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Quinolone and glycopeptide therapy for infection in mouse following exposure to mixed-field neutron-γ-photon radiation

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Abstract. The effects of increased doses of mixed-field neutron-γ-photon irradiation on bacterial translocation and subsequent sepsis, and the influence of antimicrobial therapy on these events, were studied in the C3H/HeN mouse. Animals were given 4·25, 4·50, 4·75, 5·00 and 5·25 Gy of mixed-field [n/(n+γ)=0·7] radiation. The mortality rate of mice and recovery of bacteria were directly related to the radiation dose. *Enterobacteriaceae* were mostly isolated from the livers of mice exposed to 5·00 and 5·25 Gy, and aerobic Gram-positive cocci were recovered from those exposed to 4·50 and 4·75 Gy. Oral therapy with L-ofloxacin reduced mortality of all groups of animals except those given 4·25 and 4·50 Gy. This reduction was associated with a decrease in the number of the recovered *Enterobacteriaceae*. However, the number of Gram-positive cocci was unaffected. Addition of i.m. glycopeptide therapy failed to prevent Gram-positive coccal infection, due to the development of glycopeptide-resistant *Enterococcus faecalis*. These data demonstrate a relationship between the doses of mixed-field radiation and the rates of infection due to *Enterobacteriaceae*. While L-ofloxacin therapy reduces the infection rate, prolongs survival and prevents mortality, the addition of a glycopeptide can enhance systemic infection by resistant bacteria in the irradiated host.

1. Introduction

Ionizing radiation increases a recipient's susceptibility to systemic infection due to endogenous and exogenous organisms (Kaplan *et al.* 1965, Brook *et al.* 1986). Most endogenous infections in γ-photon-irradiated animals originate in the bacterial flora of the gastrointestinal tract (Brook *et al.* 1986). Following irradiation, some members of that bacterial flora translocate to the liver and spleen, and they can be associated with fatal septicemia (Brook *et al.* 1986, Brook *et al.* 1988). The predominant organisms that can be recovered from septic animals are *Enterobacteriaceae* and *Streptococcus* spp. Preventing translocation of these bacteria and providing therapy of the subsequent sepsis can reduce mortality in experimental infection (Brook *et al.* 1990). However, antimicrobial agents that inhibit the anaerobic gastrointestinal flora can also enhance bacterial translocation and increase the mortality rate (Brook *et al.* 1988).

Previous studies (Brook and Ledney 1990, 1991, Brook *et al.* 1990) demonstrated the ability of quinolone therapy to reduce bacterial translocation and subsequent sepsis due to *Enterobacteriaceae* in the γ-photon-irradiated mouse. However, bacterial sepsis was documented in over one-quarter of the mice due to quinolone resistant *Streptococcus* spp. (Brook and Ledney 1991). The addition of an antimicrobial agent such as penicillin, which is effective against the *Streptococcus* spp., reduced the infection due to quinolone resistant *Streptococcus* spp. and increased the survival rate (Brook and Ledney 1991). Because of the growing resistance of *Streptococcus* spp. to penicillin and the occurrence of other Gram-positive pathogens such as *Staphylococcus* spp., evaluating the efficacy of a more potent antimicrobial agent was desired. Most of the past research relating to infection following irradiation was done in γ-photon-irradiated animals, and limited work was done on the bacterial aetiology and therapy of sepsis following neutron irradiation (Hammond *et al.* 1955).

Exposure to neutron radiation can occur accidentally or following therapeutic use of radiation. Although infection can occur following γ-photon as well as neutron irradiation, infection following neutron irradiation may have unique features due to the greater effect that neutrons have upon the intestinal epithelial cell lining than do γ-photons (Ledney *et al.* 1991). This study was designed to investigate the effect of increased doses of mixed-field neutron-γ-photon irradiation on bacterial translocation and subsequent sepsis and the mortality rate in mice using therapy with L-ofloxacin (a quinolone) and vancomycin or teicoplaning (glycopeptides).

2. Materials and methods

2.1. *Animals*

Female C3H/HeN mice (approximately 12 weeks old) were obtained from the National Cancer Insti-

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tute animal breeding facility (Frederick, MD, USA). All animals were kept in quarantine for about 2 weeks before transfer to a room with a 12-h 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 National Research Council guidelines and approved by an Institutional Animal Care and Use Committee.

