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Oral Ofloxacin Therapy of *Pseudomonas aeruginosa* Sepsis in Mice after Irradiation

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Death subsequent to whole-body irradiation is associated with gram-negative bacterial sepsis. The effect of oral therapy with the new quinolone ofloxacin for orally acquired *Pseudomonas aeruginosa* infection was tested in B6D2F1 mice exposed to 7.0 Gy of bilateral radiation from ^{60}Co. A dose of 10^7 organisms was given orally 2 days after irradiation, and therapy was started 1 day later. Only 4 of 20 untreated mice (20%) survived for at least 30 days compared with 19 of 20 mice (95%) treated with ofloxacin ($P < 0.005$). *P. aeruginosa* was isolated from the livers of 21 of 28 untreated mice (75%), compared with only 2 of 30 treated mice ($P < 0.005$). Ofloxacin reduced colonization of the ileum by *P. aeruginosa*; 24 of 28 untreated mice (86%) harbored the organisms, compared with only 5 of 30 (17%) with ofloxacin ($P < 0.005$). This experiment was replicated twice, and similar results were obtained. These data illustrate the efficacy of the quinolone ofloxacin for oral therapy of orally acquired *P. aeruginosa* infection in irradiated hosts.

Ionizing radiation enhances the susceptibility of the host to systemic infections due to endogenous and exogenous organisms (1, 7). *Pseudomonas aeruginosa* is one of the most frequent causes of gram-negative bacterial sepsis that develops in irradiated mice (5, 17) and is especially prevalent in immunocompromised patients (13, 19). This organism was found in patients that were therapeutically (11, 19) or accidentally (4) exposed to ionizing radiation.

Therapy of severe systemic infection due to gram-negative bacteria generally involves the use of aminoglycosides in combination with beta-lactam antibiotics (6). Such therapy reduces the mortality rate in irradiated animals (14). However, aminoglycosides are administered parenterally, and administration of such therapy requires close monitoring because of potential nephrotoxicity and ototoxicity. Simpler modes of therapy may be beneficial in situations that involve mass casualty exposure to ionizing radiation. The recently developed quinolone compounds have exhibited high in vivo bactericidal activity against most gram-negative bacteria, including *P. aeruginosa* (20). These agents can also be administered orally and are relatively free of serious side effects.

In this study, we evaluated the efficacy of oral therapy with the quinolone ofloxacin in a model of experimental septicemia due to orally administered *P. aeruginosa* in irradiated mice.

MATERIALS AND METHODS

Animals. Female B6D2F1 mice approximately 10 weeks of age were obtained from Jackson Laboratory, Bar Harbor, Maine. All animals were kept in quarantine for about 2 weeks. Representative organ samples were examined to ensure the absence of specific bacteria and common murine diseases. Animals were maintained on a 12-h light-dark cycle 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 2.5) that was changed to tap water 48 h before irradiation. This was done to facilitate colonization of the gastrointestinal tract with *P. aeruginosa* (18). All experimental procedures were performed in compliance with National Institutes of Health and Armed Forces Radiobiology Research Institute guidelines regarding animal use and care. We also followed the guidelines of the Institute of Laboratory Animal Resources, National Research Council (*Guide of the Care and Use of Laboratory Animals*).

Experimental design. Each mouse was fed 10^7 organisms 48 h after irradiation. The time of feeding was chosen after preliminary data showed that the animals became susceptible to *P. aeruginosa* sepsis following feeding with these gram-negative bacteria 48 h after irradiation (18). Antimicrobial therapy was initiated 24 h later and was administered for 15 days. A total of 100 mice were included in each of the first two experiments, and 40 were used in the third experiment; each experiment was performed three times. However, the microbial analysis of the ileal contents and livers was done only twice. Each experiment consisted of two groups: one antibiotic therapy group and one saline-treated control group. No other groups were included because preliminary work showed no recovery of *P. aeruginosa* from the livers or ileal contents of nonirradiated B6D2F1 mice. Each therapy or control group consisted of 50 mice as follows: 20 were observed for mortality, and 30 were used for cultures of liver and ileal content on the designated days.

^{60}Co irradiation. Mice were placed in Plexiglas restrainers (Rohm & Haas Co.) and given a whole-body dose of 7.0 Gy radiation at 0.4 Gy/min from bilaterally positioned ^{60}Co sources. Dose determinations were made by using a 50-ml tissue-equivalent ionization chamber (designed by the Armed Forces Radiobiology Research Institute) calibrated against a National Institute of Standards and Technology ionization chamber. The dose within the exposed field varied by 3%, as determined by thermal luminescence dosimetry conducted within tissue-equivalent mouse phantoms. The lethal dose for 50% of B6D2F1 female mice is 9.65 ± 0.36 (standard deviation) Gy 30 days after exposure in this laboratory. The dose of 7.0 Gy was, therefore, a sublethal dose and was chosen after previous studies showed that feeding the animals with *P. aeruginosa* produced mortality of 80 to 90% in 30 days (18). This dose produces significant

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neutropenia (1) but minimal translocation of enteric organisms.

