Effect of antimicrobial therapy on the gastrointestinal bacterial flora, infection and mortality in mice exposed to different doses of irradiation

Itzhak Brook and G. David Ledney

Wound Infection Management Program, Experimental Hematology Department, Armed Forces Radiobiology Research Institute, Bethesda, Maryland, USA

The effect of antimicrobial therapy on gut flora, sepsis, and mortality was investigated in C_3H/HeN female mice irradiated with 7·0, 8·0 or 8·5 Gy or ^{60}Co. The antimicrobial agents tested were metronidazole, penicillin, imipenem, gentamicin and ofloxacin. In control mice, the greatest reduction of lactose fermenting organisms (1·7–2·8 logs) occurred on day 8 after irradiation and were related directly to radiation doses. After day 8, lactose fermenting organism levels increased and the increases were associated with mortality due to Enterobacteriaceae sepsis. Irradiation reduced the populations of strict anaerobic bacteria in control mice by 2–8 logs, and these remained at low levels. Treatment with either metronidazole or penicillin resulted in greater reductions of strict anaerobic bacteria than occurred in the controls and induced earlier and greater increases in lactose fermenting organisms and associated mortality. Therapies with either gentamicin or ofloxacin resulted in lesser reductions of strict anaerobic bacteria (1·1–2·2 logs) than occurred in controls, and caused greater decreases in lactose fermenting organisms and mortality. The changes in the bacterial flora and mortality following imipenem treatment were similar to controls. These data demonstrate that in animals exposed to irradiation, antimicrobial agents effective against strict anaerobic bacteria can be deleterious, but antimicrobial agents effective against lactose fermenting organisms may be beneficial.

Introduction

Ionizing radiation increases an animal's susceptibility to systemic infection caused by endogenous and exogenous organisms (Kaplan *et al.*, 1952; Brook, Walker and MacVittie, 1986). The source of most endogenous infections is the gastrointestinal tract which is colonized by aerobic and anaerobic bacteria (Brook *et al.*, 1986). When those bacteria translocate to the liver and spleen after irradiation, they can be associated with fatal septicaemia (Brook *et al.*, 1986, 1988). The most important bacteria isolated from septic animals are from the family of Enterobacteriaceae and *Streptococcus* spp. Prevention of translocation of these organisms and control of the sepsis can prevent mortality in experimental infection (Brook, Elliott & Ledney, 1990). However, a previous study (Brook *et al.*, 1988) demonstrated that therapy with metronidazole, an antimicrobial that decreases the number of the strict anaerobic component of the gut flora, resulted in increased bacterial translocation and systemic infection caused by aerobic or facultative anaerobic bacteria and hastened mortality in lethally irradiated mice.

Correspondence: Dr Itzhak Brook, Naval Medical Research Institute, Bethesda, MD 20889-5055, USA

This study investigated the effects of several other antimicrobial agents on the gut microbial flora, on systemic infection, and on mortality in mice exposed to increased doses of radiation.

.

Materials and methods

Animals

Female C₃H/HeN mice approximately 12 weeks old were obtained from the National Cancer Institute Animal Breeding Facility (Frederick, MD, USA) and were kept in quarantine for about 2 weeks before being transferred to a room with a 12 h light/dark cycle. Representative mice were examined to ensure the absence of specific bacteria and common murine diseases. The mice were maintained in Micro-Insolator cages with hardwood chip bedding in a facility accredited by the American Association for Accredition of Laboratory Animal Care. They were given a commercial rodent diet and acidified water (pH 2·2) that was changed to tap water 48 h before irradiation. All experimental procedures were carried out in compliance with National Institutes of Health and 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 7·0, 8·0 or 8·5 Gy at 0·4 Gy/min from bilaterally positioned ⁶⁰Co sources. Before the experiment, the dose rate at the midline of an acrylic mouse phantom was measured with a 0·5 mL tissue equivalent ionization chamber manufactured by Exradin Inc. (Chicago, IL, USA). The optimal doses to be given to the mice were determined in previous studies (Brook *et al.*, 1988). The dose within the exposure field varied by 3% as determined by thermoluminescent dosimetry with acrylic mouse phantoms.

