Effect of antimicrobial therapy on bowel flora and bacterial infection in irradiated mice

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Mice exposed to 10 Gy cobalt-60 radiation were given intramuscular antimicro-
bial therapy of gentamicin, or metronidazole, or a combination of the two.
Mortality in the mice treated with metronidazole alone or in combination with
gentamicin occurred earlier than in the controls ($P < 0.001$). Microorganisms
were recovered from the blood, spleen, and liver of the metronidazole-treated
mice earlier than from other groups. The predominant organisms recovered from
these animals were Enterobacteriaceae. Quantitative cultures of the ileal flora
showed a decrease in the number of aerobic, facultative anaerobic and strict
anaerobic bacteria after irradiation, and a subsequent increase only in the number
of strict aerobic bacteria. As compared to untreated mice, a rapid decrease (by 8·8
logs) in the number of anaerobic flora occurred in the mice treated with
metronidazole 5 days after irradiation. This was followed by a rapid increase in
the number of aerobic organisms which coincided with the earlier mortality in
this group. These data suggest that antimicrobial agents that decrease the number
of the strict anaerobic component of the gut flora enhance systemic infection by
aerobic or facultative anaerobic bacteria, and this facilitates mortality after
irradiation.

1. Introduction

Animals receiving increasing doses of whole-body radiation show an increased
susceptibility to various endogenous or exogenous pathogens (Benacerraf, 1960).
The majority of the endogenous pathogens are of enteric origin and can be recovered
from lymphatic organs as well as from the bloodstream. Previous studies (Miller
et al. 1952, Hammond 1954, Hammond *et al.* 1952), demonstrated that antimicrobial
therapy using an aminoglycoside against experimental infections in mid-lethally
irradiated animals was effective in reducing the mortality. However, antimicrobial
therapy alone was not effective in treating lethally irradiated animals, without
restoration of their hematopioetic system (Miller *et al.* 1952).

Recent studies have demonstrated the recovery of aerobic, facultative and
anaerobic organisms of enteric origin in the blood of lethally irradiated animals
(Brook *et al.* 1984). No previous study has evaluated the efficacy of antimicrobial
therapy that is effective against aerobic, facultative, and anaerobic bacteria in lethally
irradiated mice. This study was designed to investigate the effects of antimicrobial
therapy on the gastrointestinal flora, systemic infection, and mortality times in
lethally irradiated mice.

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2.   Materials and methods

2.1. *Animals*

Female C3HeB/FeJ mice approximately 10 weeks of age were used. 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 assure the absence of specific bacteria and common murine diseases. Animals were maintained on chlorinated water that was changed to tap water 48 h before irradiation.

2.2. *Cobalt-60 irradiation*

Mice were placed in Plexiglas restrainers and exposed to 10 Gy whole-body radiation at 0·4 Gy/min from bilaterally positioned cobalt-60 sources. All irradiations were performed between 10 a.m. and 2 p.m. Dose determinations were made with the use of a 50 ml AFRRI-designed tissue-equivalent ionization chamber calibrated against a National Bureau of Standards ionization chamber. The dose within the exposure field varied by 3 per cent, as determined by thermal luminescence dosimetry conducted within tissue-equivalent mouse phantoms.

2.3. *Experimental design*

Four hundred irradiated mice were used in each experiment, and the experiment was performed twice. The mice were divided into four groups of 100 mice each. One group served as an irradiated control, the second received gentamicin, the third received metronidazole, and the fourth received both gentamicin and metronidazole. Twenty mice of each group were used for monitoring survival, and 80 were used for studying the gut flora and the bacteria in the blood, liver, and spleen.

2.4. *Microbiological methods*

After irradiation, the mice were observed for symptoms of disease and mortality between days 0 and 17. As long as living animals were present in a group, five animals were selected at random from each irradiation group on days 1, 3, 5, 7, 9, 11, 13, and 15. Cultures were also obtained once from 10 non-irradiated mice. Animals were killed by cervical dislocation. The spleens and livers were removed aseptically, homogenized immediately, and samples swabbed onto media for the determination of aerobic, facultative, and anaerobic bacteria (Lennette *et al.* 1980, Sutter *et al.* 1980). Through open-heart puncture 0·3–0·5 ml of blood was obtained and plated on media for the determination of aerobic and anaerobic bacteria.

