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Is Disseminated Intravascular Coagulation the Major Cause of Mortality from Radiation at Relatively Low Whole Body Doses?

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The mechanism by which radiation exposure leads to death in mammalian organisms remains unknown, although numerous hypotheses have been discussed. At the lowest total body radiation doses leading to mammalian mortality, death occurs from the hematopoietic syndrome (HS). HS is thought to result from the cell killing effects of radiation in the bone marrow that lead to low numbers of circulating blood cells and the resultant HS symptoms, such as infection [from the loss of white blood cells (WBC)] and bleeding (presumably from the loss of platelets). Over approximately the last half century, the dose of ionizing radiation that kills half of an experimental group/exposed population, known as the LD₅₀, has been used as a parameter to compare the radiation sensitivity of various mammalian species. It is well known that the LD₅₀ is highly variable for different mammalian species; however, the bone marrow cells of different species, strains and individuals are known to have remarkably similar sensitivities to the cell killing effects of ionizing radiation (1, 2). These results suggest that the lethal effects of radiation in blood cells may not be the primary mechanism by which the HS causes death. Our results have suggested that radiation induced activation of the coagulation cascade, resulting in a condition known as disseminated intravascular coagulation (DIC), could be the major mechanism by which relatively low doses of radiation could lead to animal, including human, mortality.

Our experimental work has focused on the biological effect of solar particle event (SPE) radiation in ferrets. Ferrets exposed to SPE radiation have increased clotting times and factor deficiencies, indicating hypocoagulability (3). Our current studies in ferrets have shown that SPE proton radiation exposure at 1 Gy leads to increased bleeding times, concentrations of soluble fibrin in blood, and fibrin clotting in the livers, lungs and kidneys of irradiated ferrets within 24 h postirradiation (3, 4). The measurement of soluble fibrin in the blood is a marker for DIC in the clinic (5). DIC occurs when the clotting cascade is activated and is characterized by simultaneous bleeding and clotting. Disseminated intravascular coagulation is a serious, life-threatening condition that can occur as a result of trauma, infection or cancer (6). Ferrets exposed to 2 Gy of SPE irradiation exhibit extensive hemorrhaging through organs and other signs of DIC at 13 days postirradiation (unpublished data, Krigsfeld GS, Savage AR, Lin L and Kennedy AR).

There are relatively few cases of humans (particularly in the last half century) exposed to doses near the LD₅₀ who have not received treatments for the prevention of radiation-induced death. As the commonly used treatments for potentially lethal radiation injury have beneficial effects, human LD₅₀ values are imprecise, with estimates ranging from 3–4 Gy for young adults without medical intervention. However, human LD₅₀ values for the very young or the old maybe lower (7), (between 2–3 Gy) with estimates as low as 2.43 Gy [reviewed by Lushbaugh (8)].

Remarkably different LD₅₀ values have been reported for different species. Examples of LD₅₀ values for different species (2, 9), and a range of reported human LD₅₀ values (7), are shown in Table I. The LD₅₀ values listed in Table I for the animal species indicating several strains are the average value for the different strains evaluated in the studies reviewed by Morris and Jones (2). Ferrets are the most sensitive mammalian species (9), closely followed by dogs and pigs. Pigs have a similar LD₅₀ to that of ferrets, and like the ferrets, exhibit hemorrhaging at death^{2,3} (10). The LD₅₀ in Gottingen pigs is 1.8 Gy and widespread hemorrhaging is observed (10). In this model system, at doses near the LD₅₀, there is some evidence of DIC with the “faster onset of systemic inflammation (C-reactive protein, fibrinogen) and multi-organ dysfunction”.⁴ Similarly, dogs exhibit hemorrhagic diathesis at doses near the LD₅₀, and die with signs resembling DIC (11, 12).

While DIC has not been diagnosed as a cause of radiation induced death in the pig or dog studies described above or in irradiated human populations, a hallmark of DIC, i.e., hemorrhage at death, has been frequently observed in irradiated mammals, including humans. There is evidence that humans exhibit bleeding in response to radiation at doses of 2–4 Gy (7). There is extensive evidence that widespread hemorrhages occurred in the Hiroshima and Nagasaki casualties, even in the relatively low-radiation dose groups (13); the estimated LD₅₀ values for those irradiated in Hiroshima and Nagasaki are approximately 2.5 Gy (8, 14). Other information about hemorrhaging after human radiation exposures comes from accidental whole-body irradiation (e.g., the radiation accidents in Norway (15) and Brazil (16, 17). In these cases, several people were accidentally exposed to whole-body irradiation at doses near the human LD₅₀. These patients were treated medically and bleeding was reported in many of these patients. In the Goiania, Brazil, accident, 4 people died and all 4 of these patients had symptoms resembling DIC, which included extensive internal hemorrhaging (16, 17).

