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Opinion/Position paper

Persistent depletion of plasma gelsolin (pGSN) after exposure of mice to heavy silicon ions

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ABSTRACT

Little is known about plasma proteins that can be used as biomarkers for early and late responses to radiation. The purpose of this study was to determine a link between depletion of plasma gelsolin (pGSN) and cell-death as well as inflammatory responses in the lung (one of the tissues known to be radiosensitive) of the same exposed CBA/CaJ mice after exposure to heavy silicon (^{28}Si) ions. To prevent the development of multiple organ dysfunctions, pGSN (an important component of the extracellular actin-scavenging system) is responsible for the removal of actin that is released into the circulation during inflammation and from dying cells. We evaluated the levels of pGSN in plasma collected from groups of mice (5 mice in each) at 1 week (wk) and 1 month (1 mo) after exposure whole body to different doses of ^{28}Si ions, *i.e.* 0, 0.1, 0.25, or 0.5 Gy (2 fractionated exposures, 15 days apart that totaled each selected dose). In the same mouse, the measurements of pGSN levels were coupled with the quantitation of injuries in the lung, determined by (*a*) the levels of cleaved poly (ADP-ribose) polymerase (cleaved-PARP), a marker of apoptotic cell-death, (*b*) the levels of activated nuclear factor-kappa B (NF-κB) and selected cytokines, *i.e.* tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and IL-6, from tissue-lysates of the lung. Further, the ratio of neutrophils and lymphocytes (N/L) was determined in the same mouse. Our data indicated: (*i*) the magnitude of pGSN depletion was dependent to radiation dose at both harvest times, (*ii*) a persistent depletion of pGSN up to 1 mo post-exposure to 0.25 or 0.5 Gy of ^{28}Si ions, (*iii*) an inverse-correlation between pGSN depletion and increased levels of cleaved-PARP, including activated NF-κB/pro-inflammatory cytokines in the lung, and (*iv*) at both harvest times, statistically significant increases in the N/L ratio in groups of mice exposed to 0.5 Gy only. Our findings suggested that depletion in pGSN levels reflects not only the responses to ^{28}Si-ion exposure at both harvest times but also early and late-occurring damage.

1. Introduction

The plasma gelsolin (pGSN) protein (approximately 82 kDa) is an important component of the extracellular actin-scavenging system (EASS) during cell and tissue injuries (Lee and Galbraith 1992; Silacci et al., 2004; Park et al., 2006; Bucki et al., 2008). Actin, a highly conserved cytoskeleton protein, is released to the extracellular environment after cell death resulting from normal metabolism or from tissue injury following inflammation or exposure to radiation (Chen 2005). When actin is released to the extracellular environment it polymerizes into actin filaments (F-actin) that stimulate inflammatory reactions if not removed (Osborn et al., 2008). To prevent further cell and tissue damage from inflammatory responses, the EASS is responsible for the removal of F-actin from the circulation. It has been shown that if actin filaments are not continuously removed from the circulation, they will be involved in acute tissue injury and linked to late-occurring multiple organ dysfunction syndromes (MODS) (Haddad et al., 1990; Lee and Galbraith 1992; Dahl et al., 1999; Erukhimov et al., 2000). Of note, the capacity of cell defense via the EASS can be overwhelmed by cell/tissue injury, leading to secondary organ damage and even death.

The EASS is composed of pGSN and plasma Gc-globulin (52 kD) (Lee

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and Galbraith 1992; Kułakowska et al., 2010). The role of pGSN is to bind to the sides of the F-actin filaments. After binding, pGSN ruptures F-actin (termed as "severing" step) by breaking the hydrophobic bonds holding actin monomers together, resulting in the breakage of the filaments into two monomers of actin (called G-actin). Following this severing step, pGSN remains tightly bound to one end of the polarized F-actin (referred to as a "barbed" or "plus" end), a process termed “capping”, to prevent further polymerization. Subsequently, Gc-globulin binds to the G-actin and these complexes are cleared primarily in the liver (Meier et al., 2006). The levels of pGSN and Gc-globulin are reduced during the action of the EASS. However, the reduction of Gc-globulin level is not only to a lesser extent than that of pGSN but also is transient (Dahl 2005; Peddada et al., 2012). These findings strongly suggest that the decline in levels of pGSN is a better molecular biomarker of inflammation-related tissue damage that could potentially be valuable for dose assessment and predicting early and late health risks after irradiation.

In humans, it has been well characterized that pGSN is depleted by the inflammatory response and is highly linked to the extent of disease or injury, *e.g.* acute respiratory distress syndrome, acute liver failure, myocardial infarction, septic shock, and rheumatoid arthritis (DiNubile 2008; Peddada et al., 2012). However, there are no human studies investigating the potential association of pGSN depletion and radiation-induced inflammation, which is known to be one of the deleterious effects of radiation (Schaue et al., 2015). In animal studies, there is an association between pGSN depletion and acute oxidation damage in the lung of BALB/c mice exposed to high doses of radiation, *i.e.* 12.5 to 13.5 Gy of 250 kVp x rays (Christofidou-Solomidou et al., 2002) or in the lung of C57BL/6 mice exposed to 4 or 20 Gy of ^{137}Cs γrays (Wu et al., 2013). In our laboratory, by means of 2D gel electrophoresis in combination with MALDI-TOF and/or nano ESI-LC mass spectrometry, we detected a remarkably persistent depletion of pGSN in plasma samples collected at 2 and 7 days from CBA/CaJ mice exposed to a single dose of 3 Gy of ^{137}Cs γ rays, in relation to the sham control groups (Rithidech et al., 2009a, b). Although there is no other histopathological evaluation, pGSN depletion is linked to radiation exposure since it is the only stressor used in that study.