Bacteria. The strain used in this study was a clinical isolate of *P. aeruginosa* PA220 that was isolated from a blood sample taken at the National Naval Medical Center, Bethesda, Md. The organism is serotype 1, exotoxin A and protease positive, sensitive to serum-mediated killing, and relatively virulent for normal mice. We have used this strain in previous animal studies (18). The organisms were harvested in the logarithmic phase of growth in brain heart infusion broth (BHIB). A concentration of 10^8 organisms per ml of saline was prepared, and a volume of 0.1 ml was fed to each animal by gavage by using a 20-gauge animal feeding tube fitted to a 1.0-ml syringe.

Antimicrobial agents. Ofloxacin was obtained from Ortho Pharmaceutical Corp., Raritan, N.J. A standard powder formulation with known potency was used for in vitro and in vivo studies. Ofloxacin was given every 24 h in a dose of 40 mg/kg. The antibiotic was administered in a volume of 0.1 ml of sterile distilled water by oral gavage with a 20-gauge feeding tube fitted to a 1.0-ml syringe. All control animals received 0.1 ml of sterile distilled water by oral gavage.

Antimicrobial concentrations in serum. Concentrations of the antimicrobial agents in serum were determined in six infected mice and six uninfected mice 1 and 23.5 h after oral administration of ofloxacin on day 5 of therapy. Antibiotic stock solutions were prepared volumetrically at a concentration of 50 μg/ml (5.0 mg/100 ml). Ofloxacin was solubilized by using 0.1 N NaOH followed by a sufficient quantity of H_2O. A 1:10 dilution of each stock solution was prepared by using 0.5 ml of sterile stock solution (filtered with a 0.2-μm-pore-size syringe filter) and 4.5 ml of mouse serum. Serial 1:2 dilutions were performed in mouse serum for each antibiotic tested (range, 5 to 0.3 μg/ml). The organism tested, *Escherichia coli* WYO02, was grown overnight in 50 ml of BHIB on a shaker at 100 rpm and 35°C. This was then diluted 1:10 with BHIB. At 580 nm, the 1:10 dilution gave a 36% transmission. A plate count confirmed the concentration to be 10^8 CFU/ml. One ml of the adjusted inoculum was added to 350 ml of Antibiotic Media 2 (10943, lot no. AQDRNE; BBL Microbiology Systems, Cockeysville, Md.). This was then poured into a square plate (12 by 12 cm). The agar was allowed to set at room temperature for 1 h. Wells were made in the agar with a no. 5 cork bore (diameter, ~8 mm). The wells were randomly numbered. For each number, there were four identical wells. An antibiotic solution of a differing concentration was placed in the first five sets of four wells to construct the standard curve. The remaining wells were used for the test samples (two wells per sample). The plate was then incubated at 35°C for 24 h. This procedure was repeated until all the samples could be tested.

Serum and tissue samples containing less than 0.2 μg of ofloxacin per sample were undetectable because of the limited sensitivity of the test system used. The laboratory daily correlation coefficient in determining ofloxacin levels was 0.99. All standard preparations were made in normal antibiotic-free mouse serum. The recovery of ofloxacin carboxylic acid was 99.7%.

In vitro susceptibility. MICs and MBCs were determined in Mueller-Hinton broth that was inoculated with 1.5×10^5 organisms per ml from an overnight culture.

Microbiological methods. Animals were observed for mortality and symptoms of disease. Five animals were selected at random from each group on days 4, 6, 8, 10, and 12 following irradiation. Animals were killed by cervical dislocation. Specimens of livers 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, whereas *P. aeruginosa* was concomitantly isolated in all animals that harbored the organisms in the liver (18). The livers were aseptically removed and immediately homogenized in sterile saline. The ileum was opened, and ileal content samples were obtained with swabs. The liver and stool specimens were swabbed onto blood and MacConkey agars, and the organisms were identified by conventional methods (12). The number of organisms was determined semiquantitatively.

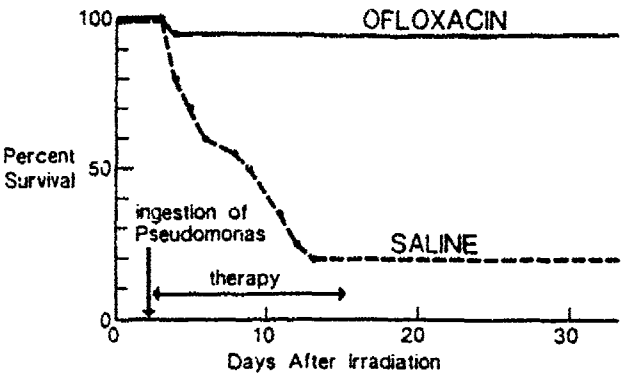

FIG. 1. Survival of 40 B6D2F1 mice irradiated with 7.0 Gy of ^{60}Co, fed with 10^7 *P. aeruginosa*, and treated orally with ofloxacin. Twenty mice were included in each group. (Data represent one experiment; two replicates of each experiment showed similar results.)