A reduction in the number of platelets can result in hemorrhaging and death; however, our results in irradiated ferrets suggest that a reduction in the number of platelets does not cause the blood clotting abnormalities leading to DIC after radiation exposure (3, 4). We have reported that platelet cell counts in ferrets exposed to a 1 Gy dose of SPE proton radiation

²Thrall KD, Murphy MK, Harkonen ME, Lovaglio J. A dose-dependent hematological evaluation of whole body gamma-irradiation in the Gottingen minipig. Poster (PS6-11), presented at the 58th Annual Meeting of the Radiation Research Society, San Juan, Puerto Rico; October 3, 2012, and Personal Communication, Dr. Karla Thrall.

³Moroni M, Owens RP, Elliott TB, Lombardini ED, Nagy V, Whitnall MH. Accelerated hematopoietic syndrome without classic GI syndrome after supralethal doses of whole body gamma radiation in Gottingen Minipigs. Poster (PS6-41) presented at the 58th Annual Meeting of the Radiation Research Society, San Juan, Puerto Rico; October 3, 2012.

⁴Moroni M, Owens RP, Elliott TB, Lombardini ED, Nagy V, Whitnall MH. Accelerated hematopoietic syndrome without classic GI syndrome after supralethal doses of whole body gamma radiation in Gottingen Minipigs. Poster (PS6-41) presented at the 58th Annual Meeting of the Radiation Research Society, San Juan, Puerto Rico; October 3, 2012.

are not significantly different from control levels over 7 days postirradiation. During this time period, the irradiated ferrets exhibit serious blood clotting abnormalities while the platelet counts are well within the normal range (4). The platelet counts are significantly reduced at times of overt or “terminal” DIC in ferrets, but they do not appear to play an important role in the early changes leading to full-blown DIC. This same phenomenon has been reported in irradiated dogs who exhibit symptoms of DIC, the “preterminal” platelet counts are not depressed at the time bleeding abnormalities are occurring, but the platelet counts in irradiated dogs with full-blown DIC are greatly reduced (12). In atom bomb casualties, widespread hemorrhages were observed in people whose level of platelets had not fallen to values when hemorrhages usually occur (13). While the primary established effect of platelets is the prevention of bleeding, platelets have numerous other functions as well (e.g., 18, 19). It has long been known that platelet injections and other factors can have beneficial effects on hemorrhaging; however, platelet infusions do not prevent all deaths from the HS (20). The correlation between platelet infusions and decreased death does not prove that the lethal effects of radiation are a consequence of reduced platelet counts, which result in bleeding.

Hemorrhaging and signs of DIC have been frequently reported in higher mammalian organisms such as dogs and pigs; however, hemorrhaging has not been reported in mice (at doses near the LD₅₀ levels), which is the species that is most often used in radiobiology studies. If mice are used in studies focused on hemorrhaging as an end point, mice with genetic deficiencies in genes associated with clotting factors, such as Factor VIII, are frequently used, as opposed to wild-type mice (e.g., 21). It has been hypothesized that to use mice as a research model in hemostasis, a “humanized” mouse model should be used that includes an immune system similar to that of humans as well as human liver/liver cells that provide components of coagulation (22). While it is generally agreed that infection and hemorrhaging are the major causes of death from the HS, one or the other of these factors may be the predominate cause of death in different species (20). For example, in mice, bacteremia is the predominant factor leading to death at doses near the LD₅₀ values (20, 23, 24). Bacteremia is not the major cause of death for radiation induced HS in dogs, rabbits, guinea pigs or pigs (20, 25). In these species, hemorrhage is thought to be the major cause of death at doses near the LD₅₀ values. The pathophysiology of the HS in pigs is thought to be like that observed in humans (10).

The mechanism by which radiation exposure results in specific hemostatic changes that eventually led to DIC remains unknown. However, we have proposed several hypotheses about the mechanisms by which radiation could cause the initial changes leading to the activation of the coagulation cascade and its progression to DIC in mammals: (1) Inflammation induces activation of coagulation and DIC through cytokine signaling that causes endothelial cells and monocytes to express tissue factor; tissue factor is a known inducer of coagulation (26); (2) Radiation is well known for its ability to cause inflammation, and rapid activation and release of cytokines (27); (3) Radiation exposure at a dose of 2 Gy can cause a loss of barrier function by the gastrointestinal tract, which results in the spread of bacteria into the bloodstream (28, 29) and these gut-derived bacteria secrete endotoxin, which is also a known inducer of DIC (30); and (4) reactive oxygen species (ROS) play a role in controlling/triggering the coagulation cascade (31), and radiation is

well known in its ability to produce ROS. Very likely, the combination of several triggers can contribute to the radiation-induced activation of the coagulation cascade. In combination, the variables that contribute to the manner in which different species handle parameters related to inflammation, infection, and ROS may explain the variability of LD₅₀ values in different species and strains.

