FINAL REPORT

Exposure to radiation above levels normally encountered on Earth can occur during wartime, accidents such as those at Three Mile Island and Chernobyl, and detonation of "dirty bombs" by terrorists. Relatively high levels of radiation exposure can also occur in certain occupations (low-level waste sites, nuclear power plants, nuclear medicine facilities, airline industry, and space agencies). Depression or dysfunction of the highly radiosensitive cells of the immune system can lead to serious consequences, including increased risk for infections, cancer, hypersensitivity reactions, poor wound healing, fibrosis and other pathologies.

The focus of this research was on the T helper (Th) subset of lymphocytes that secrete cytokines (proteins), and thus control many actions and interactions of other cell types that make up what is collectively known as the immune system. The Department of Energy (DOE) Low Dose Radiation Program is concerned with mechanisms altered by exposure to high energy photons (x- and gamma-rays), protons and electrons. This study compared, for the first time, the low-dose effects of two of these radiation forms, photons and protons, on the response of Th cells, as well as other cell types with which they communicate. The research provided insights regarding gene expression patterns and capacity to secrete potent immunostimulatory and immunosuppressive cytokines, some of which are implicated in pathophysiological processes. Furthermore, the photon versus proton comparison was important not only to healthy individuals who may be exposed, but also to patients undergoing radiotherapy. More medical centers in the United States, as well as worldwide, are turning to proton radiation than ever before.

The overall hypothesis of this study was that whole-body exposure to low-dose photons (gamma-rays) will alter CD4+ Th cell function. We further proposed that exposure to low-dose proton radiation will induce a different pattern of gene and functional changes compared to photons. These hypotheses were addressed in four Specific Aims: 1) Quantify low-dose photon effects on Th lymphocyte gene expression and signal transduction pathways. C57BL/6 mice were whole-body irradiated to total doses of 0, 0.01, 0.05 and 0.1 Gy (Co-57 gamma-rays). Spleens from a subset of animals per group were harvested for Th cytokine and relevant transcription factor gene expression immediately after irradiation; 2) Determine if Th cell function is significantly altered by low-dose photon radiation. The spleens from the remaining mice in aim 1 were evaluated at several time points after irradiation. Assays included quantification of major immunomodulatory cytokines produced by Th cells and apoptosis; 3) Quantify low dose proton effects on Th lymphocyte gene expression and signal transduction pathways. This portion of the study was performed as described for Specific Aim 1, except that low-dose proton radiation (entry region of Bragg curve) was delivered to C57BL/6 mice; and 4) Determine if Th cell function is significantly altered by low-dose proton radiation. Spleens from remaining mice irradiated with protons were evaluated in the same assays at the same time points as in Specific Aim 2.

Over the course of this research, tissues other than spleens were archived and with funding obtained from other sources, including the Department of Radiation Medicine at the Loma Linda University Medical Center, some additional assays were performed. Furthermore, groups of additional mice were included that were pre-exposed to low-dose photons before irradiating with acute photons, protons, and simulated solar particle event (SPE) protons. Hence, the original funding together with the additional support for our research led to generation of much valuable information that was originally not anticipated. Some of the data has already resulted in published articles, manuscripts in review, and a number of presentations at scientific conferences

and workshops. Difficulties in reliable and reproducible quantification of secreted cytokines using multi-plex technology delayed completion of this study for a period of time. However, final analyses of the remaining data are currently being performed and should result in additional publications and presentations in the near future.

In summary, this unique research addressed several critical questions: 1) What is the impact of low dose photon radiation on Th lymphocytes, the major immunoregulatory cells in the intact mammal? 2) Which genes and signal transduction pathways are affected in these cells by low dose exposure? and 3) Is the effect of low dose proton exposure different or similar from that of photons? The generated data should help provide answers that are vital to understanding the underlying mechanisms of low-dose radiation on cells that influence many body systems.