Antimicrobial agents

All agents were given intramuscularly to alternate thighs in 0·1 mL saline. The optimal dose to be given to each mouse was determined in previous studies (Brook *et al.*, 1988). The daily doses of the antimicrobial agents (administered in divided doses every 12 h) were 7·5 mg/kg for gentamicin (Schering Laboratories, Kenilworth, NJ, USA), 50 mg/kg for metronidazole (GD Searle & Co., Chicago, IL, USA), 50 mg/kg for imipenem-cilastatin (Merck Sharp & Dohme, West Point, PA, USA), 250 mg/kg for procaine penicillin (Wyeth Laboratories, Philadelphia, PA, USA), and 40 mg/kg for ofloxacin (Ortho Pharmaceutical Co., Raritan, NJ, USA). All control animals received im 0·1 mL normal saline.

Serum concentrations of gentamicin, imipenem, penicillin, and ofloxacin were measured by agar diffusion assays (Reeves *et al.*, 1987) with *Bacillus subtilis* ATCC 6633. Metronidazole was assayed by high-pressure chromatography (Wheeler *et al.*, 1978). These assays were performed on day 5 of therapy, 1 and 11·5 h after injection of antimicrobial agents.

Microbiological methods

Mice were observed for symptoms of disease and death for 30 days. Specimens of liver were processed for the presence of bacteria. Because of the difficulty in monitoring the mice 24 h a day, cultures from livers, which must be removed before death, were obtained from only about half of the mice. Specimens of liver were processed for the presence of bacteria as described previously (Brook *et al.,* 1988). No other organs were processed and no blood samples were obtained because previous studies showed that liver cultures correlated better with a diagnosis of sepsis (Brook *et al.,* 1986). The liver specimens were swabbed on to media able to support the growth of aerobic and anaerobic organisms for semi-quantitative determinations of bacterial counts as previously described (Brook *et al.,* 1986). Fresh specimens of stool pellets were obtained from five animals selected at random from each group on days 0, 2, 4, 6, 8, 10, 12, 14 and 16 after irradiation. When fewer than five animals survived in a group, all were examined that day. Fecal pellets were processed and total numbers of anaerobic and aerobic bacteria were determined as previously described (Brook *et al.,* 1986).

The media used to culture facultative and aerobic organisms were sheep blood agar and MacConkey agar. Plates intended to recover aerobic bacteria were incubated in air and 5% carbon dioxide. Pre-reduced anaerobic sheep-blood agar medium was used to culture anaerobic bacteria. The plates were incubated in anaerobic GasPak jars (BBL, Cockeysville, MD, USA). The jars were opened after 48 h and 96 h of incubation to assess bacterial growth and were monitored for a maximum of 10 days. Bacterial isolates were identified by standard criteria (Lennette *et al.,* 1985; Sutter *et al.,* 1985).

Experimental design

Antimicrobial therapy was initiated 48 h after irradiation and was administered for 21 days. Sixty mice were included in each of the experiments and each experiment was performed on two occasions. Each experiment consisted of five treatment groups and a saline-treated control group of ten mice.

Statistical analysis

Statistical analysis was done with the Mantel-Cox test (Lee, 1980).

Results

Survival

All mice irradiated with 7·0 Gy ^{60}Co (Figure 1(a)) survived until day 10 after irradiation. Thereafter three mice died, two that were treated with metronidazole and one that received penicillin.

All mice irradiated with 8·0 Gy (Figure 1(b)) survived until day 9 after irradiation. The subsequent rates were lower in the mice treated with metronidazole or penicillin than in those receiving saline or imipenem ($P < 0.05$). Survival rate was best following gentamicin and ofloxacin treatments ($P < 0.05$).

