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Human Research Program Elements (I) SRC Space Radiation Human Research Program Risks: None Space Biology Cross-Element None Space Biology Cross-Element None Space Biology Cross-Element None PI Email: ubderer/duci cdu Fax: FY PI Organization Type: UNIVERSITY Phone: 949-824-8081 Organization Type: UNIVERSITY Phone: 949-824-8081 Organization Name: University of California - Irvine PI Address 1: Center for Occupational and Environmental Health PI Address 2: 100 Theory, Suite 100 PI Address 2: 100 Theory, Suite 100 PI Veb Page: City: Evine Center for Occupational and Environmental Health PI Address 2: 02617-3055 Congressional District: 45 Comments: Project Type: GROUND Solicitation / Funding Source: 2013 Space Radiobiology NNI13ZSA001N Sur Date: 0301/2014 End Date: 08/31/2016 No. of Phot Despress: 1 No. of Radeleor's Degress: 1 No. of Master's Candidates: No. of Master' Degress: 1 No. of Master's Candidates: No. of Master' Degress: 1 No. of Master's Candidates: No. of Master' Degress: 1 No. of Radeleor's Degress: 1 No. of Radeleor's Degress: 1 No. of Radeleor's Degress: 1 No. of Bachelor's Degress: 1 No. of Master's Candidates: No. of Master' Degress: 1 No. of Master's Candidates: No. of Master's Degress: 1 No. of Master's Candidates: No. of Theore None: NASA JSC Contract Monitor: Sinonsen, Lisa Context Hone: NASA JSC Contract Monitor: Norfie: End date is now 2/38/2016 per NSSC information and PI (Ed. 12/1/15) Suffie End date is now 2/38/2016 per NSSC information and PI (Ed. 12/1/15) Suffie End date is now 2/38/2016 per NSSC information and PI (Ed. 12/1/15) Suffie End date is now 2/38/2016 per NSSC information and PI (Ed. 12/1/15) Suffie End date is now 2/38/2016 per NSSC information and PI (Ed. 12/1/15) Suffie End date is now 2/38/2016 per NSSC information and PI (Ed. 12/1/15) Suffie End date is now 2/38/2016 per NSSC information and PI (Ed. 12/1/15) Suffie End date is now 2/38/2016 per NSSC information and PI (Ed. 12/1/15) Suffie End date is now 2/38/2016 per NSSC information	Program/Discipline Element/Subdiscipline:			
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Task Description:	Fifteen percent of astronauts are women, but the risks of space radiation to women's reproductive health and risks of gynecological cancers remain poorly understood. Radiation treatment for cancer is known to cause temporary infertility and premature menopause. Premature menopause increases women's risks for cardiovascular disease, osteoporosis, and Alzheimer's disease. In addition, animal studies and studies of atomic bomb survivors have shown that radiation exposure increases the risk for ovarian cancer. Ovarian cancer has a high mortality rate and is the leading cause of gynecological cancer deaths in women. To best protect the health of women astronauts, it is important to understand whether space radiation has similar effects on the ovary as the types of radiation exposure that are common on Earth. We tested the effects of low dose charged particle radiation (low LET oxygen and high LET iron ions), typical of exposures in space, on ovarian follicles (the functional unit of the ovary) and on ovarian cancingenesis in adult female mice. We used histomorphometric methods to quantify the effects of charged particles on numbers of ovarian follicles and ovarian tumor multiplicity and size. We used in situ methods to assess oxidative damage, DNA damage, apoptosis, and proliferation. Our previous studies have shown that oxidative stress plays a role in and the antioxidant glutathione is protective against radiation- and chemical-induced damage to ovarian calls and ovarian effects of charged particle radiation. Our analyses provide critical insights showing that ovarian damage and tumors caused by exposure to charged particles are biologically similar to those in other mouse models of ovarian cancers and identify potential targets for preventive or therapeutic intervention. These studies help to fill important gaps in our understanding of the effects of space radiation on ovarian function and ovarian cancer and will lead to better ways to prevent ovarian cancer and protect reproductive health in women astronauts.
Rationale for HRP Directed Research	h:
Research Impact/Earth Benefits:	Charged particles are currently being used in cancer therapy. The findings of our research on the ovarian effects of charged particles have increased our understanding of the possible adverse ovarian effects of charged particle radiation used for the treatment of cancer in women. Comparisons of our data on the ovarian effects of charged particles with prior data on ovarian effects of x-rays and gamma radiation suggest that the ovary is more sensitive to charged particle radiation that to x- and gamma radiation.