Papers Published or in Press

Gridley, D.S., Coutrakon, G.B., Rizvi, A., Bayeta, E.J.M., Luo-Owen, X., Makinde, A.Y., Baqai, F., Koss, P., Slater, J.M., and Pecaut, M.J. Low dose photons modify liver response to simulated solar particle event protons. Radiat. Res. 169:280-287, 2008.

Abstract

Health consequences due to low dose radiation combined with a solar particle event (SPE) during space travel remain unresolved. The goal of this study was to determine if protracted radiation alters gene expression and oxidative burst capacity in the liver, an organ vital in many biological processes. C57BL6 mice were whole-body irradiated with 2 Gy simulated SPE protons (sSPE) over 36 h, both with and without pre-exposure to low dose/low dose rate (LDR) photons (^{57}Co, 0.049 Gy total at 0.024 cGy/h). The liver was excised from subsets/group immediately after irradiation (day 0) and on day 21 thereafter for analysis of 84 oxidative stress-related genes using RT-PCR; genes up-/down-regulated by >2-fold were noted. On day 0, genes with increased expression were: LDR – none; sSPE - *Id1*; LDR+sSPE – *Bax*, *Id1*, *Snrp70*. Down-regulated genes at this same time were: LDR - *Igfbp1*; sSPE - *Arnt2*, *Igfbp1*, *Il6*, *Lct*, *Mybl2*, *Ptx3*. By day 21, a much greater effect was noted than on day 0. Exposure to LDR+sSPE up-regulated genes completely different than those after either LDR or sSPE alone (LDR - *Cstb*; sSPE – *Dctn2*, *Khsrp*, *Man2b1*, *Snrp70*; LDR+sSPE - *Casp1*, *Col1a1*, *Hspcb*, *Il6st*, *Rpl28*, *Spnb2*). There were many down-regulated genes in all irradiated groups on day 21 (LDR – 13, sSPE – 16, LDR+sSPE – 16 genes), with very little overlap among groups. Oxygen radical production by liver phagocytes was significantly enhanced by LDR on day 21. The results demonstrate that whole-body irradiation with LDR photons, as well as time after exposure, had a great impact on liver response to a simulated SPE.

Gridley, D.S., Rizvi, A., Luo-Owen, X., Makinde, A.Y., Coutrakon, G.B., Koss, P., Slater, J.M., and Pecaut, M.J. Variable hematopoetic responses to acute photons, protons and simulated solar particle event protons. In Vivo. 22:159-170, 2008.

Abstract

Background: The goal of this study was to evaluate, for the first time, the response of bone marrow-derived cell populations to protons mimicking a space radiation environment. Materials and Methods: C57BL/6 mice were exposed to 2 Gray (Gy) simulated solar particle event protons (sSPE) over 36 h; energies ranged from 15 to 215 MeV/n and were administered in 10 MeV increments. Acute 2 Gy irradiation with photons (gamma-rays) and protons were administered to

different groups at 0.7 Gy/min and 0.9 Gy/min, respectively, for comparison with sSPE. The animals were euthanized on days 4 and 21 post-exposure for analyses. Results: Exposure to radiation, regardless of regimen, resulted in immune depression and other abnormalities in cell populations residing in the blood and spleen; the extent of the radiation damage was somewhat dependent upon body compartment and time post-exposure. However, variations were also noted among the three radiation regimens in a number of measurements: relative spleen mass, basal DNA synthesis by leukocytes, white blood cell counts and three-part differentials (lymphocytes, granulocytes, monocytes-macrophages), lymphocyte subpopulations ($CD4^+$ T, $CD8^+$T, B and NK cells) and erythrocyte and thrombocyte characteristics. Conclusion: The data demonstrate that exposure to proton radiation mimicking a solar explosion induces abnormalities in leukocytes, erythrocytes and platelets that may have adverse health consequences. However, the damaging effects of sSPE on leukocytes and platelets were generally less pronounced compared to the other radiation regimens. Results obtained with photons (gamma-rays, X-rays) and monoenergetic protons at space-relevant total doses may not necessarily predict biological responses after exposure to a solar particle event.

