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Chapter 13

Modulation of Mortality by Tissue Trauma and Sepsis in Mice after Radiation Injury

G. DAVID LEDNEY, THOMAS B. ELLIOTT, AND MARCUS M. MOORE

ABSTRACT

The nuclear disasters at Hiroshima, Nagasaki, and Chernobyl underscore the need for useful animal models to (*a*) evaluate the combined effects of radiation and tissue trauma and (*b*) develop successful therapeutic modalities for sepsis in irradiated individuals inflicted with tissue trauma. In mice, mortality subsequent to radiation and tissue trauma depends on the (*a*) timing of the trauma relative to radiation, (*b*) dose and quality of radiation, (*c*) nature of the inflicted trauma (burn or wound), (*d*) genetic makeup of the host, and (*e*) microbiologic agents associated with the host. Therapies for sepsis after wound trauma were developed in gamma ray- and neutron-irradiated mice. Single-agent therapy for infections with antimicrobials or immunomodulators is not as useful as combined modality therapy with antimicrobials and immunomodulators. Topical treatment of the injury with antimicrobials in addition to systemic therapy with antimicrobials or immunomodulators is necessary to effect survival. Sepsis in mice subsequent to neutron irradiation and wound trauma was more difficult to treat than sepsis after gamma ray exposure and wounding. The increased biological effectiveness of neutrons compared to gamma rays for radiosensitive tissues makes therapy for sepsis less successful in neutron-irradiated hosts.

INTRODUCTION

Approximately 100 years ago Roentgen discovered x-rays and Becquerel discovered radioactivity. Since that time man has attempted to harness the atom for civilian as well as military purposes. The promise of the expanded peaceful use of nuclear energies for diagnosis of disease, therapy for malignancies, and unlimited electrical power has been clouded by major accidents where radionuclides have been released into the environment from power reactors (Three Mile Island, United States, and Chernobyl, U.S.S.R.) and from abandoned medical radiation devices (Juarez, Mexico, and Goiania, Brazil). The increased risk to human health as well as the loss of life in Chernobyl and Goiania have had sobering influences upon the world.

Nearly 50 years ago nuclear weapons were developed in the United States and employed in Hiroshima and Nagasaki with devastating effects. Today many nations control nuclear weapons or reactors capable of producing weapon-grade material. In addition to these devices there are numerous medical centers and power plants using radiation for peaceful purposes. While these radiation resources are under apparent control, the possibility that accidents will occur or that terrorists will obtain and use these devices is of increasing concern.

The expanded probability of exposure to radiation environments requires the development of understanding of both the damage induced by radiation and the treatments available to counteract that damage. Further, the treatments provided must be tailored for the additional specific injuries expected or associated with radiation damage (Browne et al. 1990). For example, burn trauma in the individuals exposed to radiation at Chernobyl complicated therapeutic efforts (Baranov et al. 1989). Burns and mechanical traumas to the irradiated inhabitants of Hiroshima and Nagasaki contributed heavily to mortality (Fujita et al. 1990). In retrospective analyses of mortality after the Hiroshima event, burn and wound traumas in irradiated casualties may have resulted in underestimates of the LD_{50} (Fujita et al. 1989).

Irradiated individuals with mechanical or burn injuries are termed "combined injured." Combined injury is defined here as the infliction of tissue damage on an individual when (*a*) one of the injuries is radiation and (*b*) the recovery and repair from it or the other injury has not taken place before the occurrence of the second. In this chapter, we summarize our findings on factors contributing to mortality after combined injury in mouse models. In addition, we show that survival is possible in lethal models of combined injury in mice if hematopoietic recovery is augmented and if overwhelming sepsis is controlled.

MATERIALS AND METHODS

ANIMALS

B6CBF1/CUM female mice were obtained from Cumberland View Farms in Clinton, Tennessee. B6D2F1/J female mice were obtained from Jackson Laboratory in Bar Harbor, Maine. C3H/HeN female mice were obtained from the National Cancer Institute Animal Breeding Facility in Frederick, Maryland. The mice were maintained as previously described (Madonna et al. 1991). Research was conducted in a facility accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). All procedures involving animals were reviewed and approved by an institutional animal care and use committee.

RADIATION

Detonation of a nuclear device will result in the release of neutrons and gamma rays. The proportion of each of these received by an exposed individual will depend on such factors as type and yield of the weapon and distance from the epicenter. To simulate nuclear detonation environments, irradiations with mixed fission neutron and gamma photons were performed using the Armed Forces Radiobiology Research Institute (AFRRI) Training, Research, and Isotope, Gen-

eral Atomics (TRIGA) Mark-F research reactor. This reactor is a movable-core pool-type facility with maximum operational steady-state power of 1 MW. All reactor irradiations were performed at a total dose rate of 38 cGy/min by altering either experimental animal placement in the radiation field, reactor power, or shielding design. Desired neutron to gamma ratios were obtained by varying shielding configurations of water, lead, borated polyethylene, and paraffin at selected reactor powers. The total dose rate varied less than 2% over the entire radiation field. Mice were irradiated in aerated aluminum tubes that rotated at 1.5 rpm.

Gamma ray exposures such as that possible at a reactor site accident or in a fallout zone were simulated by irradiation with cobalt-60. B6CBF1 and B6D2F1 mice were irradiated bilaterally at 40 cGy/min in the AFRRI ^{60}Co radiation facility. C3H/HeN mice were irradiated unilaterally at 40 cGy/min with a ^{60}Co Theratron unit. All irradiations of mice were done in aerated Plexiglas restrainers. The tissue/air ratio was 0.988 for bilateral ^{60}Co irradiation and 0.98 for unilateral ^{60}Co irradiation. Dosimetric techniques for measuring reactor- and ^{60}Co-produced radiations were described elsewhere (Zeman and Ferlic 1984).

