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Adverse Effects of Pefloxacin in Irradiated C3H/HeN Mice: Correction with Glucan Therapy

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Opportunistic bacterial infections are the predominant cause of death following myelosuppressive radiation exposure. When used alone, a variety of immunomodulators and antibiotics have been reported to reduce radiation-induced death. In these studies, the combined therapeutic effects of the immunomodulator glucan and the quinolone antibiotic pefloxacin were evaluated for survival-enhancing effects in myelosuppressed C3H/HeN mice. Mice were exposed to 7.9 Gy of whole-body ^{60}Co radiation and treated with saline, glucan (250 mg/kg of body weight intravenously, 1 h after irradiation), pefloxacin (64 mg/kg/day orally, days 3 to 24 after irradiation), or glucan plus pefloxacin. Survival 30 days after irradiation in mice receiving these respective treatments was 25, 48, 7, and 85%. Evaluation of granulocyte-macrophage progenitor cell (GM-CFC) recovery in mice receiving these treatments revealed that, compared with recovery in saline-treated mice, glucan stimulated GM-CFC recovery, pefloxacin suppressed GM-CFC recovery, and glucan administered in combination with pefloxacin could override pefloxacin's hemopoietic suppressive effect.

Exposure to whole-body radiation induces mortality that is associated with bacteremia caused primarily by endogenous intestinally derived bacteria (1). Previous studies have demonstrated the usefulness of the quinolone pefloxacin in reducing mortality in irradiated B6D2F1 mice (2). The efficacy of pefloxacin was attributed to its activity against facultative intestinal bacterial flora. Pefloxacin may have exerted its antibacterial activity either directly against organisms within the intestinal lumen or systemically against translocated bacteria.

In contrast to previous work with B6D2F1 mice, preliminary studies using pefloxacin therapy in irradiated C3H/HeN mice indicated that pefloxacin therapy actually enhanced mortality after irradiation in these animals. It has been observed that endogenous gastrointestinal bacterial floras differ in these two strains of mice, with more gram-negative enteric aerobic as well as facultative and strict anaerobic organisms present in C3H/HeN mice than in B6D2F1 mice (5a), making the bacterial flora of C3H/HeN mice quantitatively and qualitatively more similar to that of humans. However, mortality following irradiation in both mouse strains results from bacteremia related to their respective intestinally derived bacterial flora. Because pefloxacin is a broad-spectrum antibiotic (14), differences in the endogenous floras of these two mouse strains did not appear to be a reasonable explanation for the survival differences observed in these irradiated mice following pefloxacin treatment. In addition to differences in endogenous flora, C3H/HeN and B6D2F1 mice differ in bone marrow and splenic hemopoietic stem and progenitor cell numbers, with fewer of these cells present in C3H/HeN mice. For example, we have observed that B6D2F1 femoral bone marrow contains approximately 6,500 multipotent spleen CFU and 11,500 granulocyte-macrophage colony-forming cells (GM-CFC) while C3H/HeN femoral bone marrow contains only about 2,000 multipotent spleen CFU and 3,500 GM-CFC (21, 28). Because quinolone antibiotics, including pefloxacin, have been reported to suppress mammalian cell proliferation (7, 8, 10, 13), we hypothesized that the increased mortality observed following pefloxacin treatment in C3H/HeN mice whose hemopoietic system has already been critically depleted by irradiation may be due to suppressed proliferation of the few surviving hemopoietic stem and progenitor cells.

We have previously demonstrated that glucan, a beta-1,3-polysaccharide immunomodulator, is capable of enhancing hemopoietic regeneration and increasing survival when administered to C3H/HeN mice following irradiation (20, 25, 27). Specifically, glucan therapy has been demonstrated to accelerate repopulation of multipotent hemopoietic stem cells, as well as hemopoietically committed granulocyte-macrophage, pure macrophage, and erythroid progenitor cells. The survival-enhancing and hemopoietic activities of glucan have been correlated with its ability to activate macrophages, resulting in the production of macrophage-derived hemopoietic growth factors as well as enhanced macrophage-mediated defense against opportunistic infections (19, 22, 24). Hence, if pefloxacin were to inhibit hemopoietic regeneration in already critically myelosuppressed mice, glucan therapy may be able to override these suppressive hemopoietic effects while permitting the beneficial antimicrobial effects of pefloxacin to be maintained.