2.2. *Mixed-field irradiation*

Mixed-field irradiation was performed using the AFRRI TRIGA Mark-F reactor. This reactor is a movable-core pool-type facility with maximum operational steady-state power of 1 MW. The reactor was operated at 45 kW. The neutron to total dose of neutrons and γ-photons ($[n/(n+\gamma)]$ dose ratio of 0·7) was achieved by irradiating mice through a 5-cm lead shield, 255 cm from the tank wall and 120 cm above the exposure room floor (Ledney *et al.* 1991). The mean energy for neutrons and γ-photons in experiments was approximately 0·8 MeV (Zeman and Ferlic 1984). Thus, mice received radiation doses with a spectrum of energies that contained a mixture of neutrons ($n = 70\%$) and γ-photons ($\gamma = 30\%$). All irradiations were performed at a total dose rate of 38 cGy/min. The total dose-rate varied $<2\%$ over the entire radiation field. Mice were irradiated in aerated aluminum tubes that rotated at 1·5 rpm.

2.3. *Antimicrobial agents*

Standard powder formulation of antimicrobials were used for *in vitro* susceptibility studies. The antimicrobials were administered orally (L-ofloxacin), or i.m. (glycopeptides) in a volume of 0·1 ml sterile saline. The daily doses of the antimicrobial agents were 50 mg/kg for vancomycin (Eli Lilly Ind., Indianapolis, IN, USA) and 50 mg/kg for teicoplanin (Dow Pharmaceuticals, Cincinnati, OH, USA) given every 12 h and 20 mg/kg for oral L-ofloxacin (Ortho Pharmaceuticals Corp., Raritan, NJ, USA) given once a day. Control animals received 0·1 ml sterile saline i.m. once a day.

Serum concentrations of all antimicrobials were measured by the agar diffusion assay (Reeves *et al.* 1987) with *Bacillus subtilis*, ATCC strain number 6633 (American Type Culture Collection, Rockville, USA). Measurements were made in non-irradiated mice on day 5 of therapy at 1 and 11·5 h after glycopeptide administration and 23·5 h after L-ofloxacin administration. The method could not detect antimicrobial concentrations $<0{\cdot}2\ \mu g/ml$.

2.4. *Microbiological methods*

Animals were killed by cervical dislocation. Specimens of livers and ilea were processed semiquantitatively 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 (Brook *et al.* 1986). About 500 mg liver tissue and about 1 cm of ileum were aseptically removed and homogenized immediately. The specimens were swabbed onto media supportive of aerobic and anaerobic bacteria.

The media used for facultative and aerobic organisms were sheep blood and MacConkey agars. Aerobic plates were incubated in air and 5% carbon dioxide. Prereduced anaerobic sheep blood agar medium was used for anaerobic bacteria. Plates were incubated in anaerobic GasPak jars (BBL, Cockeysville, MD, USA) opened after 48 and 96 h of incubation, and observed for 7 days. All media were incubated at 37°C. Isolates were identified by standard criteria (Lennette *et al.* 1985, Sutter *et al.* 1985). Susceptibility testing was done using the Kirby–Bauer method (Lennette *et al.* 1985).

2.5. *Experimental design*

Antimicrobial treatments were initiated 72 h after irradiation and administered for 21 days. Animals were observed for survival for 60 days. Terminally-ill mice were killed during the experiment. The effect of increasing doses of mixed-field irradiation on translocation and mortality rate and the effect of L-ofloxacin therapy were studied in the first set of experiments. Increased doses of radiation were administered to each group: 4·25, 4·50, 4·75, 5·00 and 5·25 Gy. An additional group that was not irradiated served as a control. Six groups of 40 mice each were included in this experiment ($n = 240$). Each group was divided into two groups of 20: one group was observed for 60 days for survival, and the second group was used for cultures of liver and ileum. (Tables 1 and 2).

