Mechanism of action for anti-radiation vaccine in reducing the biological impact of high-dose gamma irradiation

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Abstract

Ionizing radiation is a major health risk of long-term space travel, the biological consequences of which include genetic and oxidative damage. In this study, we propose an original mechanism by which high doses of ionizing radiation induce acute toxicity. We identified biological components that appear in the lymphatic vessels shortly after gamma irradiation. These radiation-induced toxins, which we have named specific radiation determinants (SRD), were generated in the irradiated tissues and then collected and circulated throughout the body via the lymph circulation and bloodstream. Depending on the type of SRD elicited, different syndromes of acute radiation sickness (ARS) were expressed. The SRDs were developed into a vaccine used to confer active immunity against acute radiation toxicity in immunologically naïve animals. Animals that were pretreated with SRDs exhibited resistance to lethal doses of gamma radiation, as measured by increased survival times and survival rates. In comparison, untreated animals that were exposed to similar large doses of gamma radiation developed acute radiation sickness and died within days. This phenomenon was observed in a number of mammalian species. Initial analysis of the biochemical characteristics indicated that the SRDs were large molecular weight (200-250 kDa) molecules that were comprised of a mixture of protein, lipid, carbohydrate, and mineral. Further analysis is required to further identify the SRD molecules and the biological mechanism by which the mediate the toxicity associated with acute radiation sickness. By doing so, we may develop an effective specific immunoprophylaxis as a countermeasure against the acute effects of ionizing radiation.

1. Introduction

Ionizing radiation is recognized as a significant environmental hazard of space travel, posing a significant health risk to human crews (Cucinotta et al., 2001b). Radiation is known to increase the occurrence of cancer, cardiovascular disease, and cataracts (Preston et al., 2003, Ivanov et al., 2001, Otake et al., 1996, Cucinotta et al., 2001a). In addition to these degenerative consequences, high doses of radiation induce acute radiation sickness (ARS) and death via well-defined pathologies (Prasad, 1995). However, the underlying cellular and molecular mechanisms that drive acute radiation-induced toxicity are not fully elucidated.

Considerable effort has been devoted to elucidate the biological consequences of ionizing radiation. A major mechanism of effect is the ionizing damage directly inflicted on the cells' DNA by radiation (Nelson, 2003, Ward, 1988). Unrepaired DNA damage is known to lead to genetic mutations, apoptosis, cellular senescence, carcinogenesis, and death (Wu et al., 1999, Oh et al., 2001, Rosen et al., 2000, Pietras et al., 1347, Gajdusek et al., 1996). The ionizing effects of radiation also generate oxidative reactions that cause physical changes in proteins, lipids, and carbohydrates, impairing their structure and/or function (Spitz et al., 2004, Lehnert and Iyer, 2002). Similarly, the hydrolysis of water molecules introduces a secondary source of oxidative stress in the form of free radicals that also induce the biochemical alteration, degradation, or cross-linking of cellular macromolecules (Martinez et al., 1997, Prasad, 1995). Physical and functional damage to the plasma membranes and mitochondria has been reported in irradiated cells (Haimovitz-Friedman et al., 1994, Lucero et al., 2003, Leach et al., 2001, Costantini et al., 1996). The radiation-induced expression of inflammatory cytokines suggests that inflammatory responses may contribute to cell death and acute radiation sickness toxicity (Mizutani et al., 2002). However, the acute toxicity that is associated with ARS is not attributed to these biological mechanisms.

Because acute radiation sickness occurs within a very short period of time, the opportunities to treat or mitigate the effects of high-dose irradiation are very limited. Instead, a prophylactic measure would be a more effective strategy to address this acute radiation-induced phenomenon. In addition, preventing the onset of ARS may also be beneficial in minimizing the other biological consequences of ionizing radiation. In this study, we describe a novel biological mechanism of acute radiation toxicity that originates in the lymphatic tissues. We also describe the development of an experimental anti-radiation vaccine against these novel radiotoxins that appear in the radiosensitive tissues after irradiation, this radiotoxins were called specific radiation determinants (SRD). Because it is directed at a biological mechanism other than DNA damage or oxidative stress, this immunologically based form of prophylaxis may be a powerful adjunct therapy that will enhance the efficacy of existing radiation countermeasures.