Skin Injuries

Mice were anesthetized by inhalation of methoxyflurane before injury. Full-thickness, nonlethal skin wounds of varying sizes, from 7% to 15% of the total body surface area (TBSA), were inflicted by removing a section of dorsal skin fold and underlying panniculus carnosus muscle with a steel punch. Details for inflicting skin wounds have been previously published (Madonna et al. 1991). Full-thickness burns were inflicted on the shaved dorsal surface area by a 12-second ignition of 95% ethanol (Steritz et al. 1982). The burn site varied in size from 7% to 15% of the TBSA.

Bacteria

All endogenously acquired bacteria found in either normal, injured, irradiated, or combined-injured mice and isolated on phenylethanol agar or MacConkey's agar were identified by using combinations of Gram's stain, colony morphology, and specific biochemical tests (Lennette et al. 1985).

Treatment Agents

Immunomodulators are substances that, when used, alter nonspecific or specific immune functions within the host. Synthetic trehalose dicorynomycolate (S-TDCM) was a gift of Ribi ImmunoChem Research, Inc., in Hamilton, Montana. S-TDCM activates nonspecific host resistance against induced bacterial infections in gamma ray- (Madonna et al. 1989) and neutron-irradiated mice (McChesney et al. 1990). Therefore, we examined its usefulness in endogenously acquired infections in combined injured mice. The method of S-TDCM preparation was previously described (Madonna et al. 1991). A dose of 200 μg S-TDCM was given intraperitoneally (ip) in 0.5 ml of 0.2% Tween-80 saline.

Oxacillin sodium, gentamicin sulfate, ofloxacin (R. W. Johnson Pharmaceutical Research Institute, Raritan, NJ), and ceftriaxone sodium (Hoffman-LaRoche,

Inc., Nutley, NJ) were used systemically. All these antimicrobials were prepared in pyrogen-free water, and 0.1 ml was injected subcutaneously (sc) above the right or left gluteus medius of each mouse daily for 10 consecutive days. The daily dose of oxacillin was 150 mg/kg; gentamicin, 7.5 mg/kg; ofloxacin, 40 mg/kg; and ceftriaxone, 75 mg/kg. Garamycin cream (0.1% gentamicin sulfate) was obtained from Schering Corp. in Kenilworth, New Jersey; a generic 0.1% gentamicin sulfate was obtained from Goldline Labs in Fort Lauderdale, Florida. The creams were applied once daily for 10 consecutive days in 0.5 to 0.7-g amounts sufficient to cover the wounded sites.

Statistical Analyses

Survival data of mice in experimental groups were obtained for 30 days after irradiation. Comparisons were made by the generalized Savage (Mantel-Cox) procedure (Lee 1980). Probit analysis of numbers of mice surviving 30 days was made on log-transformed doses (Finney 1971, 1979).

RESULTS

Influence of Radiation Quality, Radiation Dose, and the Type of Skin Trauma on Survival of Combined-Injured Mice

In uncontrolled nuclear radiation environments, individuals may be exposed to either alpha, beta, gamma, or neutron particles. External contamination of the injury site with alpha and beta emitters does not normally constitute an immediate life-threatening situation for the individual. Internal and external contamination with radionuclides, while presenting a possibility of increased risk for life shortening and malignancy, does not usually preclude immediate lifesaving treatments for personnel with combined injury. Rather, burn or wound trauma in conjunction with exposure to neutron or gamma ray irradiation or combinations thereof pose the more serious life-threatening situation.

To simulate a variety of radiation environments, groups of B6D2F1 mice were irradiated with three different neutron/gamma dose ratios (n/γ) produced by the reactor. The n/γ's employed were 0.33, 1, and 19. To simulate exposure in a high gamma ray fallout field, groups of mice were irradiated with "pure" gamma radiation either from a ^{60}Co source or the reactor. Nonlethal 2.5-cm by 3.8-cm (15% TBSA) burns or wounds were inflicted 1 to 2 hours after each exposure, and 30-day survival responses were compared to control mice that were irradiated but uninjured. Thus complete dose-response survival curves were obtained at each radiation quality with each type of injury. Radiation doses lethal to 50% of mice in 30 days ($LD_{50/30}$) are presented in Figure 13.1. In irradiated animals, the $LD_{50/30}$ is one endpoint used to compare relative sensitivity or resistance to radiations of different quality. In all groups of mice, i.e., control, irradiated, irradiated and burned, and irradiated and wounded, the $LD_{50/30}$ decreased as the proportion of neutrons in the total dose increased. At each n/γ, postirradiation burn trauma and wound trauma reduced the $LD_{50/30}$ from the comparable radiation control group about 10% and 20%, respectively.