The data discussed here from ferrets, dogs, pigs and people suggest that we need new ways of thinking about the mechanism involved in radiation induced death at the LD₅₀ level. As the human LD₅₀ is closer to the LD₅₀ of ferrets, pigs and dogs than it is to the LD₅₀ of mice, this relationship would suggest that species such as ferrets, pigs and dogs are likely to be better animal models than mice for evaluating the effects of radiation on the bleeding risks for humans. If radiation-induced DIC leads to human death at the LD₅₀ dose, it is important that a species exhibiting a bleeding phenotype at an LD₅₀ dose close to that of humans be utilized for studies related to the activation of the coagulation cascade and appropriate countermeasures for this phenomenon, as the countermeasures appropriate for use in the prevention/cure of DIC and the cell killing effects of radiation may be very different.

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References

1. Bond VP, Robinson CV. A mortality determinant in nonuniform exposures of the mammal. *Radiat Res Suppl.* 1967; 7:265–275. [PubMed: 6058663]
2. Morris MD, Jones TD. A comparison of dose-response models for death from hematological depression in different species. *Int J Radiat Biol.* 1988; 53:439–456.
3. Krigsfeld G, Sanzari J, Kennedy AR. The effects of proton radiation on the prothrombin and partial thromboplastin times of irradiated ferrets. *Int J Radiat Biol.* 2012; 88:327–334. [PubMed: 22221163]
4. Krigsfeld GS, Savage AR, Sanzari JK, Wroe AJ, Gridley DS, Kennedy AR. Mechanism of hypocoagulability in proton irradiated ferrets. *Int J Radiat Biol.* 2013 [Epub ahead of print].
5. Hay KL, Bull BS. Rapid-SF: a rapid whole-blood screen for soluble fibrin monomer. *Thromb Haemost.* 2002; 88:773–80. [PubMed: 12428093]
6. Bick RL. Disseminated intravascular coagulation: a review of etiology, pathophysiology, diagnosis, and management: guidelines for care. *Clin Appl Thromb Hemost.* 2002; 8:1–31. [PubMed: 11991236]
7. Hall, EJ.; Giaccia, AJ. *Radiobiology for the Radiologist.* 6. Philadelphia: Lippincott, Williams & Wilkins; 2006.
8. Lushbaugh, CC. *Advances in Radiation Biology.* Vol. 3. Academic Press Inc; New York: 1969. Reflections on some recent progress in human radiobiology; p. 277–314.
9. Harding, RK. 5-HT₃ receptor antagonists and radiation-induced emesis: preclinical data. In: Reynolds, DJM.; Andrews, PLR.; Davis, CJ., editors. *Serotonin and the scientific basis of anti-emetic therapy.* Oxford, London: Oxford Clinical Communications; 1995. p. 127–33.
10. Moroni M, Lombardini E, Salber R, Kazemzadeh M, Nagy V, Olsen C, Whitnall MH. Hematological changes as prognostic indicators of survival: similarities between Gottingen minipigs, humans, and other large animal models. *PLoS One.* 2011; 6:1–8.
11. Andersen AC. A substance observed within the vascular system of dogs receiving lethal exposures of whole-body x-irradiation. *Radiat Res.* 1957; 6:361–370. [PubMed: 13408478]