All mice irradiated with 8·5 Gy (Figure 1(c)) survived until the fifth day after irradiation. The survival rates were lower in the mice treated with metronidazole or penicillin than in those which received saline and imipenem ($P < 0.05$). The highest
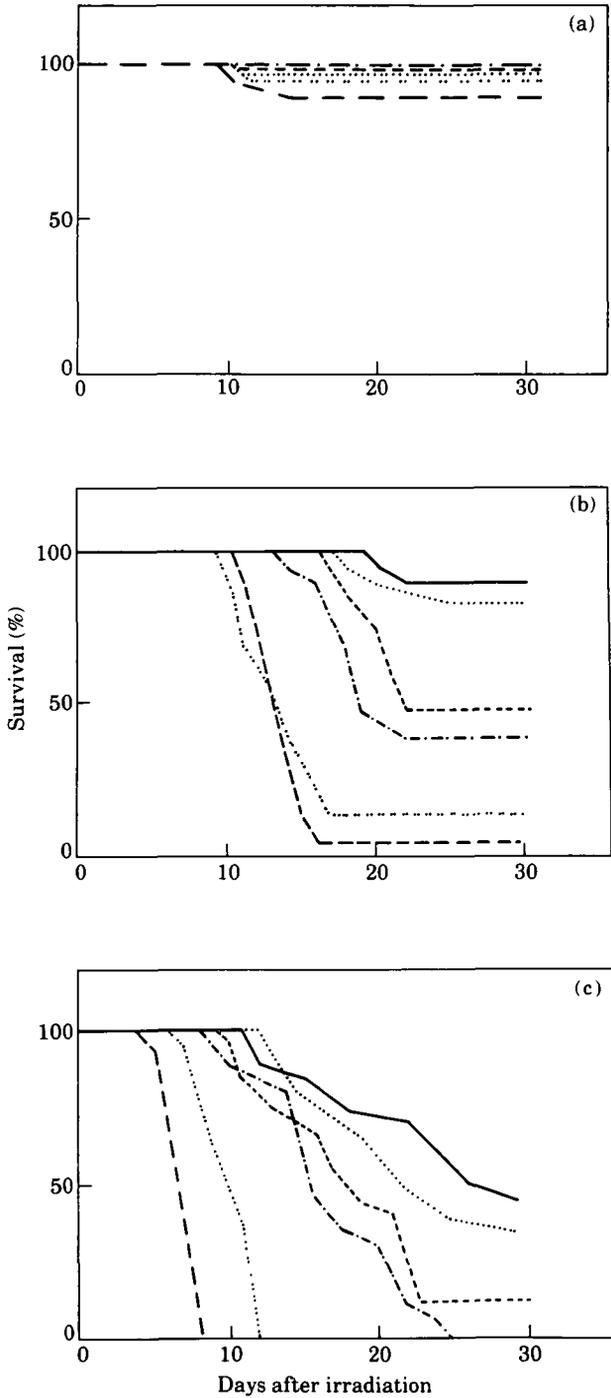
Figure 1. Survival of C3H/HeN mice exposed to 7·0 Gy (a), 8·0 Gy (b) and 8·5 Gy (c) ^{60}Co radiation and treated with saline (control) (—·—·—) and treated with an antimicrobial agent: imipenem 50 mg/kg (– – –); metronidazole 50 mg/kg (——); gentamicin 7·5 mg/kg (·· ·); ofloxacin 40 mg/kg (——); penicillin 250 mg/kg (· · · · · ·). Survival is shown as the mean of two experiments. Gentamicin data in (a) overlaps data for ofloxacin (not shown).

rates of survival recorded were following ofloxacin or gentamicin treatment ($P < 0.05$ for saline, and $P < 0.001$ for metronidazole or penicillin).

Quantitative changes in number of bacteria in stool flora

In the saline-treated control mice irradiated with 7.0 Gy, the lactose fermenting facultative anaerobic bacterial count dropped from 8.2 log to 5.4 log by day 8 and then rose to 6.8 log by day 16 (Figure 2(a)). In the same mice, the count of strict anaerobes dropped from 11.8 log to 9.6 log by day 10 and then recovered to 10.8 log by day 14. In ofloxacin-treated and gentamicin-treated mice the lactose fermenting bacterial count dropped significantly more than that in saline-treated mice ($P < 0.05$) (Figure 2(a)) and did not recover within the duration of the experiment. For metronidazole- and penicillin-treated mice, the counts initially fell slightly and then rose, compared with saline-or imipenem-treated mice ($P < 0.05$). The strict anaerobes count dropped in metronidazole- and penicillin-treated mice ($P < 0.01$ and $P < 0.005$, respectively) compared with the count in control mice.