A 2·5 cm section of the ileum ending at the cecal area, was studied on all days except on day 15. Each sample was homogenized in a Teflon grinder after dilution 1 : 10 (w : v) in prereduced saline. The resulting material was serially diluted and plated on agar supporting the growth of aerobic and anaerobic bacteria in order to determine viable counts of the bacterial flora per gram of tissue.

The time between collection and inoculation of media never exceeded 5 min. The media used for facultative and aerobic organisms were blood, chocolate, and MacConkey agar. For anaerobic bacteria, prereduced media were used, including vitamin K_1-enriched brucella blood agar and a selective blood agar plate containing kanamycin and vancomycin (Sutter *et al.* 1980).

The anaerobic plates were immediately incubated at 37°C in anaerobic GasPak jars (BBL, Cockeysville, MD) for 7 days. The aerobic plates were incubated at 37°C in air and 5 per cent carbon dioxide, and were read at 24 and 48 h. A thioglycolate broth was incubated aerobically for 14 days. Aerobic and facultative isolates were

identified on the basis of Gram's stain morphology, gas–liquid chromatography of metabolic by-products (Sutter *et al.* 1980), and the Minitek (BBL) anaerobic identification scheme.

2.5. *Antimicrobial agents*

The daily dose of the antimicrobial agents, when given singly or in combination (administered in divided doses every 12 h), was 7·5 mg/kg for gentamicin (Schering Laboratories, Kenilworth, NJ) and 50 mg/kg for metronidazole (GD Searle & Co, Chicago, IL). Treatment started on day 3 after irradiation and continued as long as the animals lived. All agents were given intramuscularly. Serum concentrations of antimicrobial agents were determined to document adequate levels effective against potential pathogens.

Serum concentrations of gentamicin were measured by agar diffusion assay (Reeves *et al.* 1978) with *Bacillus subtilis* ATCC strain 6633 (American Type Culture Collection, Rockville, MD) and metronidazole was assayed with high-pressure chromatography (Wheeler *et al.* 1978). These were performed on day 5 of therapy, 1 h after injection of antibiotics, and again 11·5 h after injection of antibiotics.

2.6. *Statistical analysis*

Statistical analysis was done using the Cox-Mantel Test (Lee 1980).

3. Results

3.1. *Mortality*

Mortality did not occur in any group until 8 days postirradiation, and all animals were dead by 17 days postirradiation (figure 1). The data presented in Figure 1 are from the first experiment; however, similar data were obtained in the second experiment. Mortality in the group that received metronidazole occurred much earlier than in any of the other groups, and all animals were dead by the 9th day ($P < 0.001$ versus irradiated control). No statistical difference was seen between the mortality times of the animals treated with gentamicin and the mortality times of the irradiated control animals. However, a significant difference was seen ($P = 0.002$) between mortality times of the animals treated with metronidazole and gentamicin and the irradiated controls.
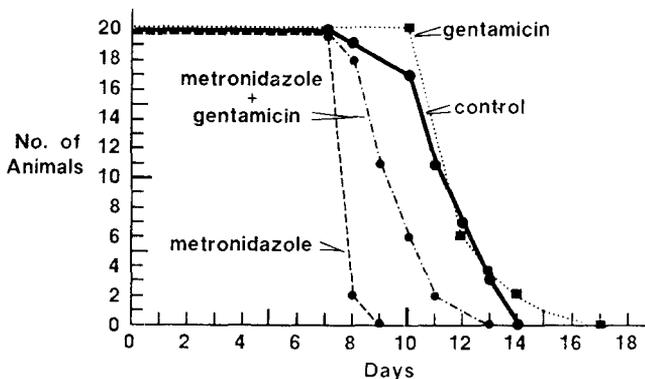

Figure 1. Survival times of mice after irradiation with 10 Gy according to therapy group.

Table 1. Bacteria recovered from irradiated mice[a] according to therapy given.