	JANUARY 2017SUPPLEMENTAL REPORT FOR FINAL REPORT "CHARGED PARTICLE EFFECTS ON THE OVARY" Introduction
	Currently about 15% percent of astronauts are women, but the risks to the ovary of exposure to galactic cosmic rays and solar particle events during space missions remain largely unknown. The ovary is highly sensitive to gamma radiation. Gamma irradiation for cancer treatment causes premature ovarian failure. Premature ovarian failure, also called premature menopause, has many adverse consequences, including early loss of fertility and increased risk of osteoporosis, cardiovascular disease, and Alzheimer's disease. Gamma irradiation has long been known to cause ovarian cancer. Sixty percent of women diagnosed with ovarian cancer will die of the disease; it is the leading cause of death from gynecological cancers. Because ovarian cancer tends to be asymptomatic until it has reached an advanced stage, treatment is often ineffective. For all of these reasons, it is important to understand the risks to the ovary of space radiation. Our own and others' work showed that gamma irradiation chronically elevates cellular reactive oxygen species (ROS) production and oxidative stress and that ROS initiate apoptotic death of ovarian follicles. We therefore hypothesize that high charge and energy (HZE) particles typical of space radiation cause ovarian oxidative stress, resulting in premature ovarian failure and that this contributes to the pathogenesis of ovarian cancer. We are testing this overarching hypothesis in two specific aims.
	Aim 1: Exposure to low dose HZE particles induces ovarian oxidative stress, which initiates apoptotic destruction of ovarian follicles, causing premature ovarian failure. Adult female C57BL/6J mice were exposed to low dose (0, 5, 30, and 50 cGy) oxygen (LET = 16.5 keV/ μ m) or iron (LET = 179 keV/ μ m) at energy of 600 MeV/u and analyzed 6 hours, 1 week, and 8 weeks after exposure. Two groups were irradiated at the highest dose for each of the two charged particles, one fed normal control rodent chow and the other fed the same chow supplemented with the antioxidant alpha lipoic acid for the duration of the experiment.
	Aim 2: Exposure to low dose HZE particles causes epithelial ovarian tumors. Adult female mice of two strains, one sensitive to radiation-induced tumors (B6C3F1) and one thought to be less sensitive to radiation-induced tumors (C57BL/6J), were irradiated with charged oxygen or iron particles at 50 cGy, 600MeV/u energy or sham-irradiated and aged to 18 months for evaluation of ovaries for tumors.
	Materials and Methods
	Three month old female mice (C57BL/6J) were divided into five groups: 1) sham-irradiated and fed with AIN-93M rodent chow (0 cGy group); 2) irradiated with 5 cGy charged iron particles at an energy of 600 MeV and fed AIN-93M rodent chow; 3) 30 cGy charged iron particles and fed AIN-93M rodent chow; 3) 30 cGy charged iron particles and fed AIN-93M rodent chow; 5) irradiated with 50 cGy charged iron particles and fed AIN-93M rodent chow; 5) irradiated with 50 cGy charged iron particles and fed AIN-93M rodent chow; 5) irradiated with 50 cGy charged iron particles and fed AIN-93M rodent chow; 5) irradiated with 50 cGy charged iron particles and fed AIN-93M rodent chow supplemented with 150 mg/kg diet alpha lipoic acid. Additional groups of mice were irradiated with charged oxygen particles at the same doses with or without dietary lipoic acid supplementation. Feeding with supplemented chow began one week before irradiation and continued until sacrifice. Ovaries were collected 6 h, 1 wk, and 8 wks after irradiation. Estrous cycling was monitored by vaginal cytology for two weeks prior to euthanasia at the 8 wk time point. Ovarian follicles were counted in serial ovarian sections stained with hematoxylin and eosin. DNA double strand breaks, oxidative lipid and protein damage, and apoptosis were assessed by immunostaining.
	To test whether charged iron particle or charged oxygen particle irradiation causes ovarian tumors, 3 month old female mice of two strains, one sensitive to radiation induced tumors (B6C3F1) and one thought to be less sensitive to radiation-induced tumors (C57BL/6J) were irradiated with 50 cGy charged iron particles at energy of 600 MeV (LET = 179 keV/ μ m) or sham-irradiated. These mice were aged to 18 months and were evaluated for gross evidence of ovarian and other tumors at necropsy, and ovaries of charged iron particle irradiated mice underwent detailed histopathological examination for the presence and types of ovarian tumors.
	Results Aim 1: Exposure to low dose HZE particles induces ovarian oxidative stress, which initiates apoptotic destruction of

