3N1	Survived	neg days 0-19, 24, 27		

TABLE 1 (Continued)
Blood Cultures from Friedlander Inhalation Anthrax Study

Group: Penicillin

Monkey #	Survival	Bacteremia	Level (Log cfu/mL)	Day of Death
85265	Survived	neg day 0,1,3,5,7,9, 11,13,15,17,19,21, 24,27,31,33		
349D	Survived	neg day 0,1,3, 5, 7 9, 11, 14, 18		
18386	Survived	neg day 0,1,3,5,6,7, 9,11,13,15,17,19, 21,24, 27,31,33		
446C	Survived	neg day 0, 1, 3, 5, 7 9, 11, 14, 18		
H-T324	Survived	neg day 0,1,3,5,7,9, 11,14,18, 21,23,25,27 29, 31,33		
B748	Survived	neg day 0,1,3,5,7 9,10,13,17		
927C	Survived	neg day 0,1,3,5,7 9, 10,13,17		
C532	Dead	neg day 0,1,3,5,7,10, 12,15,17,19, 21,24,27 31,33,34, 35, 37	2.322(2.10E02) –day 38 7.985 (9.7E07)-day39—Terminal	39
088CC	Dead	neg day 0,1,3,5,7,9, 11,14,18,21,23,25, 27,29,31,33,34,36,38	Day 40—4.113 (1.40E04) Day 42—5.807 (6.4E05)	42
L62	Dead	neg day 0,1,3,5,7 10, 13,17,20,24,27	>7.000 (>1E06)-day 50 8.204 (1.6E08)-day 50—Terminal	50

TABLE 1 (Continued)
Blood Cultures from Friedlander Inhalation Anthrax Study

Group: Vaccine

Monkey #	Survival	Bacteremia	Level (Log cfu/mL)	Day of Death
I633	Dead	neg day 0,1,2	2.695 day 3	
			3.234 day 4	
			7.34 day 5	5
C380	Dead	neg days 0,1,2	1.477 day 3	
			3.017 day 4	
			3.933 day 5	
			7.876 day 5—necropsy	5
4BD	Dead	neg day 0-4	3.011 day 5	
			3.047 day 6	
			5.587 day 7	
			6.284 day 7--necropsy	7
H-981C	Dead	neg days 0,1,2	3.519 day 3	
			3.104 day 4	
			5.893 day 5	5
853410	Dead	neg day 0,1,2	2.909 day3	
			2.251 day 4	
			3.431 day 5	
			6.149 day 6	
			8.411 day 6--terminal	6
I623	Dead	neg 0-8 days	8.275 day 10--terminal	10
T34	Dead	neg 0-8 days	8.07 day 10	
			8.303 day 10—terminal	10
468C	Dead	neg days 0-4	1.637 day 5	
			3.527 day 6	
			2.72 day 7	
			8.389 day 8--terminal	8
023E	Survived	neg 0-13 days, neg day 17		
85415	Survived	neg days 1-4	0.824 day 5	
		neg days 6-10	1.301 day 11	
			1.125 day 12	
		neg days 13-21 neg day 25		

TABLE 1 (Continued)
Blood Cultures from Friedlander Inhalation Anthrax Study

Group: Ciprofloxacin

Monkey #	Survival	Bacteremia	Level (Log cfu/mL)	Day of Death
358D	Survived	neg days 0,1,3,5 7,9,11,14,18,21, 23, 25,27,29,31,33		
82A35	Survived	neg days 0,1,3,5 7,9,11,14,18,21, 23,25,27,29,31,33		
410D	Survived	neg days 0,1,3,5 7,9,11,13,15,17 19,21,24,27,31,33		
A32	Survived	neg days 0,1,3,5 7, 9,11,14,18		
45Y	Survived	neg days 0,1,3,5 7, 9,11.14.18		
40B	Survived	neg days 0,1,3,5 7, 9,10,13,17		
T292	Dead	neg days 0,1,3,5		5 (respiratory infection)
T308	Dead	neg days 0,1,3,5 7, 9,10,13,17, 30,32,34	8.703 (5.1E08—day 36)	36
B7388	Survived	neg days 0,1,3,5 7,9,11,13,15,17 19,21,24,27,31,33 97. 103		103 (not anthrax)
84456A	Survived	neg days 0,1,3,5 7, 9,10,13,17		