Coutrakon, G.B., Benton, E.R., Gridley D.S., Hickey, T., Hubbard, J., Koss, P., Moyers, M.F., Nelson, G.A., Pecaut, M.J., Sanders, E., and Shahnazi, K. Simulation of a 36 hour solar particle event at LLUMC using a proton beam scanning system. Nucl Instrum Methods Phys Res B: Beam Interactions with Materials and Atoms 261(1-2): 791-794, 2007. Available online at http://dx.doi.org/10.1016/j.nimb.2007.03.103

Abstract

A radiation biology experiment was performed in the research room of the proton therapy facility at Loma Linda University Medical Center to simulate the proton exposure produced by a solar particle event. The experiment used two scanning magnets for X and Y deflection of the proton beam and covered a usable target area of nearly 1 m2. The magnet scanning control system consisted of Lab View 6.0 software running on a PC. The goal of this experiment was to study the immune system response of 48 mice simultaneously exposed to 2 Gy of protons that simulated the dose rate and energy spectrum of the September 1989 solar particle event. The 2 Gy dose was delivered to the entrance of the mice cages over 36 h. Both ion chamber and TLD measurements indicated that the dose delivered was within 9% of the intended value. A spot scanning technique using one spot per accelerator cycle (2.2 s) was used to deliver doses as low as 1 Gy per beam spot. Rapid beam termination (less than 5 ms) on each spot was obtained by energizing a quadrupole in the proton synchrotron once the dose limit was reached for each spot. A parallel plate ion chamber placed adjacent to the mice cages provided fluence (or dose) measurements for each beam energy during each hour of the experiment. An intensity modulated spot scanning technique can be used in a variety of ways for radiation biology and a second experiment is being designed with this proton beam scanning system to simultaneously irradiate four groups of mice with different dose rates within the 1 m2 area. Also, large electronic devices being tested for radiation damage have been exposed in this beam without the use of patch fields. The same scanning system has potential application for intensity modulated proton therapy (IMPT) as well. This paper discusses the beam delivery system and dosimetry of the irradiation.

Manuscripts in Review

Gridley, D.S., Pecaut, M.J., Rizvi, A., Coutrakon, G.B., Luo-Owen, X., Makinde, A.Y., and Slater, J.M. Low-dose, low-dose-rate proton radiation modulates $CD4^+$ T cell gene expression. Int. J. Radiat. Biol. In review, 2008.

Abstract

Purpose: To evaluate CD4+ T cell gene expression and related parameters after whole-body exposure to proton radiation as it occurs in the spaceflight environment. Materials and methods: C57BL/6 mice were irradiated to total doses of 0, 0.01, 0.05, and 0.1 Gy at 0.1 cGy/h. On day 0 spleens were harvested from a subset in the 0, 0.01 and 0.1 Gy groups; cluster of differentiation 4 (CD4+) T cells were isolated; and expression of 84 genes relevant to T helper (Th) cell function was determined using reverse transcriptase-polymerase chain reaction (RT-PCR). Remaining mice were euthanized on days 0, 4, and 21 for additional analyses. Results: Genes with >2-fold difference and $p < 0.05$ compared to 0 Gy were noted. After 0.01 Gy, 5 genes were up-regulated (*Ccr5*, *Cd40*, *Cebpb*, *Igsf6*, *Tnfsf4*) and 3 were down-regulated (*Il4ra*, *Mapk8*, *Nfkb1*). After 0.1 Gy there were 9 up-regulated genes (*Ccr4*, *Cd40*, *Cebpb*, *Cxcr3*, *Socs5*, *Stat4*, *Tbx21*, *Tnfrsf4*, *Tnfsf4*); none were down-regulated. On day 0 after 0.01 Gy, $CD4^+$ T cell counts and CD4:CD8 ratio were low in the spleen ($p < 0.05$). Spontaneous DNA synthesis in both spleen and blood was lowest in the 0.01 Gy group on day 0; on days 4 and 21 all p values were >0.1. Conclusion: The data show that the pattern of gene expression in $CD4^+$ T cells after protracted low-dose proton irradiation was significantly modified and highly dependent upon total dose. The findings also suggest that low-dose radiation, especially 0.01 Gy, may enhance $CD4^+$ T cell responsiveness.