Injury subsequent to radiation not only increases the mortality incidence (i.e.,

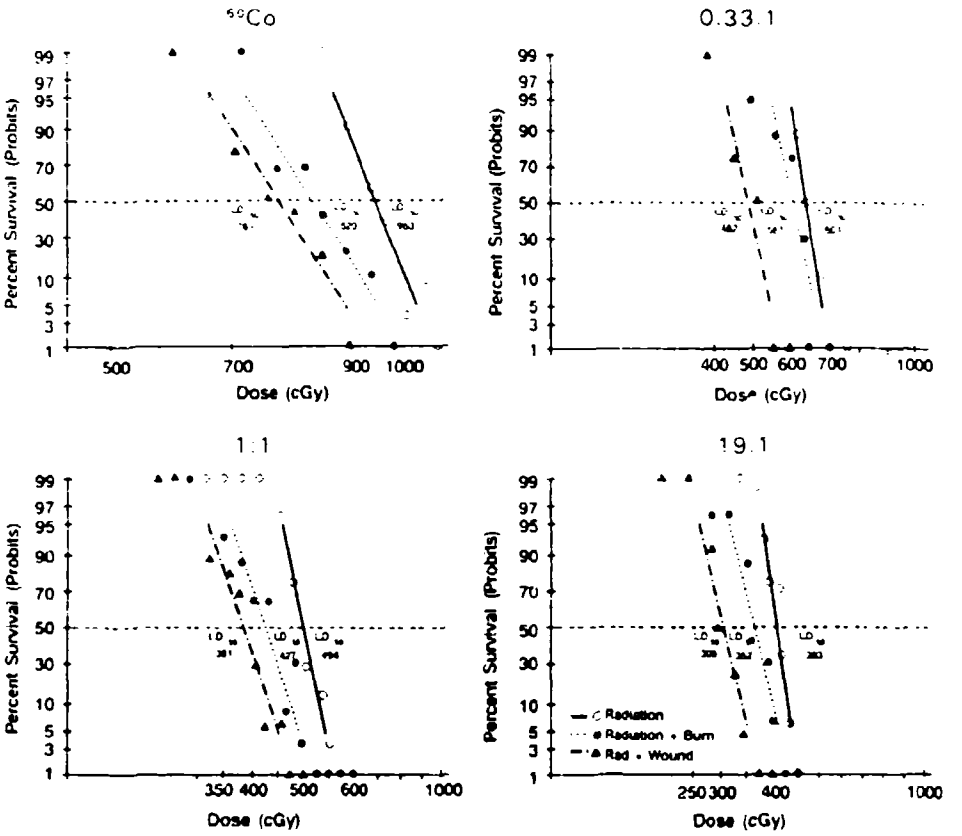

FIG. 13.1. Radiation doses required to produce 50% lethality in mice. All B6D2F1 mice were irradiated at 0.4 Gy/min, and $LD_{50/30}$ values were statistically determined from complete dose-response survival curves. The *open circles* indicate survival for irradiated mice. The *closed circles* indicate survival for mice given 15% TBSA burn trauma 1 to 2 hours after irradiation. The *triangles* indicate survival for mice given 15% TBSA wound trauma 1 to 2 hours after irradiation.

reduces the $LD_{50/30}$) but also reduces the survival time of mice that died. Plotted in Figure 13.2 are the mortality percentages of mice dying after irradiation with various proportions of neutron and gamma rays and or irradiated mice inflicted with burn or wound trauma. Except in isolated instances, the greatest mortality occurred during the 1st week after receiving a high proportion of neutrons or when wound trauma was inflicted after irradiation. When mice were irradiated with an $n/\tau = 1$ or less, or when burn trauma had been inflicted after irradiation, the majority of mortality occurred in the 2nd week postirradiation.

Influence of Time of Injury Relative to Irradiation on Survival of Combined-Injured Mice

In a series of studies, three groups of B6CBF1 mice were irradiated with three (9, 10, or 11 Gy) lethal doses of ^{60}Co, respectively. At eight time points before

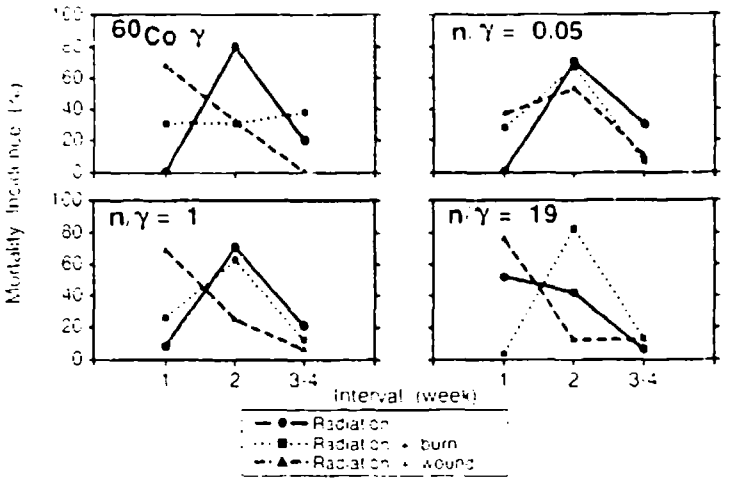

FIG. 13.2. Incidence of mortality of mice irradiated with ^{60}Co-produced gamma rays or reactor-produced neutrons and gamma rays. The mortality percentages are based on a total of 30 to 60 mice irradiated, irradiated and burned, or irradiated and wounded at their respective $LD_{50/30}$'s at the indicated gamma ray or neutron to gamma ratios.