The studies described here were designed to (i) document the deleterious effects of pefloxacin in irradiated C3H/HeN mice, (ii) investigate whether pefloxacin inhibits hemopoietic recovery in C3H/HeN mice following radiation-induced myelosuppression, and (iii) evaluate whether glucan therapy could be used to override the deleterious effects of pefloxacin in irradiated C3H/HeN mice.

MATERIALS AND METHODS

Mice. C3H/HeN female mice (about 25 g) were purchased from Charles River Laboratories (Raleigh, N.C.). Mice were maintained in Micro-Isolator cages (Lab Products, Maywood, N.J.) on hardwood-chip contact bedding and were provided commercial rodent chow and acidified water (pH 2.5) ad libitum. Three days prior to experimentation and

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throughout the experiments, tap water containing 0.03 mg of ascorbic acid per ml was substituted for acidified water. Animal rooms were equipped with full-spectrum light from 0600 to 1800 h and were maintained at 21 ± 1°C with 50% ± 10% relative humidity, with at least 10 air changes of 100% conditioned fresh air per h. Upon arrival, all mice were tested for *Pseudomonas* infection and quarantined until test results were obtained. Only healthy mice were released for experimentation. The Institute Animal Care and Use Committee approved all experiments prior to performance. Animals were maintained in a facility accredited by the American Association for the Accreditation of Laboratory Animal Care, and research was conducted according to the principles enunciated in reference 13a.

Irradiation. The ^{60}Co source at the Armed Forces Radiobiology Research Institute was used to administer bilateral total-body gamma radiation. Mice were placed in ventilated Plexiglas containers and irradiated at a dose rate of 0.4 Gy/min. Dosimetry was determined by ionization chambers (31), with standards traceable to the National Institute of Standards and Technology. The tissue-to-air ratio was determined to be 0.96, and the dose variation within the exposure field was <3%.

Pefloxacin therapy. Pefloxacin methanesulfonatedihydrate powder (henceforth referred to as pefloxacin) was obtained from Rhone-Poulenc Sante (Antony, France). Pefloxacin therapy was initiated 3 days after irradiation. In dose-response experiments, pefloxacin was prepared in tap water and administered to mice in 0.5-ml volumes at doses of 32, 64, or 320 mg/kg of body weight per day by oral gavage, using a blunt-end 20-gauge feeding needle attached to a 3-ml syringe. In all other experiments, pefloxacin was prepared in tap water at the concentration of 0.6 mg/ml and substituted for tap water in the mouse cage water bottles. As recommended by the manufacturer, 0.03 mg of ascorbic acid per ml was added to the tap water to stabilize pefloxacin activity. Preliminary studies using high-performance liquid chromatography (18) to measure serum pefloxacin levels verified pefloxacin absorption following both oral gavage and water bottle administration. One hour following administration of 64 mg of pefloxacin per kg by oral gavage, the mean serum pefloxacin concentration was 2.6 ± 0.4 μg/ml; similarly, mice maintained on pefloxacin-containing tap water for 5 days exhibited a mean serum pefloxacin concentration of 1.8 ± 0.5 μg/ml. The pefloxacin concentration to be used for water bottle administration was based on preliminary studies in which it was determined that the daily consumption of tap water in mice exposed to 7.9 Gy of ^{60}Co was approximately 2.6 ml. Hence, pefloxacin at a concentration of 0.6 mg/ml would allow ingestion of approximately 1.6 mg of pefloxacin per mouse per day, or the 64-mg/kg recommended daily dose (9). Water consumption in mice receiving pefloxacin was also monitored to verify pefloxacin intake.

Glucan therapy. Endotoxin-free glucan was purchased from Tulane University School of Medicine (New Orleans, La.). This glucan preparation was a soluble (1-3)-beta-D-glucan isolated from the inner cell wall of *Saccharomyces cerevisiae* (4). Glucan was intravenously administered 1 h after irradiation at a dose of 5 mg per mouse. This dose has previously been demonstrated to be an effective hemopoietic stimulant in irradiated C3H/HeN mice (25). Control mice were injected intravenously with saline.

Survival assays. Mice entered into survival studies were irradiated with 7.9 Gy of ^{60}Co. In preliminary studies, this radiation dose was determined to be lethal for approximately 80% of tap-water-maintained mice within 30 days postexposure. Survival of irradiated mice was checked and recorded daily for 30 days; on day 31, surviving mice were euthanized by cervical dislocation. Each treatment group consisted of 10 to 20 mice; experiments were repeated three to five times.