In the second and third experiments, the effects of

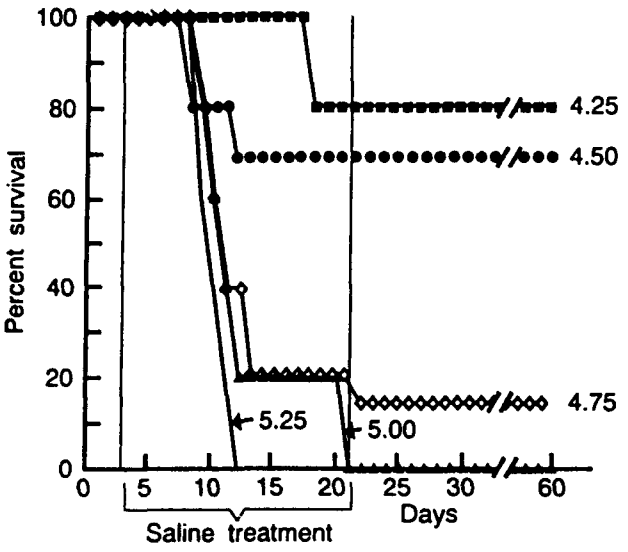

Figure 1. Effects of various doses (Gy) of mixed field irradiation on survival of the saline-treated (control) C3H/HeN mouse.

quinolone and glycopeptide therapies were studied in mice exposed to 4·75 Gy. This dose was chosen because L-ofloxacin therapy had the best efficacy on survival. A total of 88 mice were included in the second experiment and 72 in the third experiment. Each experiment consisted of four equally divided sets of mice consisting of three antimicrobial therapy groups and a saline-treated control group. The therapy groups were as follows; one group received L-ofloxacin, one a glycopeptide (vancomycin or teicoplanin), and one both L-ofloxacin and a glycopeptide. Each group consisted of 10 mice used for cultures of livers (five mice each on days 12 and 14 after irradiation) while the rest were used to monitor mortality. When fewer than five animals survived in a group, all were studied that day.

In the second experiment (Table 3) one group received oral L-ofloxacin, one teicoplanin, one the combination of the two, and one saline. In the third experiment (Table 4), one group received L-ofloxacin, one vancomycin, one the combination of the two, and one saline.

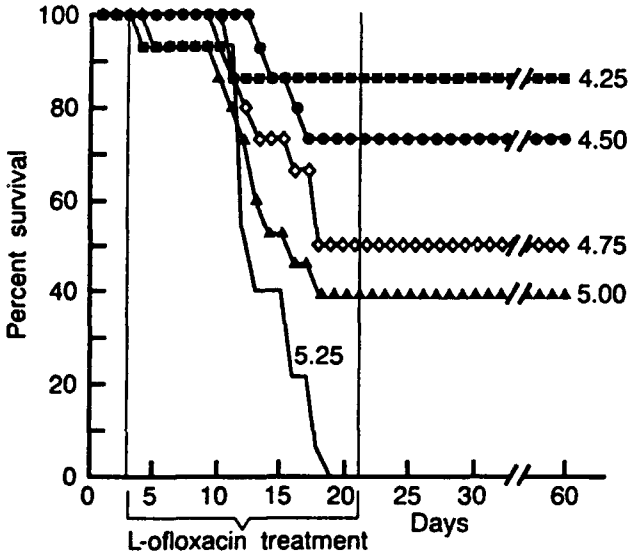

Figure 2. Effect of various doses (Gy) of mixed field irradiation on survival of the L-ofloxacin-treated C3H/HeN mouse.

2.6. *Statistical analysis*

Statistical analysis was done using the Mantel–Cox test (Lee 1980).

3. Results

3.1. *Mortality*

In the first experiments (Figures 1 and 2), mortality rate was directly related to the dose of irradiation. In saline-treated mice, all mice exposed to 5·0 and 5·25 Gy, 85% of those exposed to 4·75 Gy, 30% exposed to 4·50 Gy, and 20% exposed to 4·25 Gy expired within 21 days. Therapy with L-ofloxacin did not change the ultimate survival of those exposed to 5·25 Gy although their mean survival time improved from 8·2 to 14·2 days. However, in those exposed to 5·00 Gy, 40% survived ($p<0·05$), and in those exposed to 4·75 Gy, 50% survived ($p<0·05$). There was no improvement in survival rate in those exposed to 4·50 and 4·25 Gy.

In the second and third sets of experiments, mortality was 20 and 40% in L-ofloxacin treated mice (Figures 3 and 4), as compared with 75 and 80% in saline-treated mice ($p<0·05$). Mortality rate in those treated with either of the glycopeptides alone or a glycopeptide combined with L-ofloxacin was greater than controls ($p<0·05$) or those treated with L-ofloxacin alone ($p0·001$).

3.2. *Isolation of bacteria*

In the first experiment no bacteria were isolated from the livers of non-irradiated mice (data not shown) or those exposed to 4·25 Gy (Table 1).

