

20030225171

AD-A253 158

Approved
0704-0188

2

REPORT DOCUMENTATION

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the collection of information, reviewing the collection of information, including suggestions for reducing this burden to Washington Headquarters Suite 1204 Arlington VA 22202 4302 and to the Office of Management and Budget, Paperwork Project (0704-0188) Washington DC 20503



1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE 1992	3. REPORT TYPE AND DATES COVERED Reprint	
4. TITLE AND SUBTITLE (see title on reprint)			5. FUNDING NUMBERS PE: NWED QAXM WU: 00129	
6. AUTHOR(S) Brook, I., and Ledney, G.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Armed Forces Radiobiology Research Institute Bethesda, MD 20889-5145			8. PERFORMING ORGANIZATION REPORT NUMBER SR92-16	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Defense Nuclear Agency 6801 Telegraph Road Alexandria, VA 22310-3398			10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited.			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words)				
14. SUBJECT TERMS			15. NUMBER OF PAGES 4	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT	

Short and Long Courses of Ofloxacin Therapy of *Klebsiella pneumoniae* Sepsis following Irradiation

ITZHAK BROOK AND G. DAVID LEDNEY

Wound Infection Management Program, Experimental Hematology Department,
 Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20889-5145

BROOK, I., AND LEDNEY, G. D. Short and Long Courses of Ofloxacin Therapy of *Klebsiella pneumoniae* Sepsis following Irradiation. *Radiat. Res.* 130, 61-64 (1992).

Exposure to whole-body irradiation is associated with fatal gram-negative sepsis. The optimal length of therapy of such infection is not established. The effect of short and long courses of oral therapy with the quinolone ofloxacin for orally acquired *Klebsiella pneumoniae* infection was tested in B6D2F₁ mice exposed to 8.0 Gy of bilateral radiation from ⁶⁰Co. A dose of 10⁸ organisms was given orally 4 days after irradiation, and therapy was started 1 day later. Cultures of the ileum 7 days after irradiation showed the recovery of *K. pneumoniae* in 7 of 10 untreated mice and in 3 of 20 treated with ofloxacin. However, 14 days after irradiation *K. pneumoniae* was isolated in 5 of 6 untreated mice, in 7 of 9 that received the short course of therapy, and in one of those that received the long course of therapy ($P < 0.05$). At Day 7, *K. pneumoniae* was isolated from the livers of 6 of 10 untreated mice, and from none of those receiving ofloxacin ($P < 0.05$). At 14 days, *K. pneumoniae* was isolated in 4 of 6 untreated animals, in 4 of 9 that received the short course of therapy, and in none of the mice that received the long course of therapy ($P < 0.05$). Only 3 of 20 (15%) untreated mice survived for 30 days as compared to 11 of 20 (55%) mice treated for 7 days with ofloxacin and 18 of 20 (90%) mice treated for 21 days with ofloxacin ($P < 0.05$). These survival data illustrate the efficacy of a 21-day course over a 7-day course of ofloxacin therapy for orally acquired *K. pneumoniae* infection in irradiated hosts. © 1992 Academic Press, Inc.

INTRODUCTION

Exposure to ionizing radiation enhances the host's susceptibility to systemic infection due to endogenous and exogenous organisms (1, 2). A possible source of endogenous infection in irradiated hosts is the gastrointestinal tract (2), which is colonized by aerobic and anaerobic organisms. Following irradiation, members of that flora may translocate to the liver and spleen, and thereafter can be associated with fatal septicemia (2, 3). The most important bacterial species isolated from septic animals are gram-negative enteric bacteria (2, 3). *Klebsiella pneumoniae* is frequently linked to death from sepsis (4, 5) and is especially prevalent in im-

munocompromised patients (6). In a preliminary report it was shown experimentally that the prevention of translocation of these organisms and the control of sepsis can reduce mortality.¹

We found the quinolone antibiotics to be efficacious in reducing systemic infection due to *K. pneumoniae* following irradiation and oral feeding with *K. pneumoniae* (7, 8). The efficacy of these antibiotics may be due to their selective ability to eradicate *Enterobacteriaceae* while preserving the anaerobic gut flora (8). However, animal mortality was not completely prevented and the duration of quinolone therapy necessary to eliminate the bacteria was not determined in these studies.

This study was designed to evaluate the optimal length of therapy required to treat irradiated mice that develop septicemia due to orally ingested *K. pneumoniae*.