Hemopoietic assays. The endogenous spleen CFU (E-CFU) assay (34) was used in initial studies to screen for pefloxacin-induced inhibition of hemopoiesis. Mice were exposed to 6 Gy of ^{60}Co radiation to partially ablate endogenous hemopoietic stem cells yet ensure the survival of all mice for at least the 12 days required for E-CFU detection. On day 12 postirradiation, mice were euthanized by cervical dislocation and the spleens were removed. Spleens were fixed in Bouin's solution, and grossly visible spleen colonies, arising from the clonal proliferation of surviving endogenous multipotent hemopoietic stem cells, were counted. Each treatment group consisted of five mice. Experiments were repeated three times.

In additional studies, the effects of pefloxacin on regeneration of hemopoietic progenitor cells committed to GM-CFC development were assayed by a previously described agar GM-CFC assay (23). Mouse endotoxin serum (5%, vol/vol) was added to feeder layers as a source of colony-stimulating factors. Colonies (>50 cells) were counted after 10 days of incubation in a 37°C humidified environment containing 5% CO_2. The cell suspensions used for these assays consisted of tissues from three normal, irradiated, or treated and irradiated mice at each time point. Femurs and spleens, both major hemopoietic organs in mice, were removed from mice euthanized by cervical dislocation. Cells were flushed from femurs with 3 ml of McCoy's 5A medium (Flow Laboratories, McLean, Va.) containing 10% heat-inactivated fetal bovine serum (HyClone, Logan, Utah). Spleens were pressed through a stainless-steel mesh screen, and the cells were washed from the screen with 6 ml of medium. The numbers of nucleated cells in the suspensions were determined with a Coulter counter. Triplicate samples were plated for each cell suspension in each experiment, and experiments were repeated three times.

Microbiological assays. Mice were exposed to 7.9 Gy of ^{60}Co, and five mice from each treatment group were randomly selected for microbiological evaluation on days 11, 13, 15, 19, and 22 after irradiation. Animals were euthanized by cervical dislocation, and the entire liver of each mouse was excised aseptically and weighed to the nearest milligram. Organs were then added to a volume of sterile 0.9% NaCl solution equivalent to 1.0 ml/100 mg of tissue and homogenized in ground-glass homogenizers (Bellco, Vineland, N.J.) at a moderate speed. The homogenates were then inoculated onto media supportive for the growth of facultative and aerobic bacteria by using sheep blood, tryptic soy, and MacConkey agars. The number of bacterial CFU per gram of liver was calculated from the number of colonies that grew on each medium. Aerobic plates were incubated at 35°C in air containing 5% CO_2 and were observed after 24 and 48 h. Isolates were identified by standard criteria (16, 33).

Statistics. With the exception of survival data, all results are presented as the mean ± 1 standard error of the mean. Survival data were analyzed by the generalized Savage (Mantel-Cox) procedure (15). All other data, unless noted otherwise, were compared by the Newman-Keul's multiple comparison test following a significant one-way analysis of variance. In cases when variances among treatment groups were not uniform, comparisons were made by a Behrens-Fischer t test with P values Bonferroni corrected (32). All multiple-comparison tests were run at the 5% level.