ovarian follicles, causing premature ovarian failure. Charged iron and oxygen particles increase ovarian DNA double strand breaks, oxidative damage, and apoptosis

To define the mechanism of ovarian damage by charged iron or oxygen particles, we analyzed the localization of DNA double strand breaks using phosphorylated H2AX (gamma H2AX) immunostaining in ovarian sections. Percentages of ovarian follicles at all stages of development with gamma H2AX immunostaining were significantly higher in 50 cGy iron or oxygen-irradiated mice compared to control 0 cGy mice at 6 h after irradiation. Mice that received the lipoic acid supplemented diet along with 50 cGy charged iron particles had significantly decreased percentages of gamma H2AX immunostained follicles compared to 50 cGy charged iron particles-irradiated mice fed the normal diet; however, no such protective effect of lipoic acid was observed in oxygen irradiated mice.

Ovaries of mice irradiated with charged iron or oxygen particles had increased oxidative lipid and/or oxidative protein damage in ovarian follicles at 6 h and 1 wk after irradiation compared to control mice. Lipoic acid supplementation partially protected against these effects of iron, but not oxygen, charged particles.

We assessed the percentage of follicles undergoing death by apoptosis after irradiation using activated caspase-3 and PUMA immunostaining. Both PUMA and activated caspase 3 were significantly increased after 50 cGy charged iron particles and were partially protected by lipoic acid supplementation. PUMA was dose-dependently increased in ovarian follicles 6h after oxygen irradiation, and lipoic acid was partially protective.

Effects of charged iron and oxygen particles on ovarian follicle numbers. At 1 week after irradiation, the numbers of healthy small (primordial and primary) and growing (secondary and antral) follicles per ovary were dose-dependently reduced in the ovaries of mice treated with 50 cGy charged iron or oxygen particles compared to 0 cGy controls. At each dose, the effects of oxygen particles were more severe than those of iron particles. The lipoic acid supplemented diet partially prevented the decline in small ovarian follicle numbers at 1 week after iron particle treatment, but was not protective in oxygen particle irradiated mice.

Effects of charged iron and oxygen particles on estrous cycling. At the 8 wk time point, estrous cycling was monitored by vaginal cytology. Estrous cycling in rodents is analogous to menstrual cycling in women. 12.5% and 25%, respectively, of mice treated with 50 cGy charged iron particles or 50 cGy charged iron particles plus lipoic acid had irregular estrous cycles. 50% of the 50 cGy oxygen-irradiated mice had regular estrous cycles at 8 wk compared to 100% of the concurrent control mice.