MATERIALS AND METHODS

Animals

Female B6D2F₁ mice approximately 12 weeks old were obtained from the Jackson Laboratory (Bar Harbor, ME). All animals were kept in quarantine for about 2 weeks before being transferred to a room with a 12-h (6 AM to 6 PM) light-dark cycle. Representative samples were examined to ensure the absence of specific bacteria and common murine diseases. Animals were maintained in a facility accredited by the American Association for Accreditation of Laboratory Animal Care, in Microisolator cages on hardwood chip bedding and were provided commercial rodent chow and acidified water (pH 2.2) that was changed to tap water 48 h before irradiation. All experimental procedures were done in compliance with both the National Institute of Health and the Armed Forces Radiobiology Research Institute (AFRRI) guidelines regarding animal use and care.

⁶⁰Co Irradiation

Mice were placed in Plexiglas restrainers and given a whole-body dose of 8.0 Gy from a ⁶⁰Co source at 0.4 Gy/min. The dose is an LD₅₀ for B6D2F₁ female mice. Before the experiment, the dose rate at the midline of an acrylic mouse phantom was measured using a 0.5-cc tissue-equivalent ion-

¹ Brook and T. B. Elliott, "Ofloxacin Therapy in the Prevention of Mortality after Irradiation." Presented at the 16th International Congress of Chemotherapy, Jerusalem, Israel, 1989.



ization chamber manufactured by Exradin, Inc. (Chicago, IL). The dose rate at the same location with the phantom removed was then measured using a 50-cc ionization chamber manufactured at AFRRI. The ratio of these two dose rates, the tissue-air ratio, was then used to determine the animal doses for routine experimental procedures. In this experiment the tissue-air ratio was 0.98.

All ionization chambers used have calibration factors traceable to the National Institute of Standards and Technology. The dosimetry measurements were performed following the AAPM Task Group 21 Protocol for the Determination of the Absorbed Dose from High-Energy Photon and Electron Beams (9).

Bacteria

The strain used in this study was a human clinical isolate of *K. pneumoniae* with a type 5 capsule (AFRRI No. 7). The organisms were harvested in the logarithmic phase of growth in Bacto Brain Heart Infusion Agar (Difco, Detroit, MI). A concentration of 10^9 organisms/1 ml saline was prepared, and a volume of 0.1 ml was fed to each animal by using a 20-gauge animal feeding tube fitted to a 1.0-ml syringe. We used this number of organisms because ingestion of fewer bacteria did not produce mortality in the animals.

Antimicrobials

Ofloxacin was obtained from Ortho Pharmaceutical Corp. (Raritan, NJ) and was given once every 24 h in a dose of 40 mg/kg. Standard powder formulations with known potencies were used for *in vitro* and *in vivo* studies. Ofloxacin was given by gavage in a volume of 0.1 ml sterile saline. All control animals received 0.1 ml sterile saline by gavage.

Antimicrobial Serum Concentration

Serum concentrations of the antimicrobials were determined in six irradiated mice each 1 and 23.5 h after the administration of the antimicrobials on the fifth day of therapy. *Bacillus subtilis* ATCC 6633 was used as a test organism, and Mueller-Hinton agar (pH 7.4) was used as a test agar. This method could not detect serum concentrations below 0.2 μ g/ml.

Microbiological Methods

Mice were observed for mortality and symptoms of disease for 30 days. Ten animals were selected at random from each group on Days 7 and 14 following irradiation. When fewer than 10 animals survived in a group, all were studied at that day. Animals were killed by cervical dislocation. Specimens of liver and ileum were processed for the presence of bacteria. No other organs were processed and no blood samples were obtained, because previous studies showed that liver cultures correlated best with sepsis (2). The livers were removed aseptically and homogenized immediately. The ileum was opened, and ileal content samples were obtained using a swab. The liver and stool specimens were swabbed onto blood and MacConkey agars, and the organisms were identified using conventional methods (10).

Experimental Design

Each mouse was fed 10^8 *K. pneumoniae* organisms 4 days after irradiation. Antimicrobial therapy was initiated 5 days after irradiation, and was administered orally for either 7 or 21 days. Survival experiments were performed three times with 60 mice, 20 mice for each group in each experiment. Microbial analysis of the liver and the ileal contents for bacteria colonization was done only twice with 75 mice, 25 mice for each group in each experiment. Each survival and microbial analysis experiment consisted of two antibiotic therapy groups and the untreated control group. The first group of antibiotic-treated mice received ofloxacin for 7 days (short course), the second group was given ofloxacin for 21 days (long course), and the third group was given sterile saline for 21 days.

