

**Combined Injury: Factors with Potential to Impact Radiation Dose Assessments**  
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Combined injuries (CI) are expected after exposure to radiation dispersal devices (RDD) or from nuclear weapon detonations. CI may be defined as the combination of radiation with (1) tissue trauma and burns from blast and thermal energy, respectively, (2) microbial agents, or (3) exposure to chemical agents. Survival after CI in mice depends on (1) type and extent of tissue trauma injury, (2) dose and type of radiation injury (RI), (3) timing of injury relative to RI, and (4) infecting microbial agent found in the environment or used as a bio-warfare agent.

In CI studies with mice we evaluated (1) survival after wounding, burning, and intentional bacterial challenge combined with RI simulating an RDD or a nuclear weapon and (2) countermeasures intended to increase survival from CI. B6D2F<sub>1</sub>/J and C3H/HeN female mice 12-20 weeks of age received skin-wound or skin-burn injuries (15% total body skin surface on the shaved anterior dorsal surface) or bacterial challenges while under methoxyflurane anesthesia before or after RI. All procedures were approved by an Animal Care and Use Committee. <sup>60</sup>Co  $\gamma$ -photons (1.25MeV) and a TRIGA-reactor, operated at 45kW produced neutrons (n) (0.96 MeV) in either an enriched-field (n/n +  $\gamma$  = 0.95) or in a mixed-field (n/n +  $\gamma$  = 0.67) configuration, were used to irradiate mice at 0.4 Gy/min. The relative biological effectiveness (RBE) for mixed-field radiations approximated 2, determined by dividing the LD<sub>50/30</sub> for  $\gamma$ -photons by the LD<sub>50/30</sub> for the mixed-field radiations.

Dose modifying factors (DMF) for wounding and burning after either type of RI were similar at 1.3 and 1.2, respectively, determined by dividing the LD<sub>50/30</sub> after RI by the LD<sub>50/30</sub> for CI mice. In comparison, the RBE was synergistically increased only by wound trauma as the dose of neutrons/total radiation dose (Dn/Dt) increased. Separate skin-burn or skin-wound injuries were inflicted before or after RI; however, because wounds were more severe than burns, when provided after RI, they were used to determine responses to bacterial challenges and in countermeasure studies. CI-mice were susceptible to as little as 10<sup>2</sup> colony-forming units of *E. coli*, *S. pyogenes*, and *K. pneumoniae* when the wound was intentionally colonized.

In countermeasure studies, WR-151327 (WR, 200mg/kg i.p. 30 min before RI) increased survival from CI. DRFs for <sup>60</sup>Co- $\gamma$ -photon-irradiation were 1.53 for RI mice and 1.51 for CI mice. DRFs for mixed-field-irradiation (n/n +  $\gamma$  = 0.67) were 1.31 for RI mice and 1.22 (p  $\leq$  0.05) for CI mice. WR prior to radiation increased resistance to *K. pneumoniae* challenge up to 400-fold for  $\gamma$ -irradiated mice (p $\leq$ 0.01) and up to 30-fold for mixed-field irradiated mice (p $\leq$ 0.01). Silvadene and gentamicin antibiotic creams applied topically alone or with antibiotics given systemically increased survival from CI. Survival from CI was also increased when antibiotics were provided topically or systemically with the immune modulator, synthetic trehalose dicorynomycolate (sTDCM, 200  $\mu$ g i.p. after CI). The immune modulators, particulate glucan or IL-1, did not increase survival from CI. Syngeneic bone marrow (BM) transplantation after CI resulted in increased numbers of survivors but the BM-cell dose required for survival was 20 $\times$  greater than that needed for RI mice.

The biodosimetric impact of combined injuries need to be taken into consideration. The factors and countermeasures described may singly or in combination interfere or modify cellular and molecular endpoints used to determine prompt or protractedly delivered doses of radiation.