	Days postirradiation[b]																								
	7					9					.11					13					15				
	Ec[c]	Pr	Sa	B	Ps	Ec	Pr	Sa	B	Ps	Ec	Pr	Sa	B	Ps	Ec	Pr	Sa	B	Ps	Ec	Pr	Sa	B	Ps
Irradiated control	1					5	1		1		4		2	2	4	6	2	1	1	3		3	1	2	2[d]
Gentamicin	3	1						2		3	3		2	3		3		1	2	4		2			1[e]
Metronidazole	4	3	2			5	2	3			All dead					All dead					All dead				
Gentamicin plus metronidazole								2	3		2	3			2	1	1		1	2[d]	All dead				

[a] For purpose of analysis, an isolate had to be recovered from at least one site (blood, liver, or spleen) for mouse to be culture positive.
[b] A total of 100 mice were given 10 Gy in each group, and 10 mice were sacrificed per day from each group.
[c] Abbreviations for bacteria isolated: Ec = *E. coli*, Pr = *Proteus*, Sa = *S. aureus*, B = *Bacteroides*, Ps = *Peptostreptococci.*
[d] Only eight animals were studied in this group.
[e] Only six animals were studied in this group.

3.2. *Isolation of microorganisms in spleen, liver, and blood*

No bacterial growth was detected in all samples obtained from the unirradiated mice or in those irradiated until 7 days postirradiation. The results of bacterial cultures of blood, spleen, and liver are summarized in table 1. These results summarize the two experiments, in which cultures were obtained from 152 mice. Most organisms were recovered concomitantly in all three sites: liver, spleen, and blood. Each bacterial isolate was counted only as one, even when it was recovered from more than one site in a mouse.

A total of 45 isolates were recovered from the irradiated control group: 30 aerobic and 15 anaerobes. The predominant aerobic and facultative organisms were *Eschirichia coli* (19 isolates) and *Proteus mirabilis* (seven isolates); the predominant anaerobes were *Peptostreptococcus* sp. (nine isolates) and the *Bacteroides fragilis* group (six isolates). Organisms were first detected on day 7 in the group treated with metronidazole, and all of them were aerobic. Bacteria were detected less frequently in the group treated with gentamicin (26 isolates), and most of them were anaerobic. However, Gram-negative enteric bacteria were also detected in this group, mostly on or after the 11th day. The smaller number of isolates were recovered in the group treated with gentamicin and metronidazole; however, despite the wide antimicrobial coverage, organisms were still isolated.

3.3. *Quantitative changes in ileal flora*

For irradiated control animals the number of lactose-fermenting facultative anaerobes (LF) dropped from 7·2 to 1·9 logs by day 9 (figures 2 and 3) and then rose to 8·4 by day 13. The number of strict anaerobes (SA) also dropped from 11·4 logs to 3·4 logs at day 9, but stayed low even at day 13. For gentamicin-treated animals, both LF and SA dropped to low levels and stayed low throughout day 13. The number of SA for metronidazole-treated animals dropped more rapidly than for controls; it reached a low of 1·6 logs at day 5 and stayed low through day 9. In contrast, the number of LF rose more rapidly than for the irradiated controls. For the gentamicin- and metronidazole-treated animals, the LF and SA dropped more rapidly than for irradiated controls and they stayed low through day 13.

Statistical analysis revealed differences in the number of LF on day 7 between metronidazole and gentamicin groups ($P < 0.05$), on day 9 between metronidazole group and all other groups and ($P < 0.001$), and on days 11 and 13 between untreated
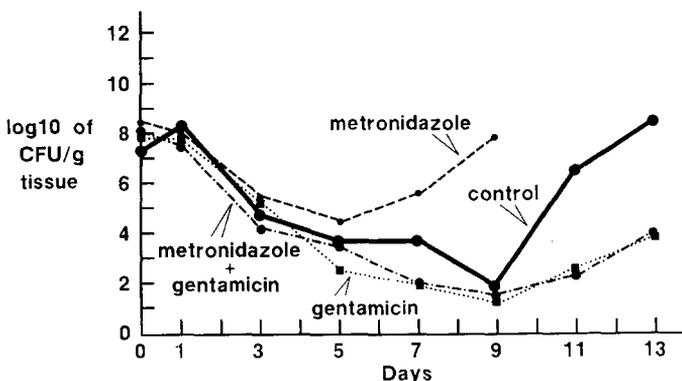