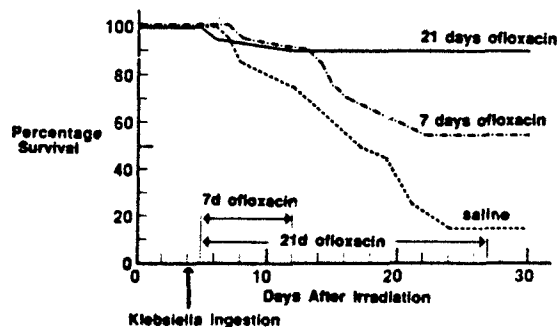


FIG. 1. Survival of 60 B6D2F1 female mice irradiated with 8.0 Gy of ^{60}Co γ rays, fed with 10^8 *K. pneumoniae*, and treated with ofloxacin. Twenty mice were included in each group. The data represent one experiment. A total of three experiments showed similar results; see text.

Samples of liver and ileum were obtained 7 and 14 days after irradiation. These sampling times correlated with mice receiving either two oral ofloxacin doses from the short course group or nine oral ofloxacin doses from the long course group. By 14 days after irradiation, mice on the short treatment schedule had not received ofloxacin for 2 days.

Statistical Methods

Statistical analyses were done using the Cox-Mantel test (11).

RESULTS

Mortality

Mortality in the groups that received ofloxacin was significantly less in all three experiments ($P < 0.05$) than in the water-treated control groups. A further significant increase in survival was noted in all three experiments in the animals treated for 21 days compared to 7 days. The survival after 30 days in the first experiment (Fig. 1) was 3 of 20 (15%) of the water-treated control mice, 11 of 20 (55%) of the animals treated with ofloxacin for 7 days, and 18 of 20 (90%) of the mice treated for 21 days. In the second experiment, 4 of 20 (20%) water-treated control mice 10 of the 20 (50%) mice treated for 7 days with ofloxacin, and 17 of 20 (85%) mice treated for 21 days with ofloxacin survived. In the third experiment, 5 of 20 (25%) water-treated control mice, 12 of 20 (60%) mice treated for 7 days with ofloxacin, and 18 of 20 (90%) mice treated for 21 days with ofloxacin survived.

Isolation of Organisms in Liver

In the first experiment, at Day 7 after irradiation, *K. pneumoniae* was isolated from the livers of 6 of 10 (60%) randomly selected control animals, and in none of those receiving ofloxacin ($P < 0.05$) (Table I). At day 14, *K. pneumoniae* was recovered in 4 of 6 (67%) water-treated animals, in 4 of 9 (45%) of those that received the short course

TABLE I
Recovery of *K. pneumoniae* from Liver and Ileum of
B6D2F₁ Mice Irradiated with 8.0 Gy ⁶⁰Co γ Rays

Therapy group	Liver		Ileum	
	Days after irradiation		Days after irradiation	
	Day 7 ^a	Day 14 ^b	Day 7 ^a	Day 14 ^b
First experiment				
Control (water)	6/10	4/6	7/10	5/6
Short course	0/10	4/9	2/10	7/9
Long course	0/10	0/10	1/10	1/10
Second experiment				
Control (water)	5/10	4/7	5/10	4/8
Short course	0/10	3/8	0/10	3/5
Long course	0/10	0/10	0/10	0/10

^a By Day 7, animals in both the short- and long-course therapy groups had received 2 days of treatment with ofloxacin.

^b By Day 14, animals in the short-course group had been treated for 7 days; animals in the long-course group had been treated for 9 days.

of therapy, and in none of those that received the long course ($P < 0.05$).

In the second experiment, *K. pneumoniae* was recovered from the livers of 5 of 10 (50%) control animals, and in none of those receiving ofloxacin ($P < 0.05$). At Day 14, *K. pneumoniae* was isolated in 4 of 7 (57%) water-treated animals, in 3 of 8 (37%) of those that received the short course of therapy, and in none of those receiving the long course.