In the saline-treated control mice irradiated with 8.0 Gy, the lactose fermenting bacterial count dropped from 8.7 log to 6.2 log by day 8 and then rose to 10.1 log by day 16 (Figure 2(b)). In the same mice, the strict anaerobe count declined from 12.2 log to 4.8 log by day 16. In ofloxacin- and gentamicin-treated mice the lactose fermenting bacterial count dropped significantly faster and further than in saline-treated mice. The count failed to recover and registered 3 to 4 log by day 16. In metronidazole- and penicillin-treated mice, the numbers of lactose fermenting bacteria were unchanged at about 9 log up to day 10; thereafter, the counts increased by 1 to 2 log to similar values to those in saline-treated mice ($P > 0.05$) (Figure 2(b)). the numbers of lactose fermenting bacteria in imipenem-treated mice were similar to those in saline-treated mice, except that after the twelfth day the recovery of the count in imipenem-treated mice was less pronounced. The numbers of strict anaerobes dropped in all groups, but were significantly lower, by 2 to 3 logs, in those mice receiving penicillin or metronidazole than in saline-treated mice. The strict anaerobe counts were significantly higher in ofloxacin- and gentamicin-treated animals.

In the control mice irradiated with 8.5 Gy, the lactose fermenting bacterial count dropped from 7.5 log to 5.8 log by day 6 and then rose to 9.5 log by day 16 (Figure 2(c)). In the same mice, the strict anaerobe count decreased from 11.9 log to 3.8 log by day 10 and stayed at this value through to day 16. In ofloxacin- and gentamicin-treated mice, the populations of lactose fermenting bacteria dropped significantly more than that in the saline-treated mice and remained low through day 16 ($P < 0.001$). In metronidazole- and penicillin-treated mice, the lactose fermenting bacterial count initially remained steady but then increased significantly after day 6 ($P < 0.05$). The lactose fermenting bacterial counts in imipenem-treated mice were similar at all times to those in saline-treated mice. The numbers of strict anaerobes dropped in all groups but, compared with the number in saline-treated mice, were significantly lower in those treated with penicillin ($P < 0.05$) or metronidazole ($P < 0.001$) and were significantly higher in those receiving ofloxacin or gentamicin ($P < 0.001$).