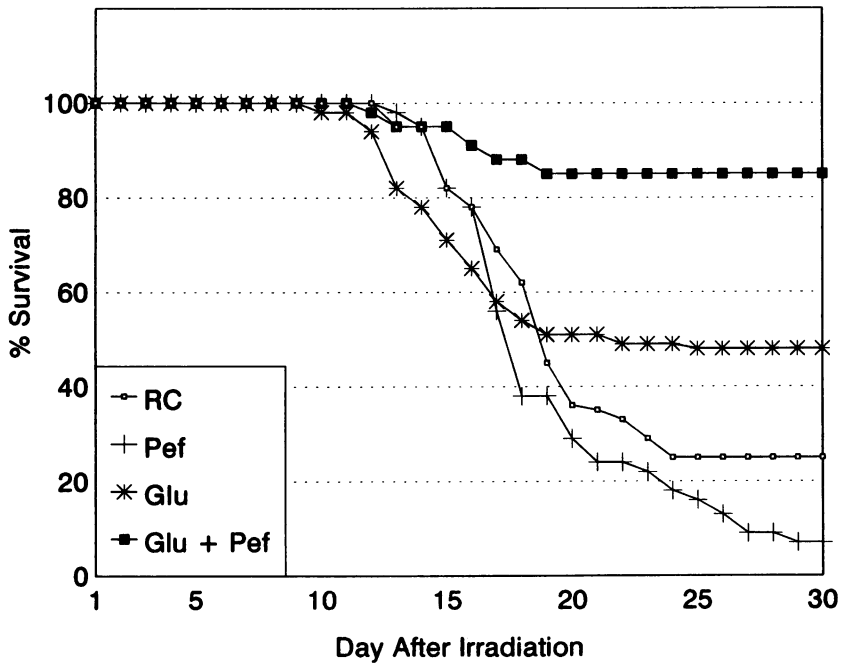


FIG. 1. Effects of pefloxacin (Pef), glucan (Glu), and glucan-pefloxacin treatments on survival of irradiated C3H/HeN mice. Mice were exposed to 7.9 Gy of ^{60}Co and administered either pefloxacin (0.6 mg/ml in drinking water on days 3 to 24 after irradiation), glucan (5 mg per mouse intravenously 1 h after irradiation), or both treatments. Survival was monitored daily for 30 days. Data are a composite from five experiments, totaling 65 mice per treatment group. RC, radiation control mice.

RESULTS

Survival studies. Mortality-enhancing effects of pefloxacin were consistently observed in irradiated C3H/HeN mice. On the basis of three experiments, the average 30-day survival in mice exposed to 7.9 Gy of ^{60}Co and treated with pefloxacin was 5.7% ± 2.9%, compared with 25.7% ± 3.0% in radiation control mice ($P < 0.05$; $n = 45$ in each treatment group).

The ability of glucan therapy to override the mortality-enhancing effects of pefloxacin therapy in irradiated mice is illustrated in Fig. 1. This figure represents composite data from five experiments totaling 65 mice per treatment group. In contrast to the reduced survival produced by pefloxacin therapy alone (5 versus 25%; $P < 0.05$), glucan therapy alone enhanced survival compared with that in radiation controls (48 versus 25%; $P < 0.05$). Most interesting, however, was the ability of glucan-pefloxacin treatment to enhance survival beyond that observed with glucan therapy alone ($P < 0.05$); survival in combination-treated mice was 85%. These phenomena were consistently observed in each of the five experiments performed.

We initially suspected that if the antibiotic-containing tap water produced a taste aversion, dehydration may adversely affect survival in pefloxacin-treated mice. Figure 2 illustrates that the average daily water consumption in pefloxacin-treated mice was significantly less than that of radiation control mice. However, combination-treated mice, which exhibited enhanced survival rather than impaired survival, also consumed significantly less water than radiation controls, indicating that changes in water consumption could not be correlated with survival effects.

Hemopoietic stem and progenitor cell studies. In the E-CFU assay, multipotent stem cells which survive the myelosuppressive 6-Gy dose of radiation should seed the spleen and clonally proliferate to form grossly visible spleen colonies by day 12 after irradiation. When this assay was used to examine pefloxacin for the ability to inhibit hemopoietic regeneration in myelosuppressed mice, a dose-dependent reduction in E-CFU numbers was observed (Fig. 3). The E-CFU assay required that animals be euthanized on day 12 after irradiation to count the spleen colonies. For this reason, mice in these experiments were exposed to pefloxacin for only 10 days (days 3 to 12) instead of the 22-day pefloxacin treatment used in survival studies (days 3 to 24). Even following this relatively short 10-day course of pefloxacin, only 10.4 ± 0.5, 9.1 ± 0.3, and 5.7 ± 0.6 colonies were observed in mice treated with 32, 64, and 320 mg of pefloxacin per kg per day, respectively, compared with 12.2 ± 0.4 colonies in radiation control mice.

Because the dose-response studies demonstrated the ability of pefloxacin to suppress hemopoiesis, the involvement of this phenomenon in enhancing mortality in irradiated mice was further evaluated. As in survival studies, mice were exposed to 7.9 Gy of ^{60}Co and provided pefloxacin in the drinking water. On days 11, 13, 15, and 19 following irradiation, evaluations were performed to determine the numbers of GM-CFC progenitors in the bone marrow and spleen. In the same experiments, glucan-treated mice that also received pefloxacin were evaluated to determine whether pefloxacin-induced hemopoietic suppression could be overridden by the use of a known hemopoietic stimulant. Figure 4 illustrates the results of these studies. Compared with those in radiation control mice, both bone marrow and splenic GM-CFC recoveries were found to be suppressed in pefloxacin-treated mice. Bone marrow GM-CFC values in pefloxacin-treated mice on days 13, 15, and 19 after irradiation, respectively, were only 60, 46, and 26% of radiation control values. These data suggest that GM-CFC suppres-