2. Materials and Methods

2.1 Animal subjects

These studies incorporated the use of statistically significant numbers of a variety of mammals (Table 1). The animals that were used were typically young adults of average weight for their species.

2.2 Irradiation protocols

The animals were exposed to high doses of gamma radiation to induce acute radiation sickness and death within 7 days. Whole body equivalent doses were typically between 6-10 Gy, and delivered at an energy of 0.66 MeV and a dose rate of 3.7-3.8 cGy/min.

2.3 Isolation and biochemical characterization of SRDs

Lethal doses of gamma radiation were administered parenterally to bovine species and their lymphatic fluid was collected several hours later. Within hours, lymphatic fluid was collected from the animals' ductus thoracicus and the SRDs were separated by size exclusion chromatography (Maliev et al., 1990). The SRDs were subjected to chromatographic separation to determine their biochemical composition and molecular weight (le Maire et al., 1987).

2.4 Radiomimetic and radioprotective properties of SRDs.

The levels of SRDs were quantitated with an SRD ELISA (Popov et al., 1989). Previously untreated animals were treated with SRDs by subcutaneous or intramuscular injection. After a minimum incubation time of 21 days, the animals were subjected to lethal doses of gamma irradiation (Popov et al., 1990). The post-irradiation survival times of control and immunized animals were recorded.

3. Results

3.1 Radiation-induced formation of SRDs.

Application of lethal doses of gamma radiation induced the appearance of SRDs in animals' lymphatic fluid within hours (Table 2). SRDs were also detected in the animals' blood shortly after their initial appearance in the lymphatic fluid. This suggests that the SRDs originate in the lymphatic tissues, circulate into the bloodstream, and then traverse the body via the circulatory system. This phenomenon was observed in rabbits, dogs, and cattle; however, the precise timeline of SRD appearance varied in each species.

3.2 Biochemical analysis of SRDs

Initial chromatographic analysis of two of the radiation toxins induced by acute doses of lethal gamma radiation revealed complex biochemical compositions (Table 3). Approximately half of the SRDs were comprised of protein, with lipids comprising an abundant fraction of the molecule. Carbohydrates comprised approximately 10% of the SRD molecules and trace amounts of minerals were also detected. The biochemical composition of SRD 3 and SRD 4/4 were similar, but distinct differences were noted. The molecular weights of the SRDs are approximately 200-250 kDa (not shown). Chromatographic analysis of the remaining SRDs remains to be completed.

Seven distinct variants of SRDs have been identified to date (Table 4). Multiple variants of SRDs were induced simultaneously, but in all cases, a single SRD comprised the majority of SRD generated with trace amounts of other SRDs detected. The primary variant detected was associated with the type or severity of ARS induced, rather than total radiation dose or animal species. For example, SRD 1 was the most abundant SRD observed in animals that developed the cerebral syndrome of ARS, while SRD 3 was the most abundant SRD observed in animals that developed the gastrointestinal syndrome of ARS, regardless of species (Table 4).

3.3 Radiomimetic and radioprotective properties of SRDs

There was a dose-dependent response to the SRDs when they were injected into previously untreated animals. In high doses, the SRDs induced the symptoms of the ARS with which it was associated, including death, in unirradiated animals. In smaller doses, the development of specific active immunity against the toxic effects of lethal doses of gamma irradiation was observed (Table 5). Untreated control animals that were exposed to lethal doses of irradiation died of ARS within 30 days. Animals that were pretreated with SRDs experienced significantly increased survival rates and survival times. This phenomenon was observed in all mammals tested (Table 5).