Isolation of bacteria from liver

A total of 153 organisms were recovered from 147 mice (Table 1). No statistically significant differences were noted in the types of organisms recovered nor in the number
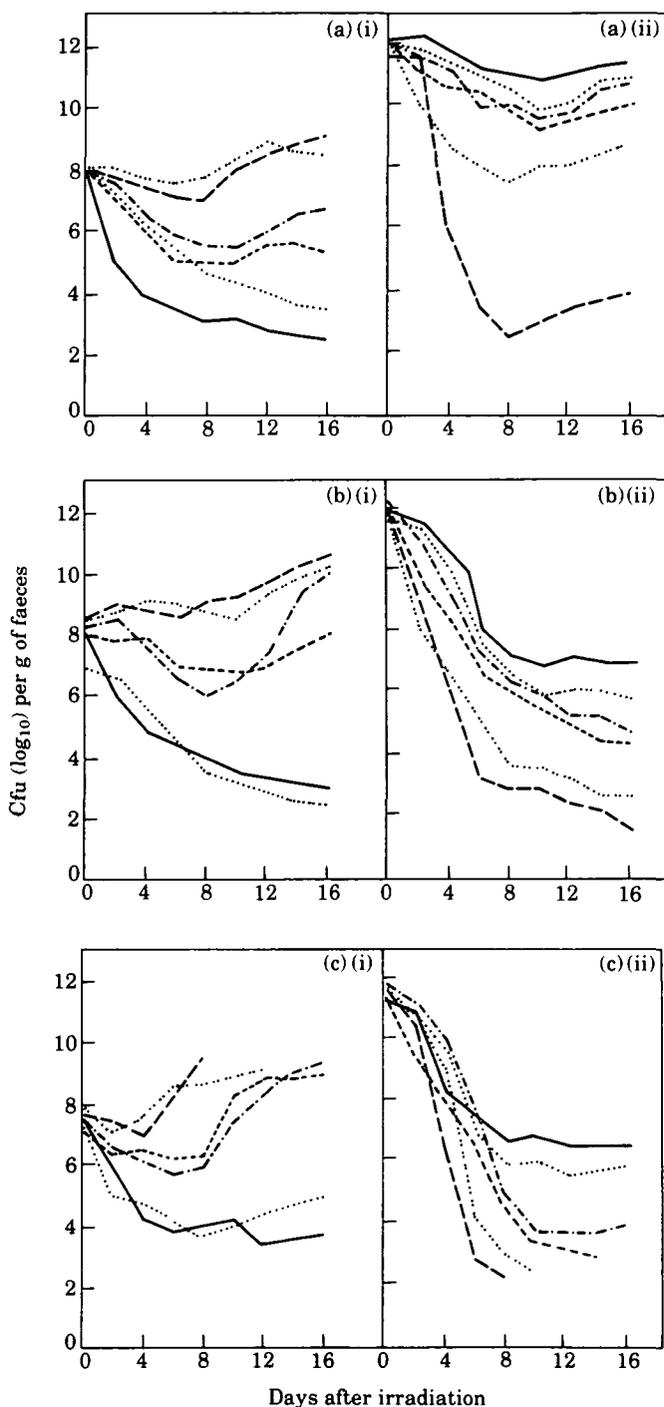
Figure 2. Numbers of lactose fermenting bacteria (i) and strict anaerobes (ii) in the stools of C3H/HeN mice exposed to 7·0 Gy (a), 8·0 Gy (b) and 8·5 Gy (c) ⁶⁰Co radiation treated with saline (control) (—·—) and treated with an antimicrobial agent: imipenem 50 mg/kg (– – –); metronidazole 50 mg/kg (— —); gentamicin 7·5 mg/kg (· · · ·): ofloxacin 40 mg/kg (——): penicillin 250 mg/kg (· · ··). Cfu are shown as the mean of two experiments.

Table. Numbers of bacteria isolated from liver of C3H/HeN mice ($n = 147$) exposed to 8·0 Gy and 8·5 Gy irradiation doses (data from two experiments

Irradiation group antibiotic	No. mice studied	α-haemolytic streptococci	S. aureus	Proteus spp.	E. coli	Peptostreptococcus spp.
8·0 Gy						
saline	9	3	2	1	3	0
metronidazole	16	4	5	2	7	0
penicillin	15	0	1	4	6	0
imipenem	11	4	3	2	1	0
gentamicin	4	2	2	0	0	0
ofloxacin	3	3	0	0	0	0
total	58	16	13	9	17	0
8·50 Gy						
saline	18	5	2	2	12	2
metronidazole	20	2	3	1	14	0
penicillin	12	1	2	0	12	0
imipenem	17	3	2	4	6	0
gentamicin	12	5	2	0	4	1
ofloxacin	10	6	1	0	5	1
total	89	22	12	7	53	4

of organisms/animal isolated from those irradiated with 8·5 Gy (1 isolate/liver) and those irradiated with 8·0 Gy (0·95 isolate/liver). No organisms were isolated from the livers of mice exposed to 7·0 Gy.

The organisms recovered were *Escherichia coli* (70 isolates), α-haemolytic streptococci (38), *Staphylococcus aureus* (25), *Proteus* spp. (16) and *Peptostreptococcus* sp. (4). *Peptostreptococcus* sp. were recovered only from mice exposed to 8·5 Gy. Although not statistically different from the number isolated from saline-treated mice, more *E. coli* were isolated from mice treated with penicillin or metronidazole than from those treated with imipenem, gentamicin or ofloxacin ($P < 0.05$). In contrast, although not statistically different, more aerobic Gram-positive cocci were recovered from mice treated with gentamicin or ofloxacin than from those treated with penicillin or imipenem.