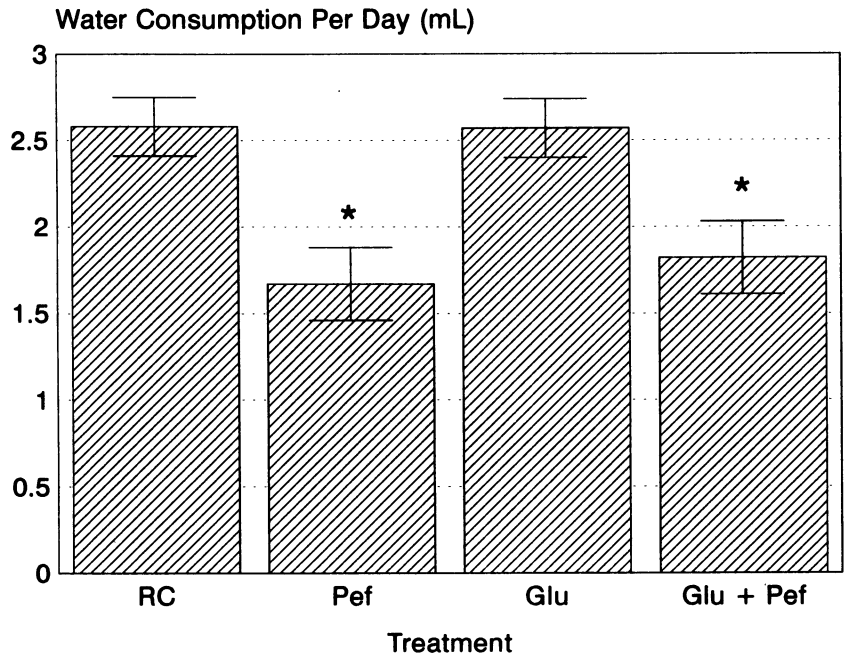


FIG. 2. Effects of pefloxacin (Pef), glucan (Glu), and glucan-pefloxacin treatments on water consumption in irradiated C3H/HeN mice. Mice were exposed to 7.9 Gy of ^{60}Co and administered either pefloxacin, glucan, or both treatments as described in the legend to Fig. 1. Water consumption was measured daily through day 24 of the experiment. RC, radiation control mice; *, $P < 0.05$, with respect to radiation control values or glucan values. Average daily water consumption in nonirradiated mice was 2.8 ± 0.6 ml.

sion increases with the duration of pefloxacin treatment. In the spleen, GM-CFC recovery was not detected until day 19 in radiation controls (11.6 ± 2.6 GM-CFC per spleen); however, no splenic GM-CFC recovery was detected at any time in pefloxacin-treated mice. In contrast to mice treated with pefloxacin alone, mice treated with glucan alone or glucan plus pefloxacin exhibited greater bone marrow and splenic GM-CFC recovery than did radiation control mice.