Figure 2.   Number (CFU/g) of lactose fermenting facultative anaerobic bacteria in the ileum of irradiated mice.
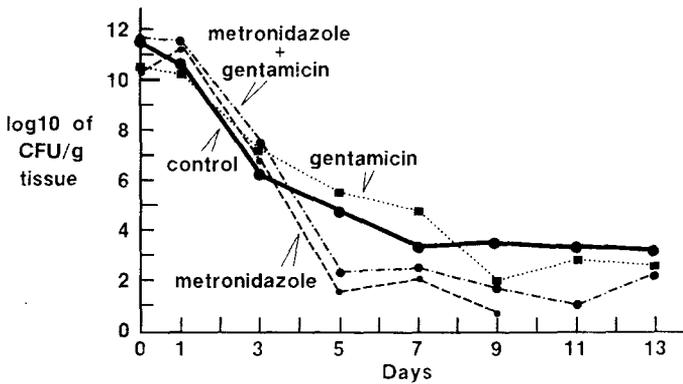
Figure 3.   Number (CFU/g) of strict anaerobic bacteria in the ileum of irradiated mice.

and gentamicin, and gentamicin and metronidazole groups ($P < 0.001$). Statistical differences in the number of strict anaerobes were noticed on day 5 between gentamicin and untreated and metronidazole groups ($P < 0.05$) and day 7 between gentamicin and metronidazole groups ($P < 0.05$).

3.4. *Antimicrobial serum concentration*

Serum concentrations were obtained in five animals in each group. The gentamicin concentration (mean + SD) at 1 h after injection was 6.2 ± 2.1 mg/l; 11.5 h after injection, it was 1.2 ± 0.5 mg/l. The metronidazole concentration at 1 h after injection was 23.8 ± 6.6 mg/l; 11.5 h later it was 12.6 ± 4.2 mg/l.

4.   Discussion

The data presented illustrate the marked reduction in the number of aerobic facultative and anaerobic flora that occurs in all animals after irradiation. The rate of decline in the anaerobic flora was more rapid in the group treated with metronidazole than in the irradiated control or those treated with gentamicin. Although the number of LF in the gut flora decreased in all groups of animals, it reached preirradiation concentrations in the group treated with metronidazole on day 9, as compared to day 13 in the untreated group.

This study confirmed the recovery of aerobic, facultative, and anaerobic bacteria in the blood of lethally irradiated animals (Brook *et al.* 1984), and demonstrated the ability of systemic antimicrobial therapy to reduce the number of these translocated intestinal organisms. Animals treated with gentamicin had less translocation with LF while therapy with metronidazole reduced translocation with anaerobic bacteria. No earlier reduction in the number of the enteric anaerobic flora was associated with gentamicin therapy; however, increased survival was not noticed, even in the group treated with the combination of gentamicin and metronidazole. Since the animals had received lethal irradiation, which suppressed the immune system, antimicrobial therapy by itself was unable to prevent mortality and completely eliminate systemic infection. Further studies are warranted to observe the protective effect of antimicrobials associated with other therapeutic modalities that replenish or enhance the immune system. Antimicrobials, however, may be effective in those exposed to sublethal irradiation (Hammond 1954).

Antimicrobial therapy with metronidazole, which has excellent antibacterial activity against anaerobic bacteria (Sutter and Finegold, 1976), was deleterious to

the survival of the animals. The mechanisms of enhancement of mortality after metronidazole therapy is postulated to be due to the effects it has on the bacterial colonization of the gut. The number of anaerobic bacteria in the ileum dropped sooner and more abruptly after metronidazole therapy compared to non-treated irradiated animals or those treated with gentamicin. This in turn was followed by an earlier increase in the number of aerobic or facultative bacteria in the bowel and their appearance in the blood, spleen, and liver. Although the number of LF that repopulates the gut never exceeds that normally present in an unirradiated animal, this repopulation was associated with translocation and lethality. This may be due to increased penetration of organisms through the damaged mucosa and/or decreased local and systemic immunity. Similar effects of antimicrobials effective against anaerobes were reported by Berg (1981), who found metronidazole and clindamycin to produce systemic translocation with enteric bacteria.

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' (Mulder *et al.* 1979). Selective decontamination of the bowel using antimicrobials that are effective only against the aerobic and facultative flora is used in immunocompromised persons (Mulder *et al.* 1979). Such therapy is aimed at the elimination of the aerobic and facultative bacteria but preserves the anaerobic bowel flora. Our data support the importance of the colonization resistance phenomenon. They also demonstrate that the administration of antimicrobial agents effective against the anaerobic gut flora can be deleterious in the irradiated host. Since the management of patients with mixed aerobic–anaerobic infections requires the administration of antimicrobials effective against both classes of organisms (Bartlett *et al.* 1978), more work is needed to develop therapeutic modalities that will effectively treat irradiated patients who develop polymicrobial infection without affecting their colonization-resistant flora.

Acknowledgements

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