Most of the organisms were recovered from mice irradiated with 5·00 and 5·25 Gy. Fifty-three differ-

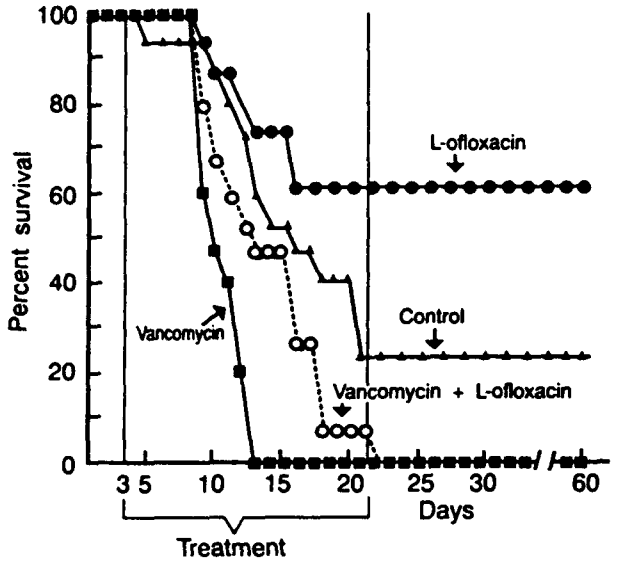

Figure 3. Survival of the 4·75 Gy mixed-field-irradiated C3H/HeN mouse treated with L-ofloxacin and vancomycin.

ent isolates were recovered from all mice: 21 (40%) from those exposed to 5·25 Gy; 18 (34%) from those exposed to 5·00 Gy; 11 (21%) from those exposed to 4·75 Gy; and 3 (6%) from those exposed to 4·50 Gy. The total number of recovered organisms increased from six organisms on day 8, to 15, 16 and 16 organisms on days 10, 12 and 14, respectively.

The predominant bacteria were *Escherichia coli* (21 isolates), alpha hemolytic *Streptococcus* (6), and *Enterobacter aerogenes* and *Acinetobacter* spp. (5 each). Most *Enterobacteriaceae* were recovered in mice exposed to 5·00 and 5·25 Gy and most *Staphylococcus* spp. were recovered in those exposed to 4·50 and 4·75 Gy. Three strains of anaerobic cocci were also isolated. In one instance anaerobic cocci were the only isolate recovered from a liver abscess on day 8 after irradiation from a mouse given 5·00 Gy.

A significant reduction in the number of the isolated *Enterobacteriaceae* was noted in the livers of mice treated with L-ofloxacin (Table 2). Of the 19 isolates recovered from these animals, only two were *E. coli* and the others were Gram-positive bacteria.

The number of isolates recovered from the livers studied in the second experiment were 26 *Enterobacteriaceae* spp. (including 14 *E. coli*, five *Klebsiella pneumoniae*, four *Acinetobacter* spp. and three *E. aerogenes*) and 22 Gram-positive aerobic bacteria (including six *E. faecalis* and five *Staphylococcus aureus*) (Table 3).

In the third experiment the isolates were 24 *Enterobacteriaceae* spp., 23 Gram-positive aerobic bacteria (including 15 gamma-hemolytic streptococcus, five *E. faecalis* and four *S. aureus*) (Table 4). More *Enterobacteriaceae* were isolated in both sets of experiments in mice receiving the glycopeptides than in those receiving L-ofloxacin ($p < 0.05$). *E. faecalis* was isolated only in mice treated with glycopeptide alone or combined with L-ofloxacin.

Organisms similar to those isolated in the liver were also recovered in the ileum as predominant bacteria. *Enterobacteriaceae* were absent in the colon of mice treated with L-ofloxacin, while the number of strict anaerobes was unchanged. In contrast, the number of *Enterobacteriaceae* was unchanged in mice treated with a glycopeptide, but the number of strict anaerobes decreased to a minimum, and the number of *Streptococcus* spp. increased.

