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Dietary modulation of the