Antimicrobial serum concentrations

Serum concentrations of the antibacterial agents tested were obtained on day 5 of therapy from five mice in each group irradiated with 7·0 Gy. the antimicrobial concentrations (mean ± S.D.) at 1 h and 11·5 h after injection were, respectively: gentamicin, 6.4 ± 1.8 mg/L and 1.2 ± 0.5 mg/L; metronidazole, 24.6 ± 5.8 mg/L and 4.6 ± 2.0 mg/L; penicillin, 18.9 ± 3.4 mg/L and 2.5 ± 1.2 mg/L; imipenem, 24.2 ± 4.8 mg/L and 3.8 ± 1.0 mg/L and ofloxacin, 2.8 ± 0.5 mg/L and 0.6 ± 0.2 mg/L.

Discussion

The data presented confirm the association between the level of exposure to irradiation and bacterial translocation and mortality associated with Enterobacteriaceae (Brook *et al.* 1986). Higher irradiation doses were found to be associated with a high recovery rate of Enterobacteriaceae, *S. aureus*, and α-haemolytic streptococci. The decrease in both aerobic and anaerobic gut flora after irradiation may be due to the loss of

damaged mucosal cells to which they attach. The 'protective' effect of the normal anaerobic bowel flora was previously recognized and was called 'colonization resistance' (van der Waaij, Hofstra & Wiegersma, 1982).

The data also confirm the previously observed reductions in the numbers of aerobes, facultative anaerobes and strict anaerobes that occur in the gut flora of animals after irradiation (Brook et al., 1988). However, this study illustrated that the reductions in the numbers of gut bacteria were directly related to the dose of irradiation—the higher the dose, the more marked the reduction. Control mice exposed to 7·0 Gy had reductions of 2·8 log of lactose fermenting bacteria and 2·2 log of strict anaerobes by day 8 and day 10, respectively, but the numbers of lactose fermenting bacteria and strict anaerobes returned almost to the previous levels by day 16. The lack of mortality in this group of mice underscores the correlation between the preservation of the anaerobic gut flora and the prevention of bacterial translocation and sepsis (van der Waaij et al., 1982). In contrast, mice irradiated with 8·0 and 8·5 Gy displayed marked decreases in the populations of lactose fermenting bacteria and strict anaerobes. However, from the ninth day after irradiation, the numbers of lactose fermenting bacteria increased to about 10 log by day 16, while the numbers of strict anaerobes stayed low. We have previously reported (Brook et al., 1988) that this increase in the lactose fermenting bacteria count was associated with the isolation of lactose fermenting bacteria such as E. coli, and Proteus spp. from the liver.

This study also confirmed the previously observed rapid decline in the strict anaerobes gut flora in lethally irradiated mice that were treated with metronidazole. This decline can be attributed to the activity of metronidazole against strict anaerobic bacteria. A similar decline in the strict anaerobe count in animals treated with penicillin is attributed to the activity of penicillin against most anaerobic gut bacteria except the Bacteroides fragilis group (Sutter & Finegold, 1976) coupled with the lack of efficacy of penicillin against Enterobacteriaceae which are able to displace the strict anaerobes.

The decrease in the numbers of strict anaerobes after metronidazole or penicillin therapy was followed by an earlier increase in the numbers of lactose fermenting bacteria than that seen in saline-treated animals, as was previously reported (Brook et al., 1988). The increase was associated with the isolation of Enterobacteriaceae, S. aureus, and α-haemolytic streptococci from the liver and the occurrence of earlier mortality. The increase in mortality after metronidazole and penicillin therapy may be due, therefore, to increased penetration of facultative anaerobic bacteria through the damaged gut mucous membranes and to a decrease in the local and systemic immunity that follows exposure to irradiation. Similar effects of antimicrobials effective against anaerobic bacteria were reported by Berg (1981) and Wells et al. (1987), who found that metronidazole and clindamycin treatment led to systemic translocation of enteric bacteria in non-irradiated rodents.