4. Discussion

In this study, we describe a novel mechanism of radiation-induced toxicity. Our experiments show that high doses of gamma radiation elicited the production of toxic compounds in the irradiated tissues, called SRDs. These SRDs arose in tissues of origin, accumulated in lymphatic vessels, and then entered the blood circulatory system via the thoracic duct. The SRDs then circulated throughout the body and ultimately induced different forms of acute radiation disease and death. In the studies presented here, the SRDs were detected in the lymphatic fluid shortly after irradiation, and then in the blood shortly thereafter. In a previous study, the removal of lymphatic fluid immediately after irradiation extended the animals survival time from a predicted seven days to sixty days (Popov et al., 1990), which supports the hypothesis that the SRDs collected in the irradiated lymph tissues and traversed the body through the general circulatory system.

The SRDs exhibited radiomimetic properties. When introduced into immunologically and radiation-exposure naïve animals, concentrated doses of SRDs stimulated a recapitulation of acute radiation sickness, including death. The SRD compounds alone induced the acute toxicity associated with high doses of irradiation, although other known physiological effects of high-dose radiation probably contributed to overall mortality. We propose that the generation of the SRDs is a contributory cause of radiation toxicity in a biological mechanism that is independent of the direct molecular damage, oxidative stress, inflammation, or mutagenesis mechanisms to

which most other radiobiological effects are attributed. Most of this work has been performed in rodents, but these immunological properties of the SRDs were also observed in larger mammals more closely related to humans. This suggests a universal mammalian response in acute radiation toxicity, and generates hope that SRDs can be effectively applied to humans to protect against ARS.

Interestingly, when administered in optimal doses to previously untreated animals, the SRDs conferred active immunity against the acute toxic effects of subsequent gamma irradiation. Optimal doses were determined individually, and depend on species, weight, and gender (Popov et al., 1990). Much work must be done to determine if this approach could be safely and effectively employed in humans. Furthermore, this immunity was highly specific; that is, each SRD protected against a single specific ARS syndrome, and the immunity conferred lasted no more than 2-3 years (not shown). Although it was not the primary focus of these experiments, no overt autoimmune reactions were observed in the experimental animals (not shown). Our studies focused on the use of the SRDs as a countermeasure against acute effects of irradiation. Because our studies suggest that the SRDs provided protection against early radiation events, they may also provide protection against the pathogenesis of long-term, degenerative effects of ionizing radiation, such as carcinogenesis, cataractogenesis, or atherogenesis. Clearly, further analyses of the long-term radioprotective effects of the SRDs are warranted.

The precise biochemical structure of the SRDs was only partially elucidated. The SRDs appeared to be a group of complex molecules generated in irradiated radiosensitive tissues. The large molecular weight of the molecules (200-250 kDa) suggests that they may be a complex of several smaller molecules. The similar composition of SRD3 and SRD 4/4 suggest that the molecules, while biochemically distinct in composition, may be related molecules. The large percentage of lipids detected in the molecules suggests that the molecule was associated with cell membranes (Dowhan, 1997). The presence of carbohydrates, which typically adorn cellular components found on the cell surface or in the extracellular matrix, particularly suggest that the plasma membrane is the origin of at least part of these molecules. We propose that the ionizing properties of gamma radiation induce physical disruption of the plasma membranes of the cells that populate or line the lymphatic tissues or other highly radiosensitive cells, possibly via lipid peroxidation (Prasad, 1995), and that the SRDs form from the degradation products or cross-linking reactions induced by ionizing radiation. Further studies are required to definitively identify the biochemical composition of the SRDs and their biological mechanism(s) of action.

In this study, we propose an original paradigm of contributory acute toxic effects of lethal doses of irradiation which is mediated through the immune system, together with the traditionally accepted cellular mechanisms of DNA damage or oxidative stress. We analyzed the biochemical properties of the SRD molecules and proposed a potentially practical application of their radioprotective properties. The SRDs may provide a powerful adjunct to other prophylactic countermeasures aimed at reducing the biological risks of ionizing radiation.