TABLE 1 (Continued)
Blood Cultures from Friedlander Inhalation Anthrax Study

Group: Doxycycline

Monkey #	Survival	Bacteremia	Level (Log cfu/mL)	Day of Death
45N	Survived	neg days 0,1,3,5 7,9,11,13,15,17, 19,21,24,27,31,33		
45W	Survived	neg days 0,1,3,5 7,9,11,13,15,17, 19,21,24,27,31,33		
DA143	Survived	neg days 0,1,3,5 7,9,11,14,18 21,23,25,27,29,31,33		
D625	Survived	neg days 0,1,3,5 7, 9, 11.14,18		
18434	Survived	neg days 0,1,3,5 7, 9,10,13,17		
46I	Survived	neg days 0,1,3,5 7,9,11,13,15,17 19, 21,24,27,31,33		
13379	Survived	neg days 0,1,3,5 7, 9, 10,13,17		
83A01	Survived	neg days 0,1,3,5 7, 9,11,14,18,20		
969C/40W	Survived	neg days 0,1,3,5 7, 9, 10,13,17		
396D	Dead	neg days 0,1,3,5 7,9,11,14,18 21,23,25,27, 29,31,33	3.158 (1.4E03)—day 56 3.392 (2.5E03)—day 57 7.257 (2E07)—day 58	58

TABLE 1 (Continued)
Blood Cultures from Friedlander Inhalation Anthrax Study

Group: Doxycycline + Vaccine

Monkey #	Survival	Bacteremia Level (Log cfu/mL)	Day of Death
H538	Dead	neg days 0,1,3,5 7, 9, 11,13,17 19,21,24,27,31,32,33 34,36, 36--terminal	36
380D	Survived	neg days 0,1,3,5 7, 9, 11,14,18 21,23,25,27,29,31,33	
85331	Survived	neg days 0,1,3,5 7, 9, 11,14,18 21,23,25,27,29,31,33	
H-A958	Survived	neg days 0,1,3,5 7, 9, 11,13,17 19,21,24,27,31,33	
T313	Survived	neg days 0,1,3,5 7, 9, 11,13,17 19,21,24,27,31,33	
H-05	Survived	neg days 0,1,3,5 7, 9, 11,14,18	
155DA	Survived	neg days 0,1,3,5 7, 9, 11,14,18	
85278	Survived	neg days 0,1,3,5 7, 9, 10,13,17	
A183	Survived	neg days 0,1,3,5 7, 9, 10,13,17	
3EX	Survived	neg days 0,1,3,5	

neg= negative culture

These data demonstrate that control animals had bacteremia at levels of 10-10^5 cfu/mL for about 1-2 days before death. Terminal bacteremia in 8 of the 9 animals that died varied from 10^4 to 10^9 cfu/mL. One animal had a low terminal bacteremia of 2 x 10^2 cfu/mL, but had higher counts in brain tissue. The animal that survived never had a positive blood culture.

In the group that received penicillin three of ten monkeys died. The animals that died had terminal bacteremia with 10^6-10^8 cfu/mL. The seven animals that survived never had a positive blood culture.

Eight of 10 animals that received vaccine alone died. Bacteremia was present at 10^6 to 10^8 cfu/mL at time of death. One of the two animals that survived had persistently negative blood cultures. The other animal had positive blood cultures on days 5, 11, and 12 at low levels of 10-20 cfu/mL, with negative blood cultures, thereafter.