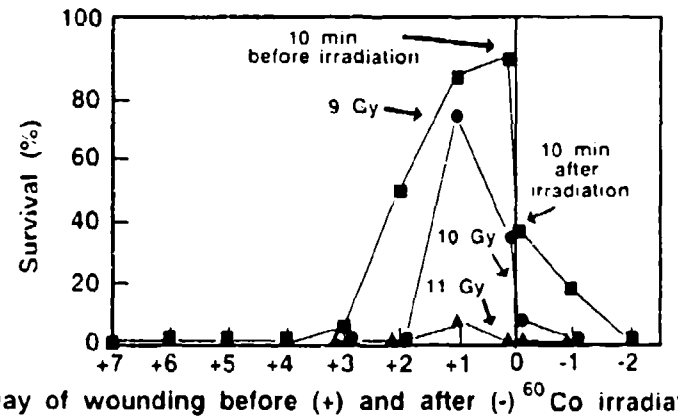

FIG. 13.3. Thirty-day survival of B6CBF1 female mice (n = 16/time point) after receiving 1.3-cm by 1.9-cm skin wounds and ^{60}Co irradiation. All control irradiated mice died; all control wounded mice lived. (From Ledney, G. D., Exum E. D. Jackson, W. E., III. Wound-induced alterations in survival of ^{60}Co irradiated mice: importance of wound timing. Experientia 1985;41:614–616.)

irradiation and three time points afterward, 1.3-cm by 1.9-cm skin wounds were inflicted. Survival data are shown in Figure 13.3. The 30-day survival of animals wounded before irradiation increased as the time interval between injury and irradiation shortened. Further, the number of 30-day survivors increased when mice were wounded within 10 minutes after a nominally lethal radiation dose (9 Gy). Survival times of all control-irradiated mice that died within the 30-day period were 11 to 14 days. However, survival was extended to 17 to 20 days for mice irradiated with nominally lethal doses (9 Gy) when wounds were inflicted

from 2 days before to 10 minutes after exposure. When wounds were inflicted 1 or 2 days after irradiation, the survival times for all groups of mice with combined injury were decreased to about 7 days.

In a second series of experiments, a comparative mortality study was done with B6D2F1 mice irradiated with sublethal doses of either ^{60}Co (7 Gy) or with an $n/\gamma = 19$ (3 Gy). Groups of mice were injured at each of three time points either before or after irradiation. Mortality data are shown in Figure 13.4. The incidence of death from combined injury was greater for animals irradiated with the high n/γ (19) than with ^{60}Co. In all irradiated mice, wound injury resulted in more deaths than burn injury. Burn or wound injury after irradiation resulted in more deaths than injuries given before exposure. As seen in the first series of experiments, injuries occurring shortly before (10 minutes) sublethal irradiation resulted in fewer mortalities than injuries inflicted 1 or 2 days before radiation exposure. In the second group of studies, a relatively similar decrease in mortality incidence was noted for animals injured soon after (10 minutes) irradiation compared to injuries inflicted 1 or 2 days later.

In a third set of experiments, we determined if there was a positive correlation between wound-induced survival and the number of endogenous colony-forming units found on the spleen (E-CFU-S). The E-CFU-S assay is based on the survival and proliferation of hematopoietic cells forming discrete nodules on the surface of the spleen in an appropriately irradiated mouse. Approximately 8 to 14 days after irradiation the spleen is removed and histochemically stained and

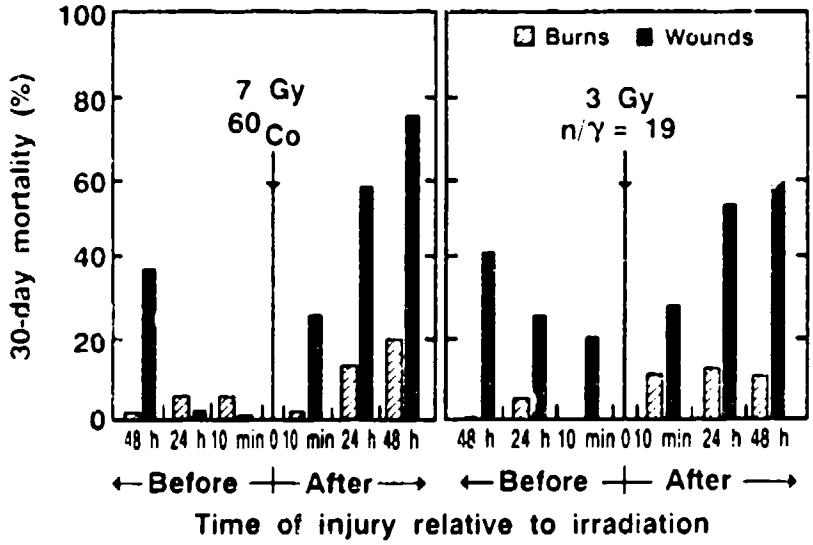

FIG. 13.4. Thirty-day mortality of B6D2F1 female mice after either 7 Gy ^{60}Co (20 mice/time point) or 3 Gy mixed field (neutron/gamma ratio of 19; 24 mice/time point) irradiation and administration of 2.5-cm by 3.8-cm skin wounds or skin burns. In these experiments, less than 4% of mice died in burned, wounded, gamma-irradiated, or neutron-irradiated control groups. (From Ledney, G. D., Madonna, G. S., McChesney, D. G., Elliott, T. B., and Brook, I. Complications of combined injury. Radiation damage and skin wound trauma in mouse models. In: Treatment of radiation injuries. New York: Plenum Press, 1990)

preserved. The number of nodules are then counted. The E-CFU-S assay has been used as an indicator for chemical agents useful for protecting against radiation-induced hematopoietic failure in mice (Kinnamon et al. 1980). Along these lines S-TDCM increased E-CFU-S in both gamma ray and neutron-irradiated mice (Stewart et al. 1991). However, no quantitative relationship between colony number and survival is suggested. Thus to determine if survival from combined injury positively correlated with increases in E-CFU-S, groups of B6CBF1 mice were irradiated with either 9, 10, or 11 Gy. Skin wounds (1.3 cm by 1.9 cm) were inflicted either 2 days, 1 day, or 10 minutes before irradiation, or 10 minutes or 1 day after irradiation. The mean values of 10-day E-CFU-S for 12 to 16 mice per treatment group are reported in Figure 13.5. A positive correlation was noted between the survival from combined injury (Fig. 13.3) and the number of spleen colonies. Thus in combined-injury situations employing nominally lethal radiation doses (9 Gy), trauma increased the hematopoietic proliferative compartments of irradiated mice.