In contrast to treatment with metronidazole or penicillin, treatment with gentamicin or ofloxacin had less effect on the strict anaerobe counts, while causing severe falls in the lactose fermenting bacterial count, which failed to recover, unlike the counts in the other groups (Figure 2). Gentamicin- and ofloxacin-treatment were also associated with reductions in the isolation of Enterobacteriaceae from livers and a decrease in the incidence of mortality, which can be attributed to prevention of overgrowth of lactose fermenting bacteria.

Previous studies have demonstrated the efficacy of aminoglycosides (Miller et al., 1952; Hammond, 1954) and quinolones (Brook et al., 1990) in reducing mortality in sublethally irradiated animals. These reductions were associated with lowered levels of

gastrointestinal colonization with Enterobacteriaceae and decreased translocation of these organisms (Brook *et al.*, 1990; Hammond, 1954). The efficacies of these two classes of agents are due to their selective activities against facultative and aerobic Gram-negative bacilli and to their lack of activity against strict anaerobes. This difference allows their use in selective removal from the gut of facultative and aerobic Gram-negative bacilli, while preserving the strict anaerobe flora.

Selective decontamination of the bowel with antimicrobials that are effective only against the aerobic and facultative anaerobic bacteria is used in immunocompromised persons (van der Waaij *et al.*, 1982). Our findings support the importance of the colonization resistance phenomenon and demonstrate that the administration of antibiotics effective against intestinal anaerobic bacteria can be deleterious to the irradiated host.

Several quinolones, including ofloxacin, have been used to selectively decontaminate the gut in attempts to prevent sepsis in immunocompromised patients (Rozenberg-Arska, Dekker & Verhoef, 1985; Pecquet, Andremont & Tancrède, 1986; Liang *et al.*, 1990). These agents have also been found to be effective in the management of septic episodes in neutropenic patients (Kelsey *et al.*, 1989). Because quinolones can be administered orally, coupled with the advantage of achieving selective inhibition of potential pathogens in gut and the ability to treat systemic infections, make them promising agents for prevention and treatment of infection after irradiation.

The changes in the intestinal bacterial flora of mice treated with imipenem were similar to those seen in the saline-treated control group (Figure 2). Although imipenem is as effective against strict anaerobes as metronidazole, it is also effective against most aerobic and facultative anaerobic bacteria. This wide spectrum of activity and the absence of the increased incidence in mortality associated with metronidazole or penicillin treatments suggest that imipenem may be a useful agent with which to treat mixed aerobic-anaerobic infection in irradiated hosts.

Our study demonstrates that the administration of antimicrobials effective against the anaerobic gut flora of mice exposed to high irradiation dosage can be detrimental. Although further studies in humans are warranted, these data illustrate the potential adverse effects of some antibiotics to the irradiated host. Hence, when treating bacterial infections in irradiated hosts, antibiotics with little or no activity against strict anaerobes may minimize the adverse effects of treatment. However, the management of patients with mixed aerobic-anaerobic infections requires the administration of antimicrobials effective against both classes of organisms (Bartlett *et al.*, 1978); as a consequence, more work is needed to develop therapeutic regimens that will effectively treat irradiated patients who develop polymicrobial aerobic-anaerobic infection while minimizing the effects on their colonization-resistant flora.

References

Bartlett, J. G., Onderdonk, A. B., Louie, T., Kasper, D. L. & Gorbach, S. L. (1978). A review. Lessons from an animal model of intra-abdominal sepsis. *Archives of Surgery* **113**, 853–7.

Berg, R. D. (1981). Promotion of the translocation of enteric bacteria from the gastrointestinal tracts of mice by oral treatment with penicillin, clindamycin, or metronidazole. *Infection and Immunity* **33**, 854–6.

Brook, I., Elliott, T. B. & Ledney, G. D. (1990). Quinolone therapy of *Klebsiella pneumoniae* sepsis following irradiation: comparison of pefloxacin, ciprofloxacin, and ofloxacin. *Radiation Research* **122**, 215–7.