Isolation of Organisms in the Ileum

In the first experiment, at Day 7 after irradiation, *K. pneumoniae* was recovered in 7 of 10 (70%) water-treated mice and in 3 of 20 (15%) mice treated with ofloxacin ($P < 0.05$) (Table I). At 14 days after irradiation, *K. pneumoniae* was isolated in 5 of 6 (83%) water-treated mice, in 7 of 9 (78%) that received the short course of therapy, and in one of those that received the long course ($P < 0.05$).

In the second experiment, at Day 7, *K. pneumoniae* was isolated in 5 of 10 (50%) water-treated mice and in none of those treated with ofloxacin ($P < 0.05$). At Day 14, the organism was recovered in 4 of 8 (50%) water-treated mice, in 3 of 5 (60%) mice that received the short course of therapy, and in none of those that received the long course.

Antibiotic Serum Concentration

The mean serum concentrations of ofloxacin were $2.4 \pm 0.3 \mu\text{g/ml}$ at 1 h and $0.4 \pm 0.2 \mu\text{g/ml}$ at 23.5 h.

DISCUSSION

The results demonstrate that a quinolone such as ofloxacin can reduce the colonization of the ileum and the devel-

opment of subsequent septicemia with *K. pneumoniae* in γ -photon-irradiated mice. The results support the findings of Trautmann *et al.* (12), who observed the efficacy of ciprofloxacin in the management of systemic *K. pneumoniae* infection in neutropenic mice.

We have developed a model of acquired *K. pneumoniae* infection in irradiated mice that may represent the mode of invasion of external pathogens into an irradiated host. We showed previously that irradiated mice develop fatal septicemia due to orally administered *P. aeruginosa* (13). We also observed that the number of endogenous gastrointestinal tract aerobic and anaerobic bacteria declined 24 h following irradiation and that the decline was maximal at 7 days after irradiation (14). The decrease in the number of endogenous bacteria in the gut may make the host more susceptible to acquisition of external pathogens such as *K. pneumoniae*.

The ability of *K. pneumoniae* to cause systemic infection in irradiated mice may be due to the following factors: (1) the bacterial void created in the gut following the decrease in the number of endogenous organisms, (2) the increased permeability of the mucosal cells damaged by irradiation, and (3) the diminution of the local and systemic immunity.

The effectiveness of quinolones in the therapy of *K. pneumoniae* infection may be attributed to local inhibition of the organism's growth within the gut lumen, while preserving the anaerobic gut flora (15), and to their systemic antibacterial activity to prevent the infection from spreading to other sites within the body. We found that the optimum duration of quinolone therapy is a prolonged one that would provide adequate coverage against the offending organism for at least 21 days. The superior efficacy of a 21-day course of therapy over a shorter course may be due to the need to provide adequate therapy until the immune system recovers, and granulocytes are present in the circulation (16). Although a complete recovery of the granulocytes requires up to 6 weeks at this dose of radiation, a sufficient number of them may be present after 3 weeks to eradicate the *K. pneumoniae* infection completely (16). Immunomodulators such as synthetic trehalose dicorynomycolate (16), glucan (17), and colony-stimulating factor (18) may facilitate this process.

Selective decontamination of the gut with orally administered quinolones is used to prevent sepsis in immunocompromised hosts (8, 15). These agents were also found to be effective in the management of septic episodes in neutropenic patients (19). The availability of an oral route of administration, the advantage of achieving selective inhibition of potential pathogens in the gut, and the ability to treat systemic infection make the quinolones promising agents for oral therapy of orally acquired *K. pneumoniae* infection in irradiated hosts. Although the exact length of therapy with quinolones is yet to be determined, and may be shorter than 21 days, therapy with quinolones should be

administered for a sufficient time, which will provide extended coverage until recovery of the immune system occurs.

ACKNOWLEDGMENTS

The authors acknowledge the secretarial assistance of Ms. Anne Bartky. This research was supported by the Armed Forces Radiobiology Research Institute, Defense Nuclear Agency, under Work Unit 4440-00129. Views presented in this paper are those of the authors; no endorsement by the Defense Nuclear Agency has been given or should be inferred. Research was conducted according to the principles enunciated in the *Guide of the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animals Resources, National Research Council.