Influence of Genetic Strain on Survival of Combined Injured Mice

The survival-mortality responses of mice to x-irradiation is controlled by their gene makeup (Kohn and Kallman 1956). We irradiated inbred C3H/HeN mice and hybrid B6D2F$_1$ mice with mixed field (n/γ = 1) or ^{60}Co gamma radiation to

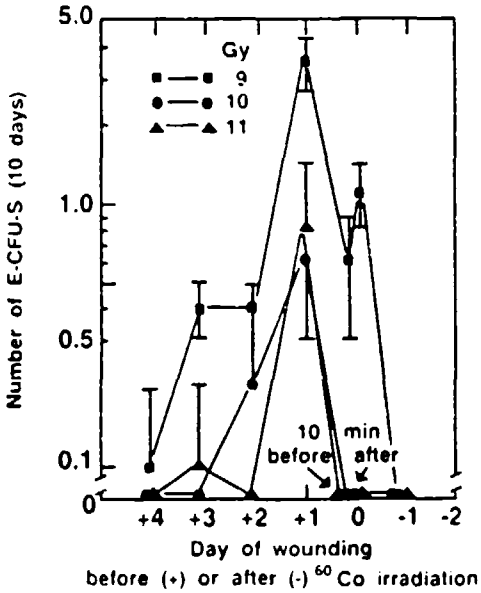

FIG. 13.5. Number of endogenous E-CFU-S in wounded irradiated mice. The time points tested were based on the survival studies in Figure 13.3. No E-CFU-S were found in control irradiated mice or in control wounded mice. Data presented in Figures 13.3 and 13.5 were obtained from all mice exposed to radiation at the same time. (From Ledney, G. D., Exum E. D., Jackson, W. E., III. Wound-induced alterations in survival of ^{60}Co irradiated mice: importance of wound timing. Experientia 1985;41:614–616.)

determine if survival responses to subsequent wound trauma were different in these two mouse strains.

Groups of B6D2F1 and C3H/HeN mice were given doses ranging from 250 cGy to 600 cGy ($n/\tau = 1$). A comparative study was done with these mouse strains given ^{60}Co gamma photons in doses ranging from 550 cGy to 1100 cGy. In both sets of experiments, additional groups of mice received skin wounds 1 hour after irradiation. Figure 13.6 presents the $LD_{50/30}$ and relative biological effectiveness (RBE) for all of these experiments. $LD_{50/30}$ values for B6D2F1 mice were higher than for C3H/HeN mice, indicating resistance to the lethal effects of radiation or radiation wounding. Wound trauma significantly ($p<.009$) decreased slope values for a $n/\tau = 1$ and gamma-irradiated B6D2F1 mice and for gamma-irradiated C3H/HeN mice. The slope for $n/\tau = 1$ irradiated C3H/HeN mice (29.1) was not changed ($p<.22$) by inflicting wound trauma. Slope values for B6D2F1 and C3H/HeN mice were similar after gamma irradiation (35.8 and 37.6, respectively) and gamma irradiation with subsequent wound trauma (22.3 and 20.8, respectively). Slope values for these strains of mice were significantly different after $n/\tau = 1$ irradiation (40.3 versus 23.4, $p = .0002$). The p value was 0.096 between the strains given $n/\tau = 1$ radiation and wound trauma.

Sepsis in Irradiated Mice after Wound Trauma

Antimicrobial defenses are compromised by radiation, and death from sepsis (i.e., spread of bacteria or their products from a focus of infection) may occur if injury is severe. We have demonstrated that antimicrobials are useful in managing endogenously derived (Brook and Elliott 1991) or exogenously induced (Brook and Ledney 1990) sepsis in irradiated mice. In addition, we recently reported our findings on the use of the immunomodulator S-TDCM for managing sepsis in gamma-irradiated mice (Madonna et al. 1989) and neutron-irradiated mice (McChesney et al. 1990). Compared to animals irradiated only, little research

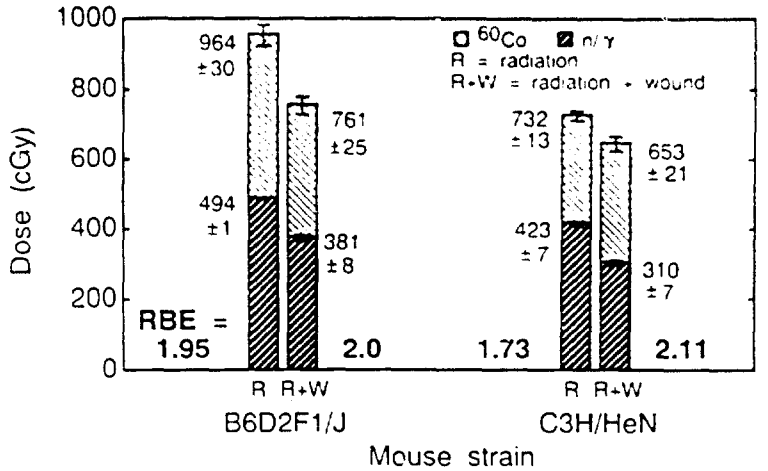

FIG. 13.6. $LD_{50/30}$'s and relative biological effectiveness (RBE) values of normal and skin-wounded mice after mixed-field ($n/\tau = 1$) or ^{60}Co irradiation. The RBE values were calculated at $LD_{50/30}$.

has been done on the management of sepsis in combined-injured animal models (Elliott et al. 1990).