Brook, I., Walker, R. I. & MacVittie, T. J. (1986). Effect of radiation dose on the recovery of aerobic and anaerobic bacteria from mice. *Canadian Journal of Microbiology* **32**, 719–22.

Brook, I., Walker, R. I. & MacVittie, T. J. (1988). Effect of antimicrobial therapy on bowel flora and bacterial infection in irradiated mice. *International Journal of Radiation Biology* **53**, 709–16.

Kaplan, H. S., Speck, R. S. & Jawetz, E. (1952). Impairment of antimicrobial defenses following total body irradiation of mice. *Journal of Laboratory and Clinical Medicine* **40**, 682–91.

Hammond, C. W. (1954). The treatment of post-irradiation infection. *Radiation Research* **1**, 448–58.

Kelsey, S. M., Wood, M. E., Shaw, E. & Newland, A. C. (1989). Intravenous ciprofloxacin as empirical treatment of febrile neutropenic patients. *American Journal of Medicine* **87**, *Suppl. 5A*, 274s–7s.

Lee, T. E. (1980). *Statistical Method for Survival Data Analysis*, pp. 127–9. Life Time Learning Publication, Belmont, CA.

Lennette, E. H., Balows, A., Hausler, W. J., Shadomy, H. J., Eds (1985). *Manual of Clinical Microbiology*, 4th edn. American Society for Microbiology, Washington, DC.

Liang, R. H. S., Yung, R. W. H., Chan, T.-K., Chau, P.-Y., Lam, W.-K., So, S.-Y. *et al.* (1990). Ofloxacin versus co-trimoxazole for prevention of infection in neutropenic patients following cytotoxic chemotherapy. *Antimicrobial Agents and Chemotherapy* **34**, 215–8.

Miller, C. P., Hammond, C. W., Tompkins, M. & Shorter, G. (1952). The treatment of post-irradiation infection with antibiotics: an experimental study on mice. *Journal of Laboratory and Clinical Medicine* **39**, 462–79.

Pecquet, S. A., Andremont, A. & Tancrède, C. (1986). Selective antimicrobial modulation of the intestinal tract by norfloxacin in human volunteers and in gnotobiotic mice associated with a human fecal flora. *Antimicrobial Agents and Chemotherapy* **29**, 1047–52.

Reeves, D. S., Phillips, I., Williams, J. V. & Wise, R., Eds (1987). *Laboratory Methods in Antimicrobial Chemotherapy*. Churchill Livingstone, Edinburgh.

Rozenberg-Arska, M., Dekker, A. W. & Verhoef, J. (1985). Ciprofloxacin for selective decontamination of the alimentary tract in patients with acute leukemia during remission induction treatment: the effect on fecal flora. *Journal of Infectious Diseases* **152**, 104–7.

Sutter, V. L., Citron, E. M., Edelstein, M. A. C. & Finegold, S. M. (1985). *Wadsworth Anaerobic Bacteriology Manual,* 4th edn. Star Publishing, Belmont, CA.

Sutter, V. L. & Finegold, S. M. (1976). Susceptibility of anaerobic bacteria to 23 antimicrobial agents. *Antimicrobial Agents and Chemotherapy* **10**, 736–52.

van der Waaij, D., Hofstra, H. & Wiegersma, N. (1982). Effect of β-lactam antibiotics on the resistance of the digestive tract of mice to colonization. *Journal of Infectious Diseases* **146**, 417–22.

Wells, C. L., Maddaus, M. A., Reynolds, C. M., Jechorek, R. P. & Simmons, R. L. (1987). Role of anaerobic flora in the translocation of aerobic and facultatively anaerobic intestinal bacteria. *Infection and Immunity* **55**, 2689–94.

Wheeler, L. A., De Meo, M., Halula, M., George, L. & Heseltine, P. (1978). Use of high-pressure liquid chromatography to determine plasma levels of metronidazole and metabolites after intravenous administration. *Antimicrobial Agents and Chemotherapy* **13**, 205–9.