Effects of charged iron and oxygen particles on reproductive hormone concentrations. If the hypothalamic-pituitary-ovarian axis were functioning normally, one would expect the depletion of ovarian follicles to result in decreased negative feedback to the hypothalamus and pituitary, decreasing the circulating levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) secreted from the anterior pituitary gland at 8 weeks post-irradiation. Serum FSH and LH concentrations were significantly and dose-dependently increased in the mice irradiated with charged iron or charged oxygen particles compared to 0 cGy control mice. Lipoic acid supplementation did not prevent these increases in FSH and LH.

Aim 2: Exposure to low dose HZE particles causes epithelial ovarian tumors. Charged iron and oxygen particles cause premature cessation of estrous cycling and increased weight gain.

C57BL/6J and B6C3F1 mice irradiated with 50 cGy charged iron or oxygen particles and respective controls were followed for 15 months after irradiation, and mice in all irradiated groups gained more weight than their concurrent controls. This may be related to early cessation of ovarian function, as none of the mice in any of the irradiated groups still had regular estrous cycles at 14-15 months of age, while most of the concurrent 0 cGy controls did.

Charged iron irradiation causes epithelial ovarian tumors in C57BL/6J mice and gross ovarian tumors in B6C3F1 mice and oxygen causes gross ovarian tumors in both strains. At necropsy, 70% of the iron irradiated B6C3F1 and 60% of the iron irradiated C57BL/6J mice had probable ovarian tumors; 0% of the concurrent control B6C3F1 mice and 13% of the control C57BL/6J controls had gross ovarian abnormalities. Histological examination of both ovaries of the C57BL/6J mice irradiated with iron showed a statistically significantly increased ovarian tumor prevalence in the irradiated mice of 60% compared to 14% in the control mice. Based on tumor histology and positive immunostaining for cytokeratin, an epithelial tumor marker, the tumors were mostly tubular adenomas. At necropsy, 43% of the B6C3F1 oxygen irradiated mice and 60% of the C57BL/6J mice and 7% of the control C57BL/6J mice.

Conclusion

Our results reveal that exposure to iron or oxygen charged particles induces ovarian follicular DNA double strand breaks, oxidative damage, and apoptosis, resulting in the destruction of ovarian follicles. With depletion of the ovarian follicles, ovarian hormone production is diminished, resulting in increased serum concentrations of FSH and LH. The results further suggest that dietary supplementation with lipoic acid is partially protective against these effects of iron exposure, but less so for oxygen exposure. Therefore, use of lipoic acid as a single agent countermeasure against the ovarian effects of space radiation is unlikely to offer sufficient protection to female astronauts. Our findings further show a very high prevalence of ovarian tumors in 18 month old mice exposed to 50 cGy iron or oxygen charged particles.

APRIL 2016--ANNUAL PROGRESS REPORT-- "CHARGED PARTICLE EFFECTS ON THE OVARY"

Introduction

Currently about 15% percent of astronauts are women, but the risks to the ovary of exposure to galactic cosmic rays and solar particle events during space missions remain largely unknown. The ovary is highly sensitive to gamma radiation. Gamma irradiation for cancer treatment causes premature ovarian failure. Premature ovarian failure, also called premature menopause, has many adverse consequences, including early loss of fertility and increased risk of osteoporosis, cardiovascular disease, and Alzheimer's disease. Gamma irradiation has long been known to cause ovarian cancer. Sixty percent of women diagnosed with ovarian cancer will die of the disease; it is the leading cause of death from gynecological cancers. Because ovarian cancer tends to be asymptomatic until it has reached an advanced stage, treatment is often ineffective. For all of these reasons, it is important to understand the risks to the ovary of space radiation. Our own and others' work showed that gamma irradiation chronically elevates cellular reactive oxygen species (ROS) production and oxidative stress and that ROS initiate apoptotic death of ovarian follicles.

We hypothesize that high charge and energy (HZE) particles typical of space radiation cause ovarian oxidative stress,

resulting in premature ovarian failure and that this contributes to the pathogenesis of ovarian cancer. We are testing this overarching hypothesis in two specific aims.

Aim 1: Exposure to low dose HZE particles induces ovarian oxidative stress, which initiates apoptotic destruction of ovarian follicles, causing premature ovarian failure. Adult female C57BL/6J mice will be exposed to low dose (0, 5, 30, and 50 cGy) oxygen (LET = $16.5 \text{ keV}/\mu\text{m}$) or iron (LET = $179 \text{ keV}/\mu\text{m}$) at energy of 600 MeV/u and analyzed 6 hours, 1 week, and 8 weeks after exposure. Two groups will be irradiated at the highest dose for each of the two charged particles, one fed normal control rodent chow and the other fed the same chow supplemented with the antioxidant alpha lipoic acid for the duration of the experiment.

Aim 2: Exposure to low dose HZE particles causes epithelial ovarian tumors. Adult female mice of two strains, one sensitive to radiation-induced tumors (B6C3F1) and one thought to be less sensitive to radiation-induced tumors (C57BL/6J) will be irradiated with charged oxygen or iron particles at 50 cGy, 600MeV/u energy or sham-irradiated and aged to 18 months for evaluation of ovaries for tumors.

Materials and Methods

Three month old female mice (C57BL/6J) were divided into five groups: 1) sham-irradiated and fed with AIN-93M rodent chow (0 cGy group); 2) irradiated with 5 cGy charged iron particles at an energy of 600 MeV and fed AIN-93M rodent chow; 3) 30 cGy charged iron particles and fed AIN-93M rodent chow; 3) 30 cGy charged iron particles and fed AIN-93M rodent chow; 5) irradiated with 50 cGy charged iron particles and fed AIN-93M rodent chow; 5) irradiated with 50 cGy charged iron particles and fed AIN-93M rodent chow; 5) irradiated with 50 cGy charged iron particles and fed AIN-93M rodent chow; 5) irradiated with 50 cGy charged iron particles and fed AIN-93M rodent chow supplemented with 150 mg/kg diet alpha lipoic acid. Additional groups of mice were irradiated with charged oxygen particles at the same doses with or without dietary lipoic acid supplementation. Feeding with supplemented chow began one week before irradiation and continued until euthanasia. Ovaries were collected 6 h, 1 wk, and 8 wks after irradiation. Estrous cycling was monitored by vaginal cytology for two weeks prior to euthanasia at the 8 wk time point. Ovarian follicles were counted in serial ovarian sections stained with hematoxylin and eosin. DNA double strand breaks, oxidative lipid and protein damage, and apoptosis were assessed by immunostaining. Analyses are complete for iron particles and nearly complete for oxygen.

To test whether charged iron particle or charged oxygen particle irradiation causes ovarian tumors, 3 month old female mice of two strains, one sensitive to radiation induced tumors (B6C3F1) and one thought to be less sensitive to radiation-induced tumors (C57BL/6J) were irradiated with 50 cGy charged iron particles at energy of 600 MeV (LET = $179 \text{ keV}/\mu\text{m}$) or sham-irradiated. These mice were aged to 18 months and were evaluated for gross evidence of ovarian and other tumors at necropsy, and ovaries of charged iron particle irradiated mice underwent detailed histopathological examination for the presence and types of ovarian tumors.

Results

Aim 1: Exposure to low dose HZE particles induces ovarian oxidative stress, which initiates apoptotic destruction of ovarian follicles, causing premature ovarian failure.

Charged iron and oxygen particles increase ovarian DNA double strand breaks, oxidative damage and apoptosis.

To define the mechanism of ovarian damage by charged iron or oxygen particles, we analyzed the localization of DNA double strand breaks using phosphorylated H2AX (gamma H2AX) immunostaining in ovarian sections. Percentages of ovarian follicles at all stages of development with gamma H2AX immunostaining were significantly higher in 50 cGy iron or oxygen-irradiated mice compared to control 0 cGy mice at 6 h after irradiation. Mice that received the lipoic acid supplemented diet along with 50 cGy charged iron particles had significantly decreased percentages of gamma H2AX immunostained follicles compared to 50 cGy charged iron particles-irradiated mice fed the normal diet; however, no such protective effect of lipoic acid was observed in oxygen irradiated mice.