In the first series of studies, we determined the species and relative incidence of bacteria on the wound and in the liver of irradiated-wounded, wounded, and irradiated mice (Table 13.1). S-TDCM alone and combined with the antimicrobial ofloxacin, a new fluoroquinolone, were evaluated as therapies for infections. We previously demonstrated that ofloxacin (40 mg/kg/day per os (po) for 7 consecutive days starting 1 day after po challenge with *Klebsiella pneumoniae* was effective against exogenously induced infection in lethally irradiated (8.0 Gy) B6D2F1 female mice (Brook and Ledney 1991).

Gram-negative and gram-positive bacteria were found on the wound site of irradiated-wounded mice treated with S-TDCM or treated with saline and were also found on the wound site of irradiated mice, while only gram-positive bacteria were found on the wound site of nonirradiated animals. No gram-negative bacteria were found either on the wound site or translocated to the liver in mice treated with ofloxacin or ofloxacin and S-TDCM–early (day 4 to 5) mortality

TABLE 13.1. ISOLATION OF BACTERIA FROM WOUNDS AND LIVERS OF GAMMA-IRRADIATED-WOUNDED MICE AFTER S-TDCM AND OFLOXACIN THERAPY[a]

Experimental Group	4-5 Days of Culture		6-11 Days of Culture	
	Wound	Liver	Wound	Liver
Treatment				
Saline or S-TDCM	*S. aureus* *S. faecium* *E. coli* *P. mirabilis*	*P. mirabilis*	[b]	[b]
Ofloxacin	*S. aureus* *S. faecium*	[c]	*S. aureus* *S. xylosus*	*S. aureus* *A. viridans* *S. faecium* *S. faecalis*
S-TDCM/ofloxacin	*S. aureus* *S. faecium*	*S. faecium* *S. aureus*	*S. aureus* *S. xylosus* *S. faecium*	*S. aureus* *S. faecium* *A. viridans* *S. faecalis*
Control				
Wounded	*S. aureus* *S. xylosus* *Streptococcus spp.* *S. epidermidis*	[c] *Streptococcus spp.*	*S. aureus* *S. xylosus*	[c]
Irradiated	[d]	[c]	[d]	*S. aureus* *E. coli* *K. oxytoca*

[a] C3H/HeN mice were wounded 1 h after 8.0 Gy gamma-irradiation, and antimicrobial therapy began 4 h later. S-TDCM (200 μg) was given i.p. 1 h after irradiation, immediately after wounding. Mice in each group were euthanized either on day 4, 5, 6, 8, or 11 after irradiation and injury, and the wound site and liver were cultured to identify the bacteria. Bacteria are listed in order of frequency of isolation in each group/time. Control mice received 9.0 Gy irradiation.

[b] No mice available for testing because of mortality.

[c] No bacteria isolated.

[d] Mice not wounded in this group.

from gram-negative sepsis was prevented by ofloxacin. However, gram-positive bacterial species were found on the wound site and in the liver of all mice treated with ofloxacin. From these findings we concluded that irradiated-wounded mice were dying with gram-positive bacterial sepsis, and that the source of the infections was the wound, because the bacteria found in the liver were similar to those bacteria colonizing the wound site.

Survival from Sepsis in Irradiated Mice Inflicted with Wound Trauma and Given Systemic Combined Modality Therapy with Antimicrobials, S-TDCM, and Topical Antimicrobials

Bacteria colonizing the wound site were systemically disseminated in irradiated mice, as noted in Table 13.1. Because topical and systemic antimicrobials applied together are more effective in treating wound infections (Stringel 1989), we evaluated several common topical antimicrobial preparations for their efficacy in treating wound infections in mice injured after irradiation. Common disinfectants were also evaluated. In normal, nonirradiated mice inflicted with wounds, treatment with several disinfectants did not alter bacterial colonization of the wound site. The agents tested were 10% povidone-iodine (Pharmadine ointment, Sherwood), 0.5% povidone-iodine (Operand aerosol, Redi-Products), and 0.25% sodium hypochlorite (diluted Dakin's solution, Clorox). Because wound colonization by bacteria was not altered by these disinfectants in normal mice, they were not evaluated in irradiated mice. The effective agents tested and the survival data obtained are presented in Figure 13.7. In this experimental series, as well as in other tests, gentamicin cream increased survival time most ($p<.05$) and hence was used in later experiments with systemic antimicrobials and S-TDCM alone or in combination.