5. References

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6. Tables

Table 1. Animals used in irradiation studies.

Species	Age	Weight	Number used in vaccine studies
Black motley cattle	2.5-3.0 years	300-350 kg	134
Ukrainian pigs	6-12 months	35-90 kg	142
Prekos sheep	3-12 months	18-23 kg	156
Mixed breed dogs	2-4 years	6.0-6.5 kg	162
Chinchilla rabbits	11-12 months	3.5-3.7 kg	180
Latvian draft horses	3-8 years	350-550 kg	32
Balp mice	2-3 months	20-22 g	2,636
Wistar rats	3-4 months	180-220 g	4,002

This table lists the number of experimental animals used, including control animals.

Table 2. Gamma radiation induces the appearance of SRDs in the circulatory system.

		Peak SRD appearance time (hours after irradiation)					
		Rabbits		Dogs		Cattle	
SRD type	ARS severity	lymph	blood	lymph	blood	lymph	blood
SRD 4/1	mild	3-8	6-10	5-8	20-40	3-12	6-15
SRD 4/2	moderate	3-10	6-10	3-10	15-50	3-14	8-15
SRD 4/3	severe	3-24	6-24	1-72	8-72	3-24	6-72
SRD 4/4	extremely severe	1-24	6-28	1-72	4-40	2-28	3-80

Rabbits, dogs, and cattle were exposed to lethal doses of gamma radiation and the SRDs were measured in lymph fluid and blood. Peak amounts of SRDs were detected first in lymph fluid within hours of irradiation and then in blood hours or days later.

Table 3. Biochemical composition of SRDs.

Component (%)	SRD 3	SRD 4/4
Protein	50.1 ± 0.1	56.2 ± 0.1
Lipid	38.2 ± 0.0	30.1 ± 0.1
Carbohydrate	10.2 ± 0.0	10.1 ± 0.1
Mineral residue	1.3 ± 0.0	3.4 ± 0.2

SRDs were isolated from γ-irradiated cattle and subjected to chromatographic analysis. The SRDs are complex molecules comprised of protein, lipid, carbohydrate, and mineral residue.

Table 4. SRD variants are associated with specific syndromes of acute radiation sickness.

Specific Radiation Determinant	ARS syndrome
SRD 1	cerebral ARS
SRD 2	toxic ARS
SRD 3	gastrointestinal ARS
SRD 4/1	mild typical ARS
SRD 4/2	moderate typical ARS
SRD 4/3	severely typical ARS
SRD 4/4	extremely severe typical ARS

ARS syndromes generated mixed populations in irradiated animals. In each case, the most abundant SRD in each population was determined by the type of ARS syndrome the animal developed, regardless of species.

Table 5. SRD vaccine extends survival times in irradiated animals.

Species	Radiation (Gy)	SDR vaccine (mg/kg)	Number of animals	Survival rate (%) 30 days	60 days	180 days	360 days
Dogs	6.5	0	17	0	0	0	0
		15	93	88	79	65	65
Pigs	7.5	0	30	0	0	0	0
		15	68	65	61	54	54
Sheep	6.5	0	23	0	0	0	0
		20	112	90	84	78	78
Horses	6.5	0	5	0	0	0	0
		20	19	14	13	13	13
Cattle	9.2	0	10	0	0	0	0
		20	60	59	57	54	51
Rats	8.5	0	250	0	0	0	0
		10	3696	3326	3142	---	---
Mice	7.0	0	300	0	0	0	0
		10	2170	1628	1628	---	---

SRD 3 was isolated from γ-irradiated cattle and then administered to immunologically naïve animals. Both untreated and immunized animals were irradiated, and their survival times were recorded. The survival rates of mice and rats were not determined after 60 days because of their shorter life expectancies compared to other species.