Gridley, D.S., Rizvi, A., Luo-Owen, X., Makinde, A.Y., and Pecaut, M.J. Low dose, low dose rate photon radiation modifies leukocyte distribution and gene expression in $CD4^+$ T cells. J. Radiat. Res. In review, 2008.

Abstract

A better understanding of low dose radiation effects is needed to accurately estimate health risks. In this study, C57BL/6 mice were γ-irradiated to total doses of 0, 0.01, 0.05, and 0.1 Gy (^{57}Co; ~0.02 cGy/h). Subsets per group were euthanized at the end of irradiation (day 0) and on days 4 and 21 thereafter. Relative spleen mass and splenic white blood cell (WBC) counts, major leukocyte populations, and spontaneous DNA synthesis were consistently higher in the irradiated groups on day 0 compared to 0 Gy controls, although significance was not always obtained. In the spleen, all three major leukocyte types were significantly elevated on day 0 ($P < 0.05$). By day 21 post-irradiation the T, B, and natural killer (NK) cell counts, as well as $CD4^+$ T cells and CD4:CD8 T cell ratio, were low especially in the 0.01 Gy group. Although blood analyses showed no significant differences in leukocyte counts or red blood cell and platelet characteristics, the total T cells, $CD4^+$ T cells, and NK cells were increased by day 21 after 0.01 Gy ($P < 0.05$). Gene analysis of $CD4^+$ T cells negatively isolated from spleens on day 0 after 0.1 Gy showed significantly enhanced expression of *Il27* and *Tcfcp2*, whereas *Inha* and *Socs5* were down-regulated by 0.01 Gy and 0.1 Gy, respectively ($P < 0.05$). A trend for enhancement was noted in two additional genes (*Il1r1* and *Tbx21*) in the 0.1 Gy group ($P < 0.1$). The data show that protracted low dose photons had dose- and time-dependent effects on $CD4^+$ T cells after whole-body exposure.

Publications for Lay Persons

Research highlighted in: LLU investigators report study of possible effect of low-dose photon irradiation against large explosions of radiation from the sun. William Preston, Ed.D., Editor. James M. Slater, M.D. Proton Treatment and Research Center Newsletter. 1(2):10-11, Summer 2008.

Presentations at Scientific Conferences/Workshops

Gridley D.S. Oral presentation during Radio-oxidative Stress and Inflammation Break-out Session. Topic: Low dose proton radiation-induced gene expression patterns in CD4+ T helper cells. Session Coordinator: Douglas Spitz, PhD. DOE Low Dose Radiation Research Investigators' Workshop VII. Washington, DC, January 21-23, 2008.

Gridley, D.S., Rizvi, A., Luo-Owen, X., Makinde, A.Y., Andres, M.L., Coutrakon, G.B., Koss, P., Slater, J.M., and Pecaut, M.J. Low dose proton radiation-induced gene expression patterns in CD4+ T helper cells. DOE Low Dose Radiation Research Investigators' Workshop VII. Washington, DC. Abstract Book page 83. January 21-23, 2008.

Luo-Owen, X., Rizvi, A., Makinde, A.Y., Coutrakon, G.B., Koss, P., Pecaut, M.J., James M. Slater, J.M., and Gridley, D.S. Effects of simulated solar particle event protons and low dose photons on Foxp3+T regulatory cells. 99th Ann. Meeting of American Association for Cancer Research (AACR), San Diego, CA. Program Book p. 391. April 12-16, 2008.