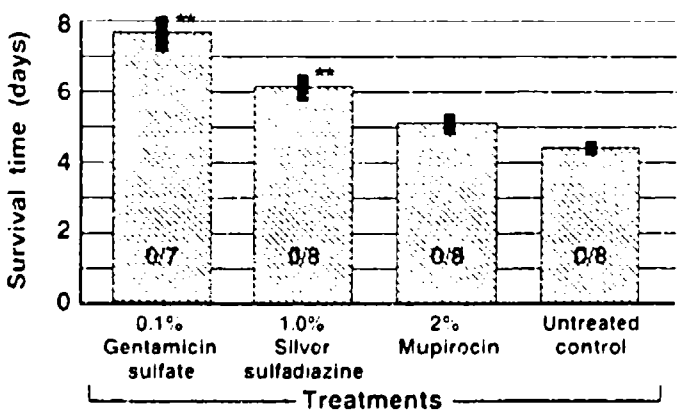

FIG. 13.7. Survival of irradiated wounded mice after topical application of antimicrobials. Mice were wounded 1 hour after 8 Gy gamma irradiation. Treatment with either gentamicin sulfate, silver sulfadiazine, or mupirocin commenced 4 hours after wounding and was applied for 5 consecutive days. Topical application of gentamicin sulfate and silver sulfadiazine significantly increased ($p<.05$) the survival of treated mice compared to untreated control mice. The mean survival time for gentamicin-treated mice differed significantly from silver sulfadiazine-treated mice ($p = .0213$).

Systemic antimicrobial therapy included sc treatments with oxacillin, ofloxacin, and gentamicin in both gamma-irradiated and $n/\tau = 1$ irradiated mice. Systemic ceftriaxone was evaluated only in $n/\tau = 1$ irradiated mice. The survival data for topical and systemic antimicrobial treatments are presented in Figure 13.8.

In mice wounded after 8.0 Gy gamma irradiation, topical gentamicin sulfate treatments with or without systemic gentamicin resulted in about 50% survival. When topical gentamicin was used with oxacillin, all mice survived. The increased survival was statistically significant at $p<.05$ when compared to the survival obtained for topical gentamicin treatments and $p<.01$ for all other comparisons. In mice wounded after 3.8 Gy $n/\tau = 1$ irradiation, topical gentamicin sulfate in combination with all the antimicrobials tested systemically increased survival significantly ($p<.01$). There were no significant differences in survival between the antimicrobial treatment groups ($p>.05$). The enhancement of survival with oxacillin and 0.1% gentamicin sulfate cream may be due to the synergistic action between the semisynthetic penicillin and the aminoglycoside gentamicin in *Staphylococcus aureus* infections (Rahal 1978). *S. aureus* was frequently found colonizing the wound and disseminating to the liver in irradiated-wounded mice (Table 13.1).

To demonstrate the efficacy of S-TDCM treatments with combination antimicrobial therapies (topical plus systemic), we used 0.1% topical gentamicin sulfate in combination with systemic gentamicin. In models of lethal $n/\tau = 1$ and gamma irradiations followed by skin wound trauma, topical gentamicin and S-TDCM therapy with or without systemic gentamicin treatment resulted in

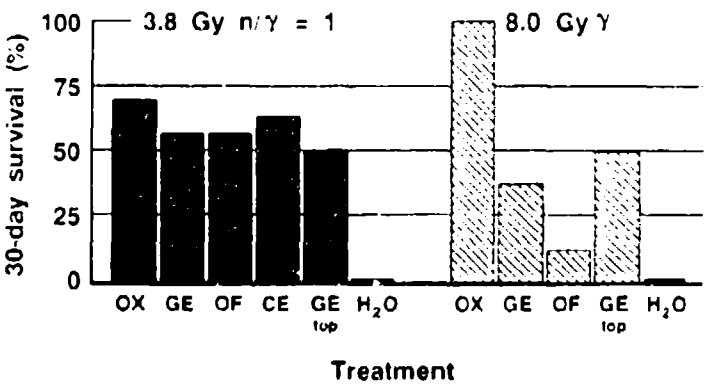

FIG. 13.8. Survival in irradiated mice inflicted with wound trauma after combined therapy with topical 0.1% gentamicin sulfate and systemic antimicrobials. C3H/HeN mice were wounded 1 hour after irradiation. Antimicrobial therapies commenced 4 hours after injury and were provided daily for 10 days. Groups of 20 mice ($n/\tau = 1$ irradiated) and groups of 16 mice (gamma irradiated) were treated topically with 0.1% gentamicin sulfate (GE, top) and systemically (sc) with either oxacillin (OX), GE, ofloxacin (OF), or ceftriaxone (CE). Control groups were treated with 0.1 ml sterile water plus gentamicin cream or were given no antimicrobial therapy. In $n/\tau = 1$ irradiated mice, all antimicrobial treatments were equally effective ($p>.05$) and increased survival ($p<.05$) over water-treated controls. In gamma-irradiated mice, topical gentamicin alone or with systemic oxacillin and gentamicin significantly increased survival ($p<.05$) compared to all other treatments.

approximately 60% survival (Fig. 13.9). S-TDCM effectively increased survival ($p<.05$) when combined with antimicrobial treatments, compared to antimicrobial treatments given without S-TDCM. Only S-TDCM combined with gentamicin produced 30-day survival approximating 50% in both $n/\tau = 1$ irradiated and gamma-irradiated mice. In this combined modality treatment series for sepsis in combined-injured mice, 0/20 survived after oxacillin treatment, 3/20 survived after ceftriaxone injection, and 1/20 survived the 30-day observation period after ofloxacin application. We did not test systemic antimicrobials without topical gentamicin sulfate in S-TDCM-treated, irradiated-wounded mice nor the combination gentamicin treatments in $n/\tau = 1$ irradiated-wounded mice.

DISCUSSION

Our data confirm the complex issues involved in developing appropriate animal models for variables associated with combined injury. In radiation casualties where there are associated tissue injuries, the primary concern is medical stabilization of the patient followed by repair of life-threatening tissue injuries. Schemes for treating victims of radiation accidents have been published elsewhere (Browne et al. 1990). Emergency care to the irradiated-traumatized patient is of greater concern than decontamination of non-life-threatening internally or externally deposited radioactive isotopes or activation products (sodium-24 $T_{1/2}$ = 15 hours; phosphorus = 32 $T_{1/2}$ = 14.3 days). Eventually, decontamination must be done to reduce the body burden of contaminating or induced isotopes. The removal of radioactive material reduces the radiation dose absorbed by the individual.

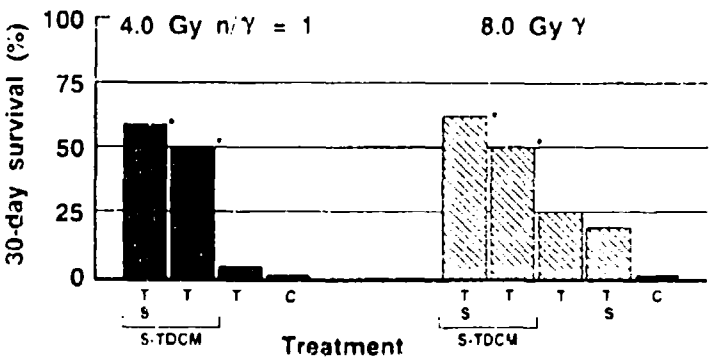

FIG. 13.9. Survival in irradiated mice inflicted with wound trauma and given combined therapy with topical 0.1% gentamicin sulfate and S-TDCM. C3H/HeN mice were wounded 1 hour after irradiation. S-TDCM (200 μg ip) was given immediately after wound trauma. Gentamicin therapy was applied topically and/or given sc 4 hours after injury and daily thereafter for 9 days. Groups of 20 mice ($n/\tau = 1$ irradiated) and groups of 16 mice (gamma irradiated) were treated as indicated. T = topical 0.1% gentamicin sulfate; S = systemic 7.5 mg/kg gentamicin sulfate; C = control. Topical gentamicin with systemic gentamicin was not tested in $n/\tau = 1$ irradiated mice. Systemic gentamicin sulfate with S-TDCM but without topical gentamicin was not evaluated in gamma-irradiated or $n/\tau = 1$ irradiated mice. In both $n/\tau = 1$ irradiated and gamma-irradiated mice, gentamicin therapy with S-TDCM and with or without systemic gentamicin therapy significantly increased (* = $p<.05$) survival, compared to all other treatment groups.

Once a radiation accident victim is stabilized, the second major concern is treatment for infection. Infection is a leading cause of death in otherwise survivable trauma incidents or radiation exposures. Severe immunological suppression induced by the combination of radiation and tissue injury makes the treatment for infection more difficult, as is noted in the present work in mice and in man (Baranov et al. 1989, Champlin 1990). Successful therapy for infections with topical antimicrobials, as reported here, suggests that bacterial colonization of the wound site must be controlled if further advances in therapy for combined injury are to be made (Kaplan 1985). Effective antibacterial therapies will allow sufficient time for induced hematopoietic proliferation or endogenous hematopoiesis to restore the aplastic condition induced by radiation. Possible increases in hematopoiesis by S-TDCM (Stewart et al. 1991) in consort with topical gentamicin sulfate increased survival from combined injury. The advantage of using specific cell growth factors, i.e., G-CSF and GM-CSF for treating sepsis and hematopoietic aplasia in combined-injured hosts remains to be determined. GM-CSF was used in several patients exposed to radiation in the accident at Goiania (Brandao-Mello et al. 1991, Butturini et al. 1988). Several patients responded to GM-CSF with increased peripheral granulocyte numbers; survival may have been increased by this cell growth factor. The animal models of combined injury discussed in this chapter could be used to evaluate the efficacy of cell growth factors prior to their use in radiation accidents.

In previous work, we observed that wound closure takes 1 to 2 weeks in unirradiated mice. Sublethal doses of 7.0 Gy ^{60}Co gamma rays further delayed wound closure by 1 week, while 2.5 Gy of reactor-produced neutrons ($n/\gamma = 19$) further delayed wound closure by 2 weeks. Thus not only does the delay in wound closure promote continued contact with bacteria but also the unhealed site may come under continued exposure to nuclear fallout.

In radiation accidents, significant time and energy may be expended in determining the absorbed dose. The immediate concern is proper triage; a later concern is effective long-term therapy. Biological dosimeters, as well as physical dosimeters, have been used to reconstruct absorbed doses. Biological dosimeters, as adjuncts to measuring radiation doses by other means, may be more difficult in the combined-injured patient than in the individual receiving radiation only. Trauma is well known to change general cell populations (white blood cells) as well as specific cell populations (macrophages and suppressor T lymphocytes). Trauma in irradiated mice significantly altered the white blood cell and platelet patterns from that observed in irradiated animals (Madonna et al. 1991). Likewise, trauma in irradiated mice reduced the possibility of using diamine oxidase (DAO) as an indicator of radiation damage (DeBell et al. 1987). DAO is an enzyme found in high concentrations in intestinal epithelial cells, a target nearly as radiosensitive as bone marrow cells. Perturbations induced by trauma in other cell systems used as biological dosimeters is a possibility and should be examined in combined injury models. In managing dose estimations of irradiated patients, staff charged with reviewing biological and physical dosimetry may need to take these concerns into account.

In conclusion, the combined-injured individual represents a greater challenge

to medical staff than the irradiated individual not compromised by additional tissue trauma. The issues of constructing radiation dose, decontamination of internal and external radioactive isotopes, activation products, and providing emergency and definitive care will all be made more difficult by the complex interactions of tissue trauma, radiation injury, and bacterial infection.

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