RECEIVED: April 3, 1991; ACCEPTED: October 2, 1991

REFERENCES

1. H. W. Kaplan, R. S. Speck, and F. Jawetz. Impairment of antimicrobial defenses following total body irradiation of mice. *J. Lab. Clin. Med.* **40**, 682-691 (1965).
2. I. Brook, T. J. MacVittie, and R. I. Walker. Recovery of aerobic and anaerobic bacteria from irradiated mice. *Infect. Immun.* **46**, 270-271 (1984).
3. I. Brook, R. I. Walker, and T. J. MacVittie. Effect of antimicrobial therapy on the gut flora and bacterial infection in irradiated mice. *Int. J. Radiat. Biol.* **53**, 709-716 (1988).
4. G. D. Maki. Nosocomial bacteremia. An epidemiologic overview. *Am. J. Med.* **70**, 719-732 (1981).
5. M. P. Weinstein, L. B. Reller, J. R. Murphy, and K. A. Lichtenstein. The significance of positive blood cultures: A comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. I. Laboratory and epidemiologic observations. *Rev. Infect. Dis.* **5**, 35-53 (1983).
6. T. Umsawadi, E. A. Middleman, M. Luna, and G. P. Bodey. *Klebsiella* bacteremia in cancer patients. *Am. J. Med.* **165**, 473-482 (1983).
7. I. Brook, T. B. Elliott, and G. D. Ledney. Quinolone therapy of *Klebsiella pneumoniae* sepsis following irradiation: Comparison of pefloxacin, ciprofloxacin, and ofloxacin. *Radiat. Res.* **122**, 215-217 (1990).
8. M. Rozenberg-Arska, A. W. Dekker, and J. Verhoef. Ciprofloxacin for selective decontamination of the alimentary tract in patients with acute leukemia during remission induction treatment: The effect of fecal flora. *J. Infect. Dis.* **142**, 104-107 (1985).
9. Task Group 21, Radiation Therapy Committee, American Association of Physicists in Medicine. Protocol for the determination of the absorbed dose from high-energy photon and electron beams. *Med. Phys.* **10**, 1-30 (1983).
10. E. H. Lennette, A. Balows, W. Hausler, and J. H. Shadomy (Eds.). *Manual of Clinical Microbiology*, 4th ed. American Society for Microbiology, Washington, DC, 1985.
11. T. E. Lee. *Statistical Methods for Survival Data Analysis*, pp. 127-129. Lifetime Learning Publications, Belmont, CA, 1980.
12. M. Trautmann, O. Bruckner, R. Marre, and H. Hahn. Comparative efficacy of ciprofloxacin, ceftizoxime, and gentamicin given alone or in combination, in a model of experimental septicemia due to *Klebsiella pneumoniae* in neutropenic mice. *Infection* **16**, 49-53 (1988).
13. R. I. Walker, I. Brook, J. W. Costerton, T. J. MacVittie, and M. L. Myhal. Possible association of mucous blanket integrity with postirradiation colonization resistance. *Radiat. Res.* **104**, 346-357 (1985).
14. I. Brook, R. I. Walker, and T. J. MacVittie. Effect of antimicrobial therapy on the gut flora and bacterial infection in irradiated mice. *Int. J. Radiat. Biol.* **53**, 709-716 (1988).
15. S. Pecquet, A. Andermont, and C. Tancrede. Selective antimicrobial modulation of the intestinal tract by norfloxacin in human volunteers and in gnotobiotic mice associated with a human fecal flora. *Antimicrob. Agents Chemother.* **29**, 1047-1052 (1986).
16. G. S. Madonna, G. D. Ledney, T. B. Elliott, I. Brook, J. T. Ulrich, K. R. Myers, M. L. Patchen, and R. I. Walker. Trehalose dimycolate enhances resistance to infection in neutropenic animals. *Infect. Immun.* **57**, 2495-2501 (1989).
17. M. L. Patchen, M. M. D'Alessandro, I. Brook, W. Blakely, and T. J. MacVittie. Glucan: Mechanisms involved in its "radioprotective" effect. *J. Leukocyte Biol.* **42**, 95-105 (1987).
18. M. L. Patchen, T. J. MacVittie, B. D. Solberg, and L. M. Souza. Therapeutic administration of recombinant human granulocyte colony-stimulating factor accelerates hemopoietic regeneration and enhances survival in a murine model of radiation-induced myelosuppression. *Int. J. Cell Cloning* **8**, 1-10 (1990).
19. S. M. Kelsey, M. Wood, E. Shaw, and A. C. Newland. Intravenous ciprofloxacin as empirical treatment of febrile neutropenic patients. *Am. J. Med.* **87**, 2745-2775 (1989).

DTIC QUALITY INSPECTED 2

Accession For	
NTIS GRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By _____	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	20