Little is known about plasma proteins that can be used as predictive biomarkers for early and late effects of exposure to radiation. A simple blood test that provides a rapid diagnosis would be a major advance in radiation protection and biodosimetry. As part of a large study directed towards improving our understanding of the biological effects of heavy ions found in space, we focused on the characterization of changes in the levels of blood pGSN after a whole-body exposure of CBA/CaJ mice to various doses of 300 MeV/n ^{28}Si ions (LET = 77 keV/μm). ^{28}Si ions were selected for study because they are important heavy ions found in space as stated by the National Aeronautics and Space Administration (NASA 2010; NASA 2017). We hypothesized that alterations in the levels of pGSN can be used as *in vivo* predictive biodosimetry for not only early but also delayed injuries (determined by cell death and inflammation) in the lung of mice exposed to ^{28}Si ions.

We measured the levels of pGSN in plasma samples collected at an early time, *i.e.* 1 wk and a late time, *i.e.* 1 mo after exposure, coupled with the levels of cleaved poly (ADP-ribose) polymerase (cleaved PARP), a marker of cell death associated with caspase activation (Cesselli et al., 2001; Los et al., 2002). It should be noted that active caspase-3 is eliminated during apoptosis via the ubiquitin/proteasome pathway (Suzuki et al., 2001; Bader and Steller 2009). Hence, it is appropriate to measure the levels of cleaved PARP (not active caspase 3) because it allows the detection of perpetual apoptosis signals even in late stages of apoptosis. Moreover, to investigate the link between pGSN depletion and inflammation in the lung, we determined the levels of activated NF-κB and selected pro-inflammatory cytokines known to be regulated by NF-κB, *i.e.* TNF-α, IL-1β, and IL-6. We selected these three key pro-inflammatory cytokines for study because the expression of these cytokines (measured at the mRNA level) in the lung of humans and rodents following high doses of x-or γ-irradiation (5–20 Gy, delivered at high dose rates) has been well documented for many years (Finkelstein et al., 1994; Rubin et al., 1995; Johnston et al., 1996; Rübe et al., 2000; Rübe et al., 2004a; Rübe et al., 2004b; Chen et al., 2005a, b; Rübe et al., 2005; Ao et al., 2009). Expression of the IL-1β gene and its receptor was also detected in the lung tissue of C57BL/6 mice after exposure to lower doses of γ rays (acute exposure, between 0.5 and 2.5 Gy) (Johnston et al., 2010), or in the spleen, or macrophages of mice exposed to varying doses of γ rays (ranging from 0.05 to 7.0 Gy) (Chang et al., 2000; Hosoi et al., 2001; Liu et al., 2003). The lung was selected for study because it is one of the tissues known to be very sensitive to radiation exposure (Coggle et al., 1986; Hill 2005). Importantly, space radiation-induced damage in the lung is one of the major concerns of NASA (Shay et al., 2011; Barcellos-Hoff et al., 2015). We also evaluated the ratio of neutrophils to lymphocytes (N/L) in the blood to determine the injury to the hematopoietic system because it has been recognized that the N/L provides a more practical parameter for biodosimetry of early responses to radiation than the reduction of lymphocytes alone (Blakely et al., 2007).

2. Materials and methods

2.1. Animals and irradiation of mice to ^{28}Si ions (300 MeV/n, LET = 70 keV/μm)

All male CBA/CaJ mice were delivered directly from the Jackson laboratory (Bar Harbor, ME) to Brookhaven National Laboratory (BNL), Upton, NY, where irradiation took place. Since all mice included in this study were part of our large study conducted to improve our understanding of the biological effects of 300 MeV/n ^{28}Si ions, details of animal husbandry and ^{28}Si-irradiation were presented elsewhere (Tungjai et al., 2013). In brief, mice were allowed two weeks to acclimatize prior to irradiation (at 10–12 weeks of age, with an average body weight of 25 g). The protocols for animal housing and care, including experimental design, were approved by both the BNL and the Stony Brook University (SBU) Institutional Animal Care and Use Committee (IACUC). ^{28}Si-irradiation was performed at the NASA Space Radiation Laboratory (NSRL, Upton, NY). A fractionated schedule, 2 exposures, 15 days apart, totaling each selected dose, *i.e.* 0, 0.1, 0.25, or 0.5 Gy, at the dose rate of 10 mGy/min by a 20 × 20 cm beam were used. Of note, to mimic the exposure to radiation in the space environment, originally we requested the ^{28}Si beam-time for a fractioned exposure scheduled with one month apart. This is because it has been estimated that every cell in the body may be hit by a heavy ion approximately once a month in space (Cucinotta et al., 1998). However, the availability of the silicon beam-time and the overall runtime of heavy-ion exposures at NSRL-BNL at the time of conducting this project dictated the schedule of the fractionated exposure. Such constraints result in two fractions of exposures with 15 days apart. Additionally, at the time of the study, the fractionated exposure was the focus of NASA. Hence, there was no single dose acute-exposure protocol in this study. Fig. 1 shows a diagram of the experimental design.

2.2. Collection of samples

There were two harvest times following exposure to varying doses of 300 MeV/n ^{28}Si ions, *i.e.* 1 wk and 1 mo post-irradiation. Biological data detected in samples collected at 1 wk post-irradiation reflect early responses to ^{28}Si-irradiation, while those detected in samples collected at 1 mo post-irradiation represent late-occurring responses. Mice included in the sham-control group were age-matched to exposed mice. Therefore, at each sacrifice time, the age of mice in each treatment group would be similar. At each harvest time for each dose of 300 MeV/n ^{28}Si ions, the following tissues from each individual mouse (5 mice per group) were collected: