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C-reactive protein and serum amyloid A as early-phase and prognostic indicators of acute radiation exposure in nonhuman primate total-body irradiation model

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ABSTRACT

Terrorist radiological attacks or nuclear accidents could expose large numbers of people to ionizing radiation. In mass-casualty radiological incidents early medical-management requires triage tools for first-responders to quantitatively identify individuals exposed to life-threatening radiation doses and for early initiation (i.e., within one day after radiation exposure) of cytokine therapy for treatment of bone marrow acute radiation syndrome.

Herein, we present results from 30 rhesus macaques total-body irradiated (TBI) to a broad dose range of 1–8.5 Gy with ^{60}Co γ-rays (0.55 Gy min^{-1}) and demonstrate dose- and time-dependent changes in blood of C-reactive protein (CRP), serum amyloid A (SAA), and interleukin 6 (IL-6) measured by enzyme linked immunosorbent assay (ELISA). CRP and SAA dose–response results are consistent with ~1 Gy and ~0.2 Gy thresholds for photon-exposure at 24 h after TBI, respectively. Highly significant elevations of CRP and SAA ($p = 0.00017$ and $p = 0.0024$, respectively) were found in animal plasma at 6 h after all TBI doses suggesting their potential use as early-phase biodosimeters. Results also show that the dynamics and content of CRP and SAA levels reflect the course and severity of the acute radiation sickness (ARS) and may function as prognostic indicators of ARS outcome.

These results demonstrate proof-of-concept that these radiation-responsive proteins show promise as a complementary approach to conventional biodosimetry for early assessment of radiation exposures and may also contribute as diagnostic indices in the medical management of radiation accidents.

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1. Introduction

Tools for rapid identification of severely irradiated individuals who require prompt medical treatment as well as to distinguish exposed vs. non-exposed individuals are needed in mass-casualty radiological incidents (Blakely et al., 2005). Early treatment of individuals exposed to life-threatening ionizing radiation requires accurate and rapid biodosimetry with a precision as high as possible to determine risk for morbidity and mortality (MacVittie et al., 2005).

The biological monitoring of radiation-responsive proteins as well as their combination with hematological biomarkers has been suggested for the use in radiation exposure assessments (Blakely et al., 2005, 2010; Ossetrova et al., 2007, 2010; Ossetrova and Blakely, 2009). Use of multiple protein targets, along with classical biodosimetric methodologies, is expected to enhance the specificity and diagnostic utility of a protein-based biomarker approach for early assessment of severe radiation exposure.

The use of nonhuman primate (NHP) model systems to validate suitable medical countermeasures and novel biodosimetric approaches is clearly recognized by the scientific community (Stone et al., 2004). Nonhuman primates exhibit a survival time–dose response similar to humans (Dixon, 1985). We recently reported results from NHP total-body irradiated (TBI) model studies and demonstrated the multiparametric approach enhancement for successful separation of samples from exposed NHPs vs. samples from the same animals before irradiation (Ossetrova et al., 2007; Blakely et al., 2010). We also showed for the first time in a murine TBI model that a protein expression profile can be developed not only to predict radiation exposure in mice but also to distinguish the level of radiation exposure, ranging from 1 to 7 Gy (Ossetrova and Blakely, 2009). The effect of combining SAA and hematological biomarkers demonstrated: i) enhanced separation of 1-Gy irradiated animals from controls and also between different combinations of doses, and ii) improvement of the threshold for γ-exposure detection up to ~1 Gy compared to the selected protein profile only (Ossetrova et al., 2010).

The physiological response to radiation injury initiates wide-ranging systemic response events. Shortly after the injury, macrophages produce a wide range of cytokines and growth factors.

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Cytokines, via the central nervous system and blood circulation, initiate a wide range of systemic responses including fever, increasing production and differentiation of bone marrow cells, and dramatic expression of acute-phase proteins CRP and SAA (Steel and Whitehead, 1993). CRP and SAA are well known multifunctional apolipoproteins involved in modulating numerous immunological responses during the acute-phase response to injury, infection, and stress (Gabay and Kushner, 1999). Acute-phase proteins, whose concentrations are significantly increased during the acute-phase response, have been extensively investigated and shown to play an essential role in injuries caused by radiation in animals (Petrov, 1963; Wood et al., 1960; Tukachinski and Moiseeva, 1961; Mal'tsev et al., 1978; Agay et al., 1997) and humans (Koc et al., 2003; Mal'tsev et al., 2006). SAA has been reported to have an important pro-inflammatory and immunostimulating role by recruiting neutrophils, monocytes, and T-lymphocytes into inflammatory lesions (Badolato et al., 1994; Xu et al., 1995). However, SAA has not received as much attention, as CRP did in the past, and specifically in injury caused by irradiation (Wu et al., 2007). Numerous studies in nonhuman primates (Petrov, 1963; Mal'tsev et al., 1978) and rabbits (Tukachinski and Moiseeva, 1961) show that dynamics and content of CRP exactly reflect the course and severity of the acute radiation sickness (ARS), as well as demonstrating its ability to play a role as a prognostic ARS indicator. They demonstrated that periods of appearance of CRP in the blood of irradiated animals correlate with periods of expressed development of the cytolytic and destructive processes induced by irradiation. Indexes of CRP content in peripheral blood of 147 Chernobyl nuclear power plant accident patients during primary reaction to the irradiation (1–2 d after) and in the latent period of radiation disease (3–9 d after) were correlated with a prognosis of ARS outcome (Mal'tsev et al., 2006).

