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0014-4754/85/050612-03$1.50 + 0.20/0

Wound-induced alterations in survival of ^{60}Co irradiated mice: importance of wound timing[1,2]

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Summary. Wounding mice shortly before or shortly after lethal ^{60}Co irradiation enhances survival. Survival of wounded-irradiated mice may be due to enhanced hematopoietic recovery as measured by endogenous spleen colony (E-CFU-s) formation.
Key words. Trauma; radiation; combined injury; endogenous spleen colonies.

In previous publications[3-5] we established that skin-wound trauma 24 h prior to graded doses of ^{60}Co radiation resulted in survival of mice that ordinarily would die from radiation-induced hematopoietic failure. We also examined the repopulation of hematopoietic centers with early proliferative cells [colony forming unit-spleen-(CFU-s)] and committed progenitor cells [granulocyte-monocyte colony forming cell (GM-CFC) and monocyte-macrophage colony forming cell (M-CFC)] of sublethally irradiated-wounded mice.

While radiation has been employed in mice[6,7] and rats[8] in combination with surgical or wound trauma, the timing of wounding prior to or after whole body lethal radiation doses has not been comprehensively studied in both sexes of a single species. Our previous work[9] in combined injury (radiation wound trauma) and that of others dealing with the recovery from radiation injury[10] suggests the use of the endogenous-colony forming unit spleen (E-CFU-s) assay as a potential indicator of survival and recovery from radiation damage in mice. Additionally, wound trauma alone[11] perturbs the clonogenic cell compartments of the hematopoietic system. Since hematopoietic cells are involved in restoration after lethal radiation doses, we posited that wound timing relative to radiation exposure would effect survival from the combined injury. Therefore, in the present study we determined 1) the survival of mice wounded prior to or after lethal irradiation and 2) the number of E-CFU-s in mice wounded prior to or after lethal irradiation.

Materials and methods. Female and male (C57BL/6 X CBA)F1 Cum BR mice were obtained from Cumberland View Farms, Clinton, Tennessee. All mice were acclimated to laboratory conditions as previously described[11].

Between the hours of 10.00 and 14.00, groups of mice were given 4% skin surface wounds under light methoxyflurane anesthesia. The technique for wounding was previously described[11].

Whole-body irradiations of 40 rad/min (midline tissue) from bilaterally-positioned ^{60}Co elements were performed on mice placed in plexiglass restrainers. An ionization chamber calibrated against a National Bureau of Standards ionization chamber was used for dose determinations. Radiation exposures were performed between 10.00 and 14.00 h. Irradiations of traumatized animals were appropriately timed in relation to skin wounding (see figs 1 and 2).

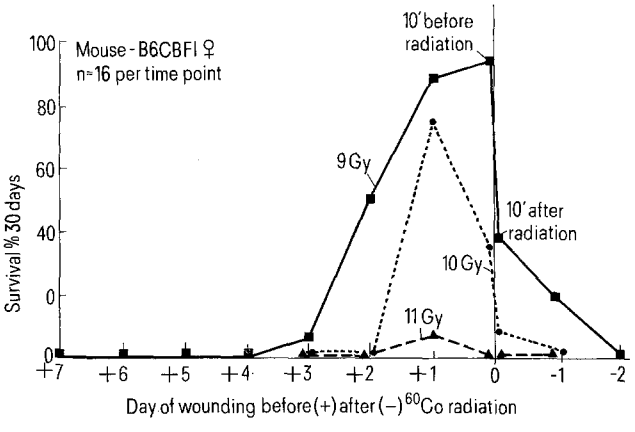

Figure 1. The 30-day survival percentages of wounded irradiated B6CBF1 female mice. At each time point indicated wounding was performed on 16 mice with the exception that 47 mice were injured 1 day after irradiation. All control-irradiated mice died (data not shown) with the exception that one mouse lived after 9.0 Gy. All control-wounded mice survived. 9.0 Gy ■—■; 10.0 Gy ●-----●; 11.0 Gy ▲– – –▲.

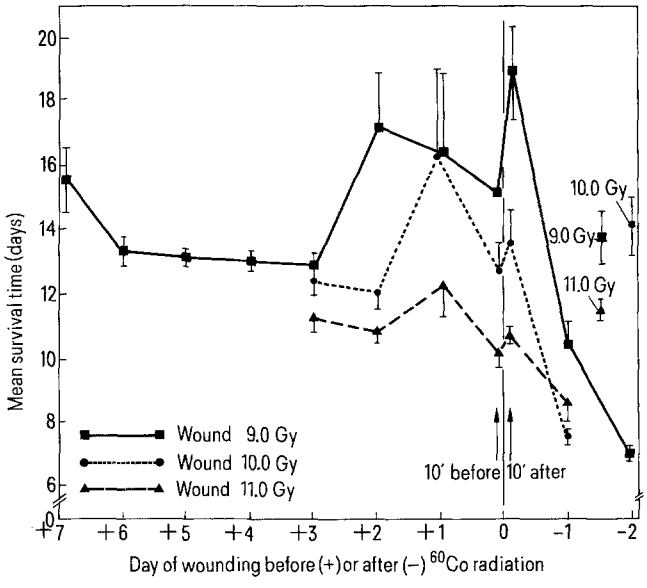

Figure 2. The mean survival times (days) of control irradiated and wounded-irradiated mice. These data reflect the survival of mice only within the 30-day observation period. See figure 1 legend for symbols.

In E-CFU-s studies, spleens were removed from mice 10 days after irradiation, placed in Bouin's solution for 4 h and the colonies counted (double-blind determinations). Presented in figure 3 are the mean values (± SEM) of two independent counts for each group of spleens.

30-day survival and E-CFU-s experiments were simultaneously performed at 9.0, 10.0, and 11.0 Gy on mouse groups given either 1) wound trauma and radiation, 2) radiation only or 3) wound trauma only. Approximately 700 mice were used in all three experiments. Survival percentages were based on groups of 16 mice each, while E-CFU-s studies were done with eight mice per group.

The survival data were analyzed as follows. The Kruskal-Wallis test[12] was used when all mice died within 30 days. The Cox F-test[12] was used when some mice within an experimental group survived (observations censored, i.e., terminated at 30 days). The chi-square test was used when comparing surviving proportions within radiation doses.

Results. Figure 1 illustrates the 30-day survival percentages of female mice wounded before or after irradiation. The mean survival times of mice dying within each of the treatment groups are depicted in figure 2.

At 9.0 Gy, wound injury performed 2 days, 1 day, 10 min before or 10 min after irradiation resulted in significant ($p < 0.01$) enhancement of survival. This was true for both survival times ($p < 0.01$) as measured by the Cox F test and for survival percentages ($p < 0.01$ or $p < 0.05$) measured by the chi-square test. Importantly, wounding 1 day after irradiation (38 of 47 died) did not significantly contribute to the mortality percentage when compared to that of the irradiated controls (15 of 16 died). In mice given 10.0 Gy, injury sustained either 1 day or 10 min prior to irradiation produced a significant ($p < 0.01$ and $p < 0.05$, respectively) increase in survival percentage. Survival times were also significantly ($p < 0.01$) increased for injury 1 day before irradiation. Injury 1 day after 10.0 Gy significantly ($p < 0.01$) reduced the survival time from that observed for irradiated controls (14.2±0.9 vs 7.6±0.2 days). Injury either before or after 11.0 Gy had no significant positive or negative influence on survival percentages or survival times.

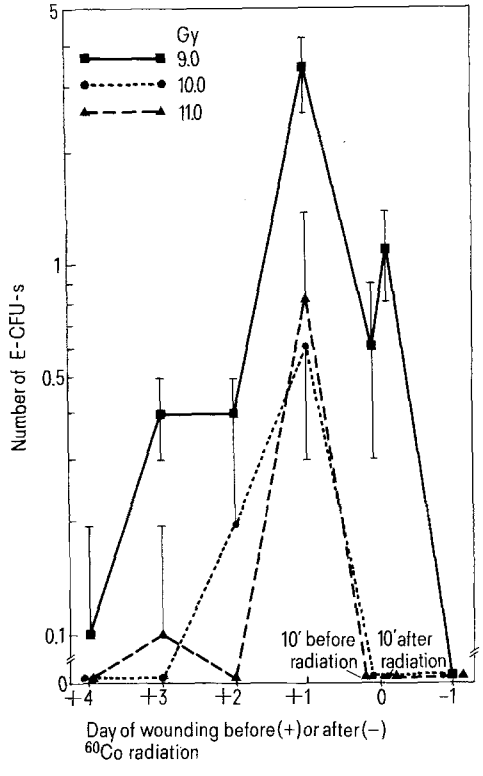

Figure 3. The number of endogenous colony forming units formed per spleen in wounded-irradiated mice. The time points tested were based on the survival studies depicted in figure 1. No E-CFU-s were found in control-irradiated mice or in control-wounded mice. The data presented in figures 1–3 were obtained from mice all exposed to radiation at the same time. See figure 1 legend for symbols.

The results of E-CFU-s analysis for female mice are illustrated in figure 1. In all survival and E-CFU-s experiments, mice were irradiated simultaneously. E-CFU-s were detected after wound-irradiation time intervals and radiation doses that were associated with statistically significant increases in survival percentages or survival times. The single exception to this was when mice were wounded 10 min before 10 Gy. E-CFU-s were not detected even though survival was observed. In most instances where all the combined-injured animals died or where survival times were significantly decreased compared to irradiated controls, none or very few E-CFU-s colonies were found. However, in the case of mice wounded 1 day prior to 11.0 Gy where no enhancement of survival was found, E-CFU-s were present.

Discussion. The foregoing data support the contention that strategically timed wound trauma results in 1) an increase in the number of mice surviving lethal irradiation and 2) an increase in endogenous spleen colonies in irradiated mice which may account for the increased survival. Three important factors may be considered in interpreting our data. They are 1) sex differences in response to radiation, 2) wound contamination with bacteria and 3) mechanisms of action leading to survival or mortality. First, the responses depicted graphically (figs 1–3) were obtained with female mice. In other experiments (data not shown) male mice of the same genetic lineage were used. Wounding before or after 9.0, 10.0, or 11.0 Gy ^{60}Co resulted in similar survival parameters. The main quantitative difference noted was that both survival percents and survival times were generally greater in male mice versus that found in female mice. This may be due to the radioresistance of male mice ($LD_{50/30}$ 8.8 Gy) compared to that of the female mice ($LD_{50/30}$ 7.9 Gy) in our laboratory (Ledney, unpublished observations). In E-CFU-s studies with male mice, wound trauma 24 h before and 10 min after 9.0–9.5 Gy resulted in significantly more colonies than that found in irradiated control mice. Thus, while male mice are more radioresistant, our data indicate that trauma-enhanced survival or mortality is similar in both sexes.

Secondly, it should be noted that all wounds can be potentially contaminated with bacteria. However, wound sepsis indicated by pus formation edema, or lymph node swelling was not observed[11]. Since irradiation depletes the antimicrobial defenses of the host, it is possible that wounding after exposure to radiation provides a portal of entry into the immune compromised host. The decreased survival fractions and survival times of mice wounded 1–2 days after irradiation are in accord with this idea. However, it is difficult to ascribe any beneficial or detrimental effect to wounds made (and potentially contaminated with bacteria) prior to radiation. Perhaps wounding prior to irradiation activates antibacterial defenses. Histologic examination of the wound site indicated granulocyte and macrophage infiltration into the margins of the wound. A 50% increase in peripheral blood granulocyte numbers was observed 1–2 days after trauma. These cellular events potentially enhance antibacterial activity. However, the beneficial value of such cellular responses would be abrogated by the subsequent radiation.

Lastly, the mechanism of action for the increases in survival or mortality are of paramount importance. These include the release of bacterial endotoxin and the elevation of prostaglandin (PGE_2) levels. In the combined injured animal, these mediators may be operating independently or concomitantly. Endotoxins are known modifiers of survival and hematopoietic responses in irradiated animals[13]. The two primary sources of endotoxin are the wound site and the intestinal tract[14]. Endotoxin given prior to irradiation has been demonstrated to afford a degree or radioprotection[15], but higher doses given after radiation enhances

mortality[16]. Prostaglandins are known modulators of hematopoiesis[17] and elevated levels are seen after both trauma[18] and radiation[19]. Thus, increased PGE_2 levels induced by trauma shortly before or shortly after radiation may enhance hematopoiesis and therefore survival, while increased amounts induced both by radiation and subsequent late trauma may result in death associated with sepsis[20]. We are currently directing our research efforts toward delineating the role of these biological modifiers in trauma-enhanced survival/mortality with radiation exposure.