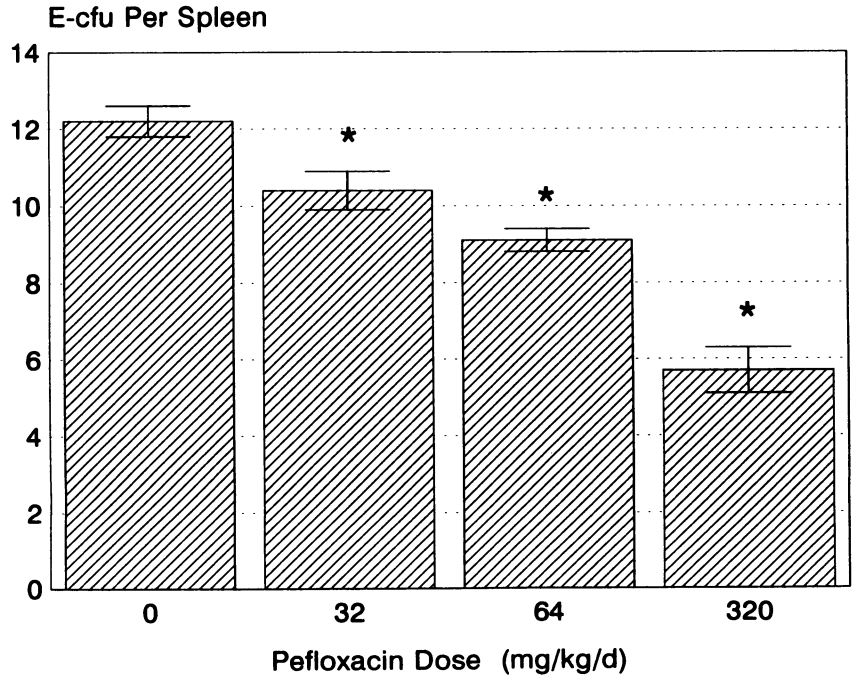
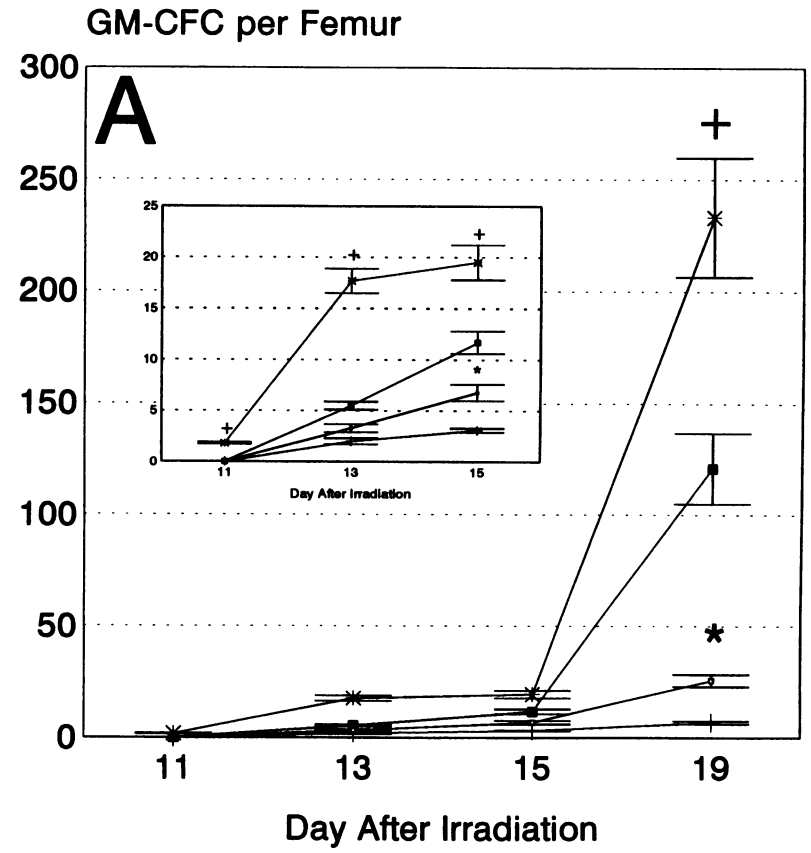
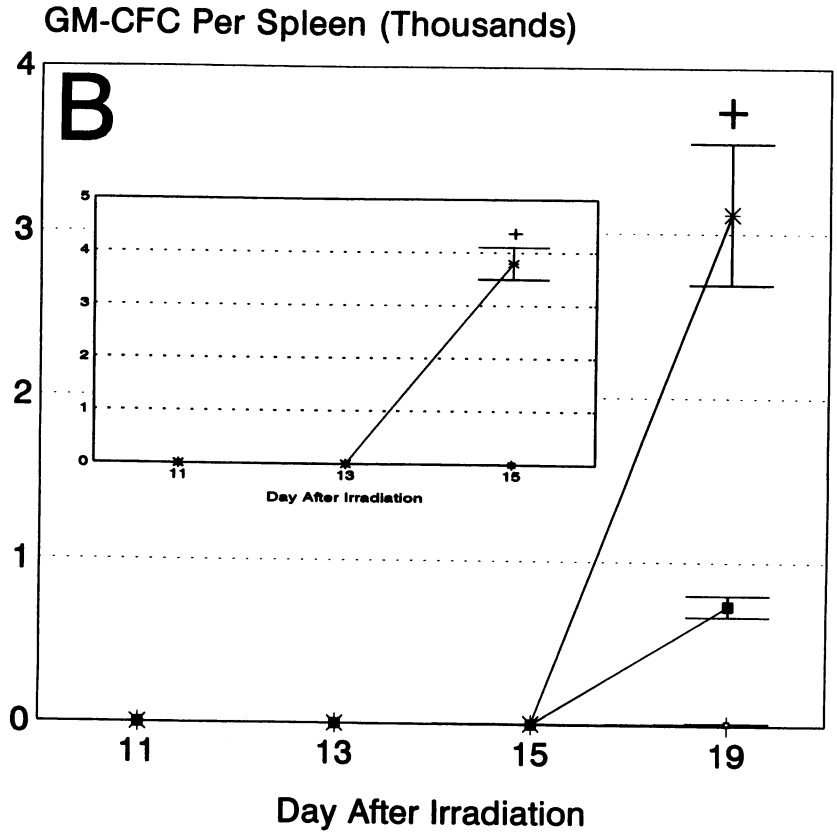