Rizvi, A., Pecaut, M.J., Andres, M.J., Coutrakon, G.B., Luo-Owen, X., Slater, J.M., and Gridley, D.S.. Low dose/low dose radiation normalizes pro-survival proteins in CD4+ T cells from mice exposed to solar particle event. 54th Ann. Meeting of Radiation Research Society (RRS) held in conjunction with meeting of American Society for Therapeutic Radiology and Oncology (ASTRO). Boston, MA. Abstract Book p. 115 (poster PS3636). Sept. 21-24, 2008.

Gridley, D.S., Rizvi, A., Makinde, A.Y., Luo, X., Tian, J., Andres, M., Coutrakon, G.B., and Pecaut, M.J. Low-dose rate photons and simulated solar particle event protons: gene expression in liver. 13th Intl. Congress of Radiation Research (ICRR) hosted by the Radiation Research Society (RRS). San Francisco, CA. Abstract Book p. 244, July 7-12, 2007.

Tian, J. and Gridley, D.S. Expression of extracellular matrix regulators in lung tissue of mice exposed to different types of radiations. 98th Annual meeting of the American Association for Cancer Research (AACR). Los Angeles, CA. April 14-18, 2007. Poster #5063. Available online at http://www.aacr.org

Gridley, D.S., Pecaut, M.J., Coutrakon, G., Rizvi, A., Luo, X., Koss, P., Benton, E., and Slater, J.M. Immune modification after a simulated solar particle event with and without low dose photon exposure. Conference of the Particle Therapy Co-Operative Group (PTCOG) 45-2006. Poster P9. Houston, TX. Oct. 7-11, 2006.

Tian, J., Andres, M.L., and Gridley, D.S. Expression of transforming growth factor-beta1 in lung tissue of mice exposed to different types of radiations. 53rd Ann. Meeting of Radiation Research Society (RRS). Abstract Book p. 111 (poster PS225). Philadelphia, PA, Nov. 6-9, 2006.

Luo, X., Rizvi, A., Coutrakon, G., Makinde, A.Y., Perez, C., Andres, M.L., Rightnar, S., Slater, J.M., Pecaut., M.J., and Gridley, D.S. Blood and spleen analyses after whole-body low dose photon and solar flare proton irradiation. 53rd Ann. Meeting of Radiation Research Society (RRS). Abstract Book p. 109-110 (poster PS218). Philadelphia, PA, Nov. 6-9, 2006.

Rizvi, A., Coutrakon, G., Luo, X, Makinde, A.Y., Jian, T, Slater, J.M., Pecaut, M.J., and Gridley, D.S. Protracted low dose photon and simulated solar flare proton effects on gene expression after whole-body irradiation. 53rd Ann. Meeting of Radiation Research Society (RRS). Abstract Book p. 109 (poster PS217). Philadelphia, PA, Nov. 6-9, 2006.

Rizvi, A., Coutrakon, G., Slater, J.M., Pecaut, M.J., and Gridley, D.S. Protracted low dose photon and simulated solar flare proton effects on cytokine/chemokine expression after whole-body irradiation. DOE Low Dose Radiation Research Program Workshop VI, Washington, D.C. Sponsored by U.S. Dept. of Energy Office of Biological and Environmental Research. Abstract Book, p. 67-68. July 31-Aug.2, 2006.

Internal Presentations at LLU/LLUMC

Eleventh Annual Research Symposium, Basic Science Departments, Loma Linda University, Loma Linda, CA. September 10, 2008.

Annual Postgraduate Conference (APC), Loma Linda University School of Medicine, Loma Linda, CA. Feb. 29 - March 4, 2008.

Tenth Annual Research Symposium, Basic Science Departments, Loma Linda University, Loma Linda, CA. September 10, 2007.

Annual Postgraduate Conference (APC), Loma Linda University School of Medicine, Loma Linda, CA. March 2-5, 2007.

Ninth Annual Research Symposium, Basic science Departments, Loma Linda University, Loma Linda, CA. Sept. 12, 2006.

Annual Postgraduate Conference (APC), Loma Linda University School of Medicine, Loma Linda, CA. March 1-6, 2006.