1 Supported by the Armed Forces Radiobiology Research Institute, Defense Nuclear Agency, under research Work Unit 00129. Views presented in this paper are those of the authors; no endorsement by the Defense Nuclear Agency has been given or should be inferred.
2 Research was conducted according to the principles enunciated in the 'Guide for the Care and Use of Laboratory Animals' prepared by the Institute of Laboratory Research, National Research Council.
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0014-4754/85/050614-03$1.50 + 0.20/0

The susceptibility to exercise-induced muscle damage increases as rats grow larger

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Summary. Glucose-6-phosphate dehydrogenase and N-acetyl-β-glucosaminidase activities were both elevated after eccentric exercise indicating that this type of exercise causes muscle damage. Muscle damage as measured by glucose-6-phosphate dehydrogenase activity in the vastus intermedius was greater and occurred later in larger rats indicating that the susceptibility to muscle damage is increased and the repair process delayed in older and larger animals.
Key words. Rat muscle; eccentric exercise; delayed muscle soreness; glucose-6-phosphate dehydrogenase; N-acetyl-β-glucosaminidase.

Heavy physical exercise performed by one unaccustomed to exercise generally results in muscular pain and stiffness. This 'delayed muscle soreness' has been mainly attributed to eccentric work[1-3]. Eccentric work, which is a component of most normal exercise, results from lengthening a muscle against a force. Although the prime physiological cause of this soreness is not known, Hough's suggestion[4] that this soreness was due to muscle damage has been substantiated. Muscle exhibited Z-band disorganization two days after eccentric exercise (running down stairs) in human subjects[5], and I-band widening was observed immediately after eccentric exercise in rats[3]. The extent of exercise-induced muscle damage has been assessed biochemically by measuring the activity of either lysosomal enzymes[6], or glucose 6-phosphate dehydrogenase (G6PDH)[3], the first enzyme in the pentose phosphate pathway. The purpose of this study was to determine which biochemical marker of muscle damage was the most sensitive and then to use this marker to determine the effect of animal size on the extent of muscle damage.

Experimental. Male Sprague-Dawley rats obtained from the East Carolina University School of Medicine animal facilities, were used in these experiments. The rats were individually caged in a temperature-controlled room (20–23 °C) with lights on from 07.00 to 19.00 h, and were allowed free access to food and water. They were exercised by running down an 18° grade on a motor driven treadmill. Running downhill biases the work towards the eccentric type thus causing increased muscle damage. The time and intensity of exercise was adjusted for each group such that about 90% of the animals could complete the exercise protocol. The large rats (411 ± 6 g) rat at 16 m/min for 90 min, the 269 ± 5 g rats ran at 25 m/min for 200 min, and the small rats (79 ± 2 g) rats at 16 m/min for 120 min. The rats in each size group were randomly subdivided into groups that were unexercised (control) or exercised. The control group (0) remained sedentary in their cages until they were sacrificed. The rats in the exercise groups were sacrificed 1 day after exercise (group 1), 2 days after exercise (groups 2), etc. Immediately after sacrifice the muscles were removed, frozen between liquid nitrogen cooled aluminum blocks, and stored for enzyme analysis. In the first experiment both G6PDH and N-acetyl-β-glucosaminidase (NAG) were assayed in the soleus and vastus intermedius. In subsequent experiments G6PDH was assayed in the vastus intermedius. The frozen muscles were homogenized (1/10, w/v) using a Polytron at a setting of 5 for 5 s in 50 mM Tris/HCl buffer, pH 7.6, containing 0.3% triton X-100. After centrifugation for 2 min in an Eppendorf model 5212 centrifuge, the supernatant was assayed for enzymatic activity. The activity of G6PDH was measured by following the reduction of $NADP^+$ at 340 nm in a 50 mM glycine assay buffer, pH 9.2, initially containing 0.4% bovine