FIG. 3. Effect of pefloxacin treatment on E-CFU formation in C3H/HeN mice. Mice were exposed to 6 Gy of ^{60}Co and on days 3 to 12 after irradiation were administered pefloxacin by oral gavage. Data are a composite from three experiments, totaling 15 spleens per treatment group. *, $P < 0.05$, with respect to radiation control values (0 mg/kg/day) based on Dunnett's test.


GM-CFC per Femur
A
300
250
200
150
100
50
0
11
13
15
19
Day After Irradiation
25
20
15
10
5
0
11
13
15
Day After Irradiation


GM-CFC Per Spleen (Thousands)
B
4
3
2
1
0
11
13
15
19
Day After Irradiation
5
4
3
2
1
0
11
13
15
Day After Irradiation

However, recovery in glucan-pefloxacin-treated mice was less than that in mice treated with glucan only, indicating an ability of pefloxacin to dampen glucan-induced GM-CFC recovery. For example, on day 19 after irradiation, bone marrow and splenic GM-CFC recoveries in glucan-pefloxacin-treated mice, respectively, were only 52 and 24% of recoveries observed in mice treated with glucan only.

Microbiological studies. A *Streptococcus* sp., *Lactobacillus* spp., and occasional members of the *Enterobacteriaceae* were the predominant microorganisms recovered from the livers of mice at all time points after irradiation. The presence of these organisms may represent bacterial translocation that could lead to sepsis. Total numbers of organisms in individual mice varied greatly, and bacteria were not detected in all specimens. On days 11 and 13 after irradiation, the total number of microorganisms per gram of liver in mice treated with glucan, pefloxacin, or glucan plus pefloxacin ($\log_{10}$ 0.10 to $\log_{10}$ 0.60) was less than the total number of organisms per gram of liver in radiation control mice ($\log_{10}$ 1.61 to $\log_{10}$ 2.16). In all mice, the total number of organisms per gram of liver increased from day 15 through day 19 after irradiation and then decreased on day 22. However, on day 22 after irradiation, the total number of organisms per gram of liver in glucan-treated and glucan-pefloxacin-treated mice ($\log_{10}$ 0.10 to $\log_{10}$ 0.11) was lower than in radiation control mice or mice treated with pefloxacin only ($\log_{10}$ 0.69 to $\log_{10}$ 2.32). Interestingly, no members of the *Enterobacteriaceae* were found in mice that received pefloxacin treatment. Fungi were not detected.

DISCUSSION

This study demonstrates that pefloxacin therapy enhances mortality following exposure of C3H/HeN mice to midlethal radiation doses. The deleterious effect of this drug appears to be due to an inhibition of hemopoietic progenitor cell proliferation, which could be corrected through the administration of the hemopoietic stimulant glucan. Although pefloxacin appeared to reduce the number of gastrointestinal members of the *Enterobacteriaceae* that translocated through the bloodstream to the liver, the overall effect of this antimicrobial action alone was not beneficial in continually myelosuppressed mice. It may be that the eradication of gram-negative bacteria by pefloxacin reduced the normal continuous release of endotoxin into the circulation. Endotoxin in small amounts induces macrophages to produce interleukin-1 and other cytokines, including hemopoietic growth factors, that lead to increased hemopoiesis. The decreased hemopoiesis observed following pefloxacin treatment could therefore be caused indirectly by its antibacterial activity that eliminates the slow release of endotoxin. The exact cause of the mortality in mice treated with pefloxacin could not be elucidated, but suppression of hemopoietic progenitor cells would ultimately inhibit the generation of cells essential in the clearance of not only invading organisms but also cellular debris from the body. Furthermore, renal failure or electrolyte imbalance cannot be excluded as possible complications leading to mortality.

It is interesting to note that although greatest hemopoietic recovery was observed in mice treated with glucan alone, combination-treated mice exhibited the best survival. It may be that glucan in combination with pefloxacin was able to override the pefloxacin-induced hemopoietic suppression just enough to allow the production of a small but critical number of cells necessary for survival after irradiation. Because glucan also activates endogenous macrophage populations, which tend to be fairly radioresistant (30) and hence survive radiation exposures such as that used in our studies, it may also be that in combination-treated mice pefloxacin reduced the bacterial load while glucan-activated macrophages assisted in controlling the residual bacterial burden and scavenging debris even prior to the generation of new leukocytes.