12. Winchell HS, Anderson AC, Pollycove M. Radiation-induced hemorrhagic diathesis in dogs unassociated with thrombocytopenia: association with an intravascular protein-polysaccharide particle. *Blood*. 1964; 23:186–192. [PubMed: 14122243]
13. Liebow AA, Warren S, Decoursey E. Pathology of atom bomb casualties. *Am J Pathol*. 1949 Sep; 25(5):853–1027. [PubMed: 18147964]
14. Fujita S, Kato H, Schull WJ. The LD50 associated with exposure to the atomic bombing of Hiroshima and Nagasaki. *J Radiat Res*. 1991; 32 (Suppl):154–61. [PubMed: 1762100]
15. Reitan, JB.; Stavem, P.; Kett, K.; Hoel, PS. The 60Co accident in Norway 1982: A Clinical Reappraisal. Ricks, RC.; Fry, SA., editors. New York: Elsevier Science Publishing Co., Inc; 1990. p. 3-11.
16. Valverde, NJ.; Cordeiro, JM.; Oliveira, AR.; Brandao-Mello, CE. The acute radiation syndrome in the 137Cs Brazilian Accident, 1987. In: Ricks, RC.; Fry, SA., editors. *The Medical Basis for Radiation Accident Preparedness II Clinical Experience and Follow-up Since 1979*. New York: Elsevier Science Publishing Co., Inc; 1990. p. 89-107.
17. The Radiological Accident in Goiania. International Atomic Energy Agency; Vienna: 1988. (http://www-pub.iaea.org/mtcd/publications/pdf/pub815_web.pdf)
18. McNicol A, Israels SJ. Beyond hemostasis: the role of platelets in inflammation, malignancy and infection. *Cardiovasc Hematol Disord Drug Targets*. 2008; 8:99–117. [PubMed: 18537597]
19. Smyth SS, McEver RP, Weyrich AS, Morrell CN, Hoffman MR, Arepally GM, et al. Platelet functions beyond hemostasis. *J Thromb Haemost*. 2009; 7:1759–66. [PubMed: 19691483]
20. Lorenz E, Congdon CC. Radioactivity; biologic effects of ionizing radiations. *Annu Rev Med*. 1954; 5:323–338. [PubMed: 13181380]
21. Levy M, Dorffler-Melly J, Reitsma P, Buller H, Florquin S, Van der Poll T, et al. Aggravation of endotoxin-induced disseminated intravascular coagulation and cytokine activation in heterozygous protein-C-deficient mice. *Blood*. 2003; 101:4823–7. [PubMed: 12609841]
22. Tatsumi K, Ohashi K, Tateno C, Yoshizato K, Yoshioka A, Shima M, Okano T. Human hepatocyte propagation system in the mouse livers: functional maintenance of the production of coagulation and anticoagulation factors. *Cell Trans*. 2012; 21:437–45.
23. Miller CP, Hammond CW, Tompkins M. The role of infection in radiation injury. *J Lab Clin Med*. 1951; 38:331–334. [PubMed: 14880746]
24. Boone IU, Woodward KT, Harris PS. Relation between bacteremia and death in mice following x-ray and thermal column exposures. *J Bacteriol*. 1952; 71:188–195. [PubMed: 13295192]
25. Eisele GR, West JL. Bacteriological evaluations of swine exposed to lethal levels of gamma radiation. *J Anim Sci*. 1973; 37:27–32. [PubMed: 4198388]
26. Gando S, Sawamura A, Hayakawa M. Trauma, shock, and disseminated intravascular coagulation: lessons from the classical literature. *Ann Surg*. 2011; 254:10–9. [PubMed: 21368657]
27. Schae D, Kachikwu EL, McBride WH. Cytokines in radiobiological responses: a review. *Radiat Res*. 2012; 178:505–23. [PubMed: 23106210]
28. Ni H, Balint K, Zhou Y, Gridley DS, Maks C, Kennedy AR, Weissman D. Effect of solar particle event radiation on gastrointestinal tract bacterial translocation and immune activation. *Radiat Res*. 2011; 175:485–491. [PubMed: 21294608]
29. Zhou Y, Ni H, Li M, Sanzari JK, Diffenderfer ES, Lin L, Kennedy AR, Weissman D. Effect of solar particle event radiation and hindlimb suspension on gastrointestinal tract bacterial translocation and immune activation. *PLOS One*. 7(9):e44329.10.1371/journal.pone.0044329 [PubMed: 23028522]
30. Hook KM, Abrams CS. The loss of homeostasis in hemostasis: new approaches in treating and understanding acute disseminated intravascular coagulation in critically ill patients. *Clin Tran Sci*. 2012; 5:85–92.
31. Gorlach A. Redox regulation of the coagulation cascade. *Antioxid Redox Signal*. 2005; 7:1398–404. [PubMed: 16115045]

TABLE 1LD₅₀ Values for Human Populations for Number of Different Animal Species

Species	LD ₅₀	Reference
Ferret	<2 Gy	Harding (9)
Pigs ^a (4 strains)	2.57 Gy	Morris and Jones (2)
Dogs ^a (11 strains)	2.62 Gy	Morris and Jones (2)
Primates ^a (8 strains)	4.61 Gy	Morris and Jones (2)
Mice ^a (49 strains)	8.16 Gy	Morris and Jones (2)
Humans	3–4 Gy ^b	Hall and Giaccia (7)

^a The actual LD₅₀ values for the animal strains were determined experimentally in the studies reviewed by Morris and Jones (2), averaged values are presented here.

^b For young adults without medical invention; may be less for the very young or the old.