Ovaries of mice irradiated with 50 cGy charged iron particles had increased oxidative lipid and oxidative protein damage in ovarian follicles at 6 h and 1 wk after irradiation compared to control mice. Lipoic acid supplementation partially protected against these effects. Analyses of these endpoints in oxygen irradiated mice are nearly complete.

We assessed the percentage of follicles undergoing death by apoptosis after irradiation using activated caspase-3 and PUMA immunostaining. Both PUMA and activated caspase 3 were significantly increased after 50 cGy charged iron particles and were partially protected by lipoic acid supplementation. Analyses of these endpoints in oxygen irradiated mice are nearly complete.

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At 1 week after irradiation, the numbers of healthy small (primordial and primary) and growing (secondary and antral) follicles per ovary were dose-dependently reduced in the ovaries of mice treated with 50 cGy charged iron or oxygen particles compared to 0 cGy controls. At each dose, the effects of oxygen particles were more severe than those of iron particles. The lipoic acid supplemented diet partially prevented the decline in small ovarian follicle numbers at 1 week after iron particle treatment, but was not protective in oxygen particle irradiated mice.

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At the 8 wk time point, estrous cycling was monitored by vaginal cytology. Estrous cycling in rodents is analogous to menstrual cycling in women. 12.5% and 25%, respectively, of mice treated with 50 cGy charged iron particles or 50 cGy charged iron particles plus lipoic acid had irregular estrous cycles. 50% of the 50 cGy oxygen-irradiated mice had regular estrous cycles at 8 wk compared to 100% of the concurrent control mice.

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If the hypothalamic-pituitary-ovarian axis were functioning normally, one would expect the depletion of ovarian follicles to result in decreased negative feedback to the hypothalamus and pituitary, decreasing the circulating levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) secreted from the anterior pituitary gland at 8 weeks post-irradiation. Serum FSH and LH concentrations were significantly and dose-dependently increased in the mice irradiate with charged iron or charged oxygen particles compared to 0 cGy control mice. Lipoic acid supplementation did not prevent these increases in FSH and LH.

Aim 2: Exposure to low dose HZE particles causes epithelial ovarian tumors.

	Charged iron and oxygen particles cause premature cessation of estrous cycling and increased weight gain
	C57BL/6J and B6C3F1 mice irradiated with 50 cGy charged iron or oxygen particles and respective controls were followed for 15 months after irradiation, and mice in all irradiated groups gained more weight than their concurrent controls. This may be related to early cessation of ovarian function, as none of the mice in any of the irradiated groups still had regular estrous cycles at 14-15 months of age, while most of the concurrent 0 cGy controls did.
	Charged iron irradiation causes epithelial ovarian tumors in C57BL/6J mice and gross ovarian tumors in B6C3F1 mice and oxygen causes gross ovarian tumors in both strains.
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	At necropsy, 43% of the B6C3F1 oxygen irradiated mice and 60% of the C57BL/6J oxygen irradiated mice had probable ovarian tumors, compared to 13% of the concurrent control B6C3F1 mice and 7% of the control C57BL/6J mice.
	Conclusion
	Our results reveal that exposure to 50 cGy iron or oxygen irradiation induces ovarian follicular DNA double strand breaks and apoptosis, resulting in the destruction of ovarian follicles. With depletion of the ovarian follicles, ovarian hormone production is diminished, resulting in increased serum concentrations of FSH and LH. The results further suggest that dietary supplementation with lipoic acid is partially protective against these effects of iron exposure, but not oxygen exposure. Therefore, use of lipoic acid as a single agent countermeasure against the ovarian effects of space radiation is unlikely to offer sufficient protection to female astronauts. Our findings further show a very high prevalence of ovarian tumors in 18 month old mice exposed to 50 cGy iron or oxygen charged particles.
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