Statistical methods. Statistical analyses were done by using the Cox-Mantel test (10).

RESULTS

Mortality. Mortality in the groups that received ofloxacin was significantly lower from day 5 onward ($P < 0.05$ in all experiments) than that of the mice treated with water. In the first experiment (Fig. 1), only 4 of 20 mice treated with water (20%) survived, compared with 19 of 20 mice treated with ofloxacin (95%). In the second run of the experiment, 6 of the 20 mice treated with water (30%) survived whereas 18 of 20 mice treated with ofloxacin (90%) survived. In the third run, 5 of 20 mice treated with water (25%) survived, compared with 17 of 20 mice treated with ofloxacin (85%).

Isolation of organisms in liver. There was no correlation between the time following irradiation and the isolation of *P. aeruginosa*. More than 10 colonies of *P. aeruginosa* were isolated from each culture-positive liver sample. In the first experiment, *P. aeruginosa* was isolated in 21 of 28 randomly selected mice treated with water (75%) and in 2 of 30 mice treated with ofloxacin (7%). In the second experiment, *P. aeruginosa* was recovered in 14 of 26 mice treated with water (54%) and in no mice treated with ofloxacin. In the third experiment, *P. aeruginosa* was recovered in 17 of 25 mice treated with water (68%) and in no mice treated with the quinolone. ($P < 0.005$ in all experiments.)

Isolation of organisms in ileal contents. More than 10 colonies of *P. aeruginosa* were recovered from each culture-positive ileal content specimen. In the first experiment, *P. aeruginosa* was isolated in ileal content specimens of 24 of 28 mice treated with water (86%), compared with only 5 of 30 mice treated with ofloxacin (17%). In the second experiment, *P. aeruginosa* was recovered from the ileal contents of 19 of 26 mice treated with water (73%), compared with only 3 of 30 mice treated with quinolones (10%). ($P < 0.005$ in all experiments.)

Antibiotic concentrations in serum. The mean concentrations of ofloxacin were 2.6 ± 0.4 mg/liter at 1 h and 0.4 ± 0.2 mg/liter at 23.5 h. No difference was noted between infected and uninfected animals.

DISCUSSION

This study demonstrates that the quinolone ofloxacin can prolong survival and reduce colonization of the ileum and recovery of *P. aeruginosa* in the livers of irradiated mice.

We have developed a model of acquired *P. aeruginosa* infection in irradiated mice that may represent the mode of acquisition of external pathogens into an irradiated host (18). We also observed that the number of endogenous gastrointestinal tract aerobic and anaerobic bacteria declined 24 h after irradiation, and the decline was maximal at 7 days (2). The decrease in the number of endogenous bacterial flora may make the host more susceptible to the acquisition of external pathogens, such as *P. aeruginosa*.

The ability of *P. aeruginosa* to cause systemic infection in irradiated mice may be due to the following factors: (i) the bacterial void created in the gut following the decline in the number of other organisms (2), (ii) the increased permeability of the mucosal cells damaged by irradiation, and (iii) the decrease in local and systemic immune defenses.

The effectiveness of ofloxacin in the therapy of *P. aeruginosa* infection may be attributed to local inhibition of the growth of the organism within the gut lumen while the anaerobic gut flora was preserved (16) and to its systemic antibacterial activity to prevent the infection within the body.

The intraluminal concentration of ofloxacin achieved in our mouse model is estimated to be about 1,000 μg/ml, which is similar to that observed in humans (Ortho Pharmaceuticals, unpublished data). Although this initial concentration is reduced as the initial antimicrobial dose is absorbed, diluted, and excreted, it is probably capable of depressing the intraluminal growth of even resistant organisms, such as *Pseudomonas* species.

The optimal duration of quinolone therapy has not been determined. Although ofloxacin was administered for 15 days in the present study, a shorter course may be as efficacious. Further studies are under way to determine the optimal duration of therapy. Although ofloxacin was administered twice a day in studies of humans (9), administration of this drug once a day was efficacious in our animal model and achieved concentrations in serum equal to those found in humans. Coprophagia, however, might have augmented the ingestion of ofloxacin. Further studies are warranted to evaluate the therapy of infection due to more resistant pathogens by using twice-daily administration of ofloxacin.

Selective decontamination of the gut with orally administered quinolones is used to prevent sepsis in immunocompromised hosts (3, 8, 9). These agents were also found to be effective in the management of septic episodes in neutropenic patients (11). The availability of an oral route of administration, the long half-life of ofloxacin that allows once-daily administration (15), the advantage of achieving selective inhibition of potential pathogens in the gut, and the ability to treat systemic infection make this quinolone a promising agent for oral therapy of orally acquired *P. aeruginosa* infection in irradiated hosts.

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