In the ciprofloxacin treated group one animal died 5 days after exposure from an aspiration pneumonia 24 hours after the inadvertent introduction of drug into the trachea. All blood cultures were negative for anthrax in this animal. All other animals survived the 30 days of drug administration and all blood cultures were negative during this 30 day period. One animal died six days (Day 36) after ciprofloxacin administration ended. This animal had bacteremia at the time of death with bacterial levels greater than 10^8 cfu/mL. Another animal in the ciprofloxacin group died 73 days (Day 103) after ciprofloxacin was discontinued. All blood cultures in this animal were negative. It appears that this animal died from urinary tract problems. All the animals that survived had persistently negative blood cultures.

In the group treated with doxycycline alone, all animals survived during the therapy and had negative blood cultures. One animal died 28 days (Day 58) after drug administration ended. This animal had positive blood cultures on day 56 and day 57 at a level of 10^3 –10^4 cfu/mL. At the time of death the blood culture was positive at a level of 10^7 cfu/mL.

In the group treated with doxycycline plus vaccine, no animals had a positive blood culture. One animal died 6 days (Day 36) after treatment but all blood cultures were negative.

CONCLUSIONS

In a study performed by Friedlander et al., *Bacillus anthracis* Vollum 1B spores were prepared and Rhesus monkeys were exposed in a head-only chamber to an aerosol generated with a nebulizer. The median diameter of the particles generated was 1.2 μm. Animals were exposed to an inhaled dose of around 5.5 x 10^5 spores (~ 11 LD_{50}). Animals were randomly distributed into six groups of ten animals each. One group was administered saline and used as the control group. Another group received human anthrax vaccine. One group received penicillin, another ciprofloxacin, and a third group was given doxycycline. The final group received doxycycline and vaccine. All groups were dosed for 30 days. Survivors from the first experiment were rechallenged with an inhaled dose of about 30 LD_{50} on day 131 to 142.

Blood cultures were performed on various days during the study. All surviving monkeys had blood cultures that were negative at all tested times, except for one animal in the vaccine alone group that had low level bacteremia (10-20 cfu/mL) on days 5,11, and 12 but negative counts on days 1-4, 6-10, 13-21, and day 25. Almost all of the monkeys that died had bacteremia at the time of death with bacterial levels of 10^4 to 10^9 cfu/mL. One animal in the control group that died had a low bacterial level of 10^2 cfu/mL at the time of death on Day 7 of treatment. There were two monkeys that died in the ciprofloxacin group and one in the doxycycline plus vaccine group with negative blood cultures. These deaths were attributed to other factors.

Blood cultures contained large gram-positive rod-shaped organisms (bacilli). The vegetative cell width was greater than 1 μm, which distinguishes the species from *Bacillus subtilis, B. brevis, B. alvei* and many other saprophytic species. The cells were also non-motile which distinguished them from *B. cereus.* The cells were non-hemolytic which distinguishes *Bacillus anthracis* from many other *Bacillus* species. The animals in the study were exposed to *Bacillus anthracis.* The initial inoculum was examined for mucoidness (capsule formation) and immunoprecipitin halos (toxic formation) by stab inoculation onto "Halo agar". All batches of the inoculum used were mucoid and were surrounded by halos. The formation of capsules and the production of toxin distinguish *Bacillus anthracis* from other *Bacillus* species. It can thus be concluded that these positive blood cultures were *Bacillus anthracis* and not another *Bacillus* species.

REFERENCE

1. Friedlander AM, Welkos SL, Pitt MLM, et al. Postexposure prophylaxis against experiemental inhalation anthrax. J Infect Dis 167:1239-1242, 1993.

/S/
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CONCURRENCES:

HFD-590/Div Dir /S/ Signature 9/29/00 Date
HFD-590/TLMicro /S/ Signature 8/27/2000 Date

CC:
HFD-590/Original NDAs19-537/S-038; 19-847/S-024; 19-837/S-027;
19-858/S-021;20-780/S-008
HFD-590/Division File
HFD-590/Micro/PDionne
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