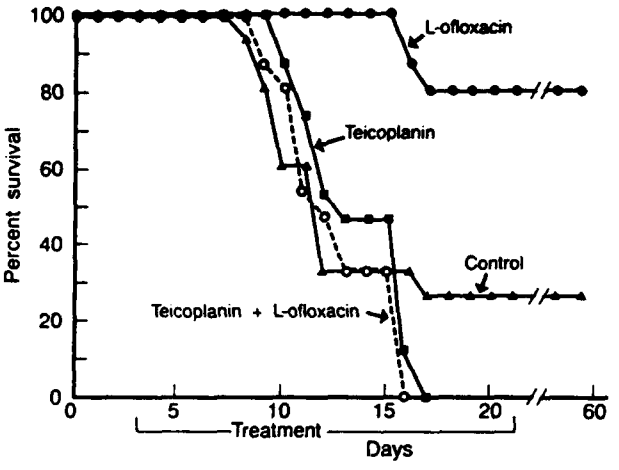

Figure 4. Survival of the 4·75 Gy mixed-field-irradiated C3H/HeN mouse treated with L-ofloxacin and teicoplanin.

3.3. *Antimicrobial serum concentrations*

Antimicrobial serum concentrations were obtained in five animals in each antimicrobial group on day 5 of therapy in unirradiated mice. The antimicrobial concentrations (mean ± SD) for L-ofloxacin at 1 and 23·5 h after injection were 2·4 ± 0·5 and 0·5 ± 0·2 μg/ml; for vancomycin at 1 and 11·5 h, 57·9 ± 6·4 and 12·5 ± 3·1 μg/ml; and for teicoplanin 49·6 ± 4·3 and 10·2 ± 2·3 μg/ml.

3.4. *Antimicrobial susceptibility*

All *E. faecalis* isolates were resistant to L-ofloxacin, teicoplanin and vancomycin as determined by the Kirby–Bauer method (Reeves *et al.* 1987).

Table 1. Bacteria recovered from livers of saline-treated mice irradiated with different doses (five mice per group were studied each day[a])

Irradiation dose (Gy):	Days after irradiation: 8					10					12					14					Total number of organisms
	4·25	4·50	4·75	5·00	5·25	4·25	4·50	4·75	5·00	5·25	4·25	4·50	4·75	5·00	5·25	4·25	4·50	4·75	5·00	5·25	
Aerobic bacteria																					
Escherichia coli					2			1	3	2			1	3	4				3	2	21
Klebsiella pneumoniae				1					1											1	3
Enterobacter aerogenes								1		1				1						2	5
Acinetobacter spp.					1					1					1				1	1	5
Staphylococcus epidermidis								1					1				1				3
Staphylococcus aureus							1						1					1	1		4
Alpha hem. streptococcus				1				1					1		1			2			6
Enterococcus faecalis									1			1			1						3
Anaerobic bacteria																					
Peptostreptococcus spp.				1						1									1		3
Total	0	0	0	3	3	0	1	4	5	5	0	1	4	4	7	0	1	3	6	6	53

[a] Only three mice were studied on day 14.

4. Discussion

These data demonstrate a relationship between the dose of mixed-field radiation to which the animals were exposed and the number and type of bacteria recovered. *Enterobacteriaceae* were more often isolated from mice exposed to 5·00 and 5·25 Gy, as compared with lower sublethal dosages, while aerobic Gram-positive cocci were mostly recovered from mice exposed to 4·50 and 4·75 Gy. These data are similar to those observed in γ-photon-irradiated mice (Brook *et al.* 1986) amd suggest common effects of neutron and γ-photon irradiation on the host gut flora and defenses. However, since the animals received a mixture of neutrons and γ-photons in this study, it is difficult to separate the effects of each type of radiation. The beneficial effect of quinolone therapy on survival of mice exposed to a higher dose of irradiation may be related to its activity against *Enterobacteriaceae*. These organisms were mostly recovered from livers of untreated animals and were eliminated from mice treated with quinolone. However, infection due to quinolone-resistant Gram-positive aerobic bacteria still occurred, and probably contributed to the mortality of those exposed to a high dose of radiation. The effect of quinolone

Table 2. Bacteria recovered from livers of L-ofloxacin-treated mice irradiated with different doses (5 mice per group were studied each day)

Irradiation dose (Gy):	Days after irradiation: 10					12					14					Total number of organisms
	4·25	4·50	4·75	5·00	5·25	4·25	4·50	4·75	5·00	5·25	4·25	4·50	4·75	5·00	5·25	
Aerobic bacteria																
Escherichia coli										1					1	2
Staphyloccus aureus			1										1			2
Staphyloccus epidermidis			1				1					1	1			4
Alpha hem. streptococcus		1		1			2	1	1			1				7
Streptococcus faecalis			1											1		2
Anaerobic bacteria																
Peptostreptococcus spp.					1									1		2
Total	0	1	3	1	1	0	3	1	1	1	0	2	2	2	1	19