Herein, we present results from the on-going NHP TBI studies demonstrating dose- and time-dependent changes in IL-6, CRP and, for the first time, in SAA. We expect that results from these studies provide additional necessary proof-of-concept that radiation-responsive protein biomarkers bridge the current gap in biodosimetry tools for rapid and effective assessment of radiation exposure early after an incident, especially after a mass-casualty radiological event.

2. Materials and methods

2.1. Model system, radiation exposure, and peripheral blood biosampling

Domestic-born, nonhuman primates, male and female rhesus monkeys, *Macaca mulatta*, (~5.5 kg; ~4-yr old at the time of exposure) were housed in stainless steel cages in conventional holding rooms at the Veterinary Research Department in an animal facility of the Armed Forces Radiobiology Research Institute (AFRRI) accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. 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 Animal Resources, National Research Council, and under an Institutional Animal Care and Use Committee (IACUC) approved protocol.

NHPs in dose cohorts ($n = 6$), received TBI to a midline tissue dose of 1.0, 3.5, 6.5, and 8.5 Gy ^{60}Co γ-rays at 0.55 Gy min^{-1} at the AFRRI's ^{60}Co facility, which provides a highly uniform radiation field. Ketamine anesthetized animals (Ketaset [10 mg kg^{-1}, i.m.], Fort Dodge Laboratories; Fort Dodge, IA) were placed in a Plexiglass restraint chair and irradiated bi-laterally. Animals representing sham ($n = 6$) were also treated the same except they were not exposed to radiation.

At designated sampling time points (i.e., from 6 h to 60 d) after exposure, peripheral blood was drawn from NHPs while under anesthesia. Drawn blood was collected into a serum separator tube and potassium EDTA vacutainer tubes (BD, Franklin Lakes, NJ). Blood in EDTA tubes for white blood cell count measurements was analyzed within several hours after biosampling. For protein measurements, tubes with collected peripheral blood were centrifuged at 800*g* (4 °C) for 10 min to isolate the supernatant (plasma) and cell pellets. Blood sample aliquots were stored frozen (−80 °C) until analysis.

2.2. Protein bioassays

Sandwich ELISA for monkey CRP was performed using a commercially available kit (Life Diagnostics, Inc., West Chester, PA) according to the manufacturer's instructions. The limit of detection (LOD) for the assay was 0.8 ng mL^{-1}. Sandwich ELISA for monkey SAA was performed using a newly developed commercially available kit (Life Diagnostics, Inc., West Chester, PA). The LOD for the assay was 0.5 ng mL^{-1}. Sandwich ELISA for monkey IL-6 was performed using a commercially available kit (Cell Sciences, Inc., Canton, MA). The sensitivity for the assay was 1 pg mL^{-1}. Three replicate measurements were determined for each sample and standards. The optical density was measured using a spectrophotometer (BIO-TEK Instruments, Inc., Winooski, VT). The CRP, SAA, and IL-6 concentrations in plasma samples were determined via use of Table Curve 2D software (Systat Software Inc., San Jose, CA).

2.3. Peripheral blood cell counts

Complete blood cell counts and differentials were determined using a clinical hematology analyzer (Bayer Advia 120, Bayer, Tarrytown, NY), as previously described (Blakely et al., 2007).

2.4. Data analysis

The analysis of variance (ANOVA) was used when comparing more than two groups and the two-sided Student's *t* test was used when comparing two groups to determine significant differences among sampling time- and dose-points. Values of $p < 0.05$ were considered statistically significant. Values are expressed as mean ± standard error (SE). Receiver Operating Characteristic curve (or ROC curve) (MedCalc Software, Broekstraat, Belgium), was used to demonstrate the sensitivity and specificity of the proposed protein biomarkers to reflect subgroup differences.

3. Results and discussion

The radiation-induced interleukin 6 (IL-6) levels peaked at 6 h in a dose-dependent manner (Fig. 1). Baseline level of IL-6 in plasma of non-exposed animals was log-normally-distributed with a highest probability IL-6 value of 1.8 (±0.56) pg mL^{-1}. In 86% of all observations/cases, IL-6 ranged from 0.72 (±0.52) pg mL^{-1} to 6.12 (±0.94) pg mL^{-1}. In the remaining three animals IL-6 level was 14.81 (±1.73) pg mL^{-1}, 17.84 (±1.78) pg mL^{-1}, and 24.44 (±1.90) pg mL^{-1}. A highly significant elevation of IL-6 ($p = 0.000012$) was found after TBI to 1 Gy compared to a sham cohort. IL-6 levels were examined because this cytokine was found to play a role in the cascade of events after irradiation (Neta et al., 1992; Wang and Clark, 1988). This cytokine was shown to be an essential contributor to natural resistance to lethal irradiation and its expression *in vivo* showed a significant effect on hematopoietic recovery after radiation.