In contrast to the results of this study performed with C3H/HeN mice, a previous study demonstrated the ability of pefloxacin to enhance survival in irradiated B6D2F1 mice (2). Because C3H/HeN mice contain fewer stem and progenitor cells than B6D2F1 mice (21, 28), following any given radiation dose, these cells will be reduced to a more critical level in C3H/HeN mice than in B6D2F1 mice. Therefore, it is tempting to attribute the detrimental effects of pefloxacin on survival in C3H/HeN mice to a radiation-induced reduction in stem and/or progenitor cell numbers to such a critical level that coupled with reduced regeneration, survival is further impaired. For example, the gamma radiation dose that reduces murine femoral GM-CFC numbers to 37% (D_0) within 24 h after exposure is approximately 1.4 Gy (17, 26). Since B6D2F1 mice contain approximately 11,500 GM-CFC per femur while C3H/HeN mice contain only about 3,500 per femur, a 1.4-Gy radiation dose would theoretically decrease femoral GM-CFC numbers in B6D2F1 mice to 4,255 and those in C3H/HeN mice to 1,295. Following the 7.9-Gy radiation dose used in the studies presented in this paper, only about 15 GM-CFC per femur would be expected to survive the radiation exposure. Even a slight inhibition of the proliferative potential of these few progenitor cells may produce a survival disadvantage. The effect of pefloxacin in B6D2F1 mice after exposure to a radiation dose biologically equivalent to that used in our C3H/HeN mice in terms of hemopoietic injury may clarify this issue.

Although pefloxacin-induced hemopoietic inhibition and mortality were demonstrated in our studies, it turned out that mice consumed less pefloxacin than had been anticipated; consumption of pefloxacin-treated water was about 1.75 ml/day as opposed to the anticipated 2.6 ml/day. Hence, the average pefloxacin dose per mouse was only 1.05 mg/day (about 42 mg/kg/day). Since the recommended daily pefloxacin dose is 64 mg/kg (9), even greater suppressive effects might have been observed had the recommended daily pefloxacin dose actually been consumed.

In addition to the hemopoietic inhibitory effects we observed in C3H/HeN mice, deleterious hemopoietic effects have been reported following quinolone administration to humans (11). It has been known from in vitro studies that quinolone antibiotics can penetrate and accumulate within

FIG. 4. Effects of pefloxacin (+), glucan (*), and glucan-pefloxacin (■) treatments on bone marrow (A) and spleen (B) GM-CFC recovery in irradiated C3H/HeN mice. Mice were exposed to 7.9 Gy of ^{60}Co and administered either pefloxacin, glucan, or both treatments as described in the legend to Fig. 1. □, radiation control mice. Insets expand GM-CFC responses observed at early time points. Above the standard error bars are indicated significant differences between radiation control and pefloxacin treatments (*) and significant differences between glucan and glucan-pefloxacin treatments (+). Average GM-CFC values per femur and per spleen from nonirradiated mice were 1,924 ± 80 and 1,360 ± 93, respectively.

mammalian cells (5). Although less toxic to mammalian cells than to bacteria, high concentrations (above 25 mg/l) of quinolones have been shown to inhibit mammalian DNA replication (7, 10, 13). Additionally, decreased production of superoxide anion by human polymorphonuclear leukocytes, decreased production of immunoglobulins by human lymphocytes, reduced lymphocyte proliferation in response to phytohemagglutinin, decreased interleukin-2 production by lymphocytes, and decreased interleukin-1 production by monocytes have been demonstrated to occur following in vitro exposures to quinolones, including pefloxacin (6, 8, 9, 29).

Since in some instances quinolones have shown promise for the prevention (2) and/or treatment (3) of bacterial sepsis in irradiated mice, their use in humans exposed to radiation is being considered (12). However, because our studies in animals demonstrate that the quinolone pefloxacin can suppress bone marrow recovery and inhibit survival following severe radiation-induced myelosuppression, caution in the use of this agent in severely myelosuppressed patients may be warranted until it is determined whether such effects can also occur in humans. Further studies are needed to explore the hemopoietic effects of other quinolones in irradiated animals and whether in addition to broad-spectrum immunomodulators such as glucan, hemopoietic growth factors such as granulocyte or granulocyte-macrophage colony-stimulating factor could be used to overcome detrimental hemopoietic effects, should they occur.

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