Table 3. Recovery of organisms from livers of C3H/HeN mice irradiated with 4·75 Gy and treated with oral L-ofloxacin and i.m. teicoplanin

Organism	Therapy	Days after irradiation[a] 12	14	Total
Enterobacteriaceae spp	L-Ofloxacin	1/10	0/8	1/18
	Teicoplanin	6/7	3/5	9/12
	L-Ofloxacin and teicoplanin	4/8	2/3	6/11
	Saline	6/10	4/6	10/16
Aerobic Gram-positive bacteria	L-Ofloxacin	4/10	2/8	6/18
	Teicoplanin	5/7	2/5	7/12[b]
	L-Ofloxacin and teicoplanin	2/8	1/3	3/11[c]
	Saline	4/10	2/6	6/16

[a] Number of animals with bacteria/number of animals studied.
[b] *Enterococcus faecalis* resistant to teicoplanin (4 of 7).
[c] *Enterococcus faecalis* resistant to teicoplanin (2 of 3).

therapy is similar to the one observed in γ-photon-irradiated mice (Brook *et al.* 1990, Brook and Ledney 1990).

The addition of parenteral glycopeptide therapy, which was provided to improve the survival of the animals by suppressing the emerging Gram-positive coccal organisms in the gut, was surprisingly deleterious. Instead of reducing streptococcal and staphylococcal infection, mortality rate was enhanced and was associated with the emergence of a resistant *Enterococcus*. The observation of the deleterious effect of i.m. glycopeptide may be due to the excretion of the agent through the bile into the gastrointestinal tract (Geraci 1977) where it can reduce the number of strict anaerobic bacteria (Kennedy and Volz 1985a,b) while selecting a resistant *Enterococcus*.

The reduction in the number of anaerobic bacteria in the gut flora may promote the proliferation of fungal species as well as antimicrobial-resistant bacteria. The findings of this study suggest that oral glycopeptide therapy can select an *E. faecalis*, resistant to both glycopeptides and quinolones. An increased frequency of recovery of these organisms was also reported in immunocompromized patients treated with vancomycin (Judeja *et al.* 1983, Green *et al.* 1991). Although quinolone therapy reduced the number of *Enterobacteriaceae* in the gut, the emergence of new pathogens was detrimental to the host. The emergence of a glycopeptide-resistant *E. faecalis* is an alarming observation, and if this phenomenon occurs in patients it will undoubtedly be of grave clinical consequence and should discourage overuse of glycopeptide therapy.

Vancomycin therapy was found to suppress the number of enteric bacilli population levels in mice and to promote *Candida albicans* fungemia (Kennedy

Table 4. Recovery of organisms from livers of C3H/HeN mice irradiated with 4·75 Gy and treated with oral L-ofloxacin and oral vancomycin

Organism	Therapy	Days after irradiation[a] 12	14	Total
Enterobacteriaceae spp.	L-Ofloxacin	0/10	0/8	0/18
	Vancomycin	7/10	4/7	11/17
	L-Ofloxacin and vancomycin	2/10	0/3	2/13
	Saline	6/10	5/9	11/19
Aerobic Gram-positive bacteria	L-Ofloxacin	2/10	4/8	6/18
	Vancomycin	3/10	2/7	5/17[c]
	L-Ofloxacin and vancomycin	4/10	3/3	7/13[c]
	Saline	3/10	2/9	5/19

[a] Number of animals with bacteria/number of animals studied.
[b] *Enterococcus faecalis* resistant to vancomycin (3 of 5).
[c] *Enterococcus faecalis* resistant to vancomycin (2 of 7).

and Volz 1985a,b, Green *et al.* 1991). Administration of vancomycin to patients with acute lymphocytic leukaemia promoted proliferation of *Candida* organisms in the gastrointestinal tract and increased the risk of candidemia (Ricket *et al.* 1991).

Although parenteral glycopeptide therapy has an important role and has been used for over two decades in the management of febrile neutropenic patients (Hathorn *et al.* 1987), this present study highlights the risk associated with administration of glycopeptide therapy alone or in combination with a quinolone in an effort to prevent or abort bacterial infection following mixed-field irradiation. An increased risk of secondary bacterial infection was recently observed in patients receiving oral vancomycin therapy (Winston *et al.* 1990). Further studies are warranted to elucidate the mechanisms of emergence of bacterial resistance in response to such therapy. This animal model of failure of glycopeptide therapy in an immunocompromized host may be useful in understanding and overcoming the problem.

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References

Brook, I., Elliott, T. B. and Ledney, G. D., 1990, Quinolone therapy of *Klebsiella pneumoniae* sepsis following irradiation: comparison of pefloxacin, ciprofloxacin, and ofloxacin. *Radiation Research*, **122**, 215–217.

Brook, I. and Ledney, G. D., 1990, Oral ofloxacin therapy of *Pseudomonas aeruginosa* sepsis in mice after irradiation. *Antimicrobial Agents and Chemotherapy*, **34**, 1387–1389.

Brook, I. and Ledney, G. D., 1991, Ofloxacin and penicillin G combination therapy in the prevention of bacterial translocation and animal mortality after irradiation. *Antimicrobial Agents and Chemotherapy*, **35**, 1685–1687.

Brook, I., Walker, R. I. and MacVittie, T. J., 1986, Effect of radiation dose on the recovery of aerobic and anaerobic bacteria from mice. *Canadian Journal of Microbiology*, **32**, 719–722.

Brook, I., Walker, R. I. and MacVittie, T. J., 1988, Effect of antimicrobial therapy on bowel flora and bacterial infection in irradiated mice. *International Journal of Radiation Biology*, **53**, 709–716.

Geraci, J. E., 1977, Vancomycin. *Mayo Clinic Proceedings*, **52**, 631–634.

Green, M., Barbadora, K. and Michaels, M., 1991, Recovery of vancomycin-resistant Gram-positive cocci from pediatric liver transplant recipients. *Journal of Clinical Microbiology*, **29**, 2503–2506.

Hammond, C. W., Vogel, H. H. Jr., Clark, J. W., Cooper, D. B. and Miller, C. P., 1955, The effect of streptomycin therapy on mice irradiated with fast neutrons. *Radiation Research*, **115**, 354–360.

Hathorn, J. W., Rubin, M. and Pizzo, P. A., 1987, Empirical antibiotic therapy in the febrile neutropenic cancer patient: clinical efficacy and impact of monotherapy. *Antimicrobial Agents and Chemotherapy*, **31**, 971–977.

Judeja, L., Kantanjian, H. and Bolivar, R., 1983, *Streptococcus bovis* septicemia and meningitis associated with chronic radiation enterocolitis. *Southern Medical Journal*, **76**, 1588–1589.

Kaplan, H. W., Speck, R. S. and Jawetz, F., 1965, Impairment of antimicrobial defenses following total body irradiation of mice. *Journal of Clinical Medicine*, **40**, 682–691.

Kennedy, M. J. and Volz, P. A., 1985a, Effect of various antibiotics on gastrointestinal colonization and dissemination by *Candida albicans*. *Journal of Medical Veterinary Mycology*, **23**, 265–273.

Kennedy, M. J. and Volz, P. A., 1985b, Ecology of *Candida albicans* gut colonization. Inhibition of *Candida* adhesion colonization, and dissemination from the gastrointestinal tract by bacterial antagonism. *Infection and Immunity*, **49**, 654–663.

Ledney, G. D., Madonna, G. S., Elliott, T. B., Moore, M. M. and Jackson, W. E. III, 1991, Therapy of infections in mice irradiated in mixed neutron/photon fields and inflicted with wound trauma: a review of current work. *Radiation Research*, **128**, S18–28.

Lee, T. E., 1980, *Statistical Method for Survival Data Analysis* (Belmont, CA, Lifetime Learning), pp. 127–129.

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

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

Ricket, H. M., Andremont, A., Tancrede, C., Pico, J. L. and Jarvis, W. R., 1991, Risk factors for candidemia in patients with acute lymphocytic leukemia. *Reviews in Infectious Diseases*, **13**, 211–215.

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

Winston, D. J., Winston, G. H., Buckner, D. A., Gale, R., and Champlin, R. C., 1990, Ofloxacin versus vancomycin/polymyxin for prevention of infection in granulocytopenic patients. *American Journal of Medicine*, **88**, 36–42.

Zeman, G. H. and Ferlic, K. P., 1984, Paired Ion Chamber Constants for Fission Gamma-Neutron Fields. Technical Report TR84-8. Armed Forces Radiobiology Research Institute, Bethesda, MD. National Technical Information Services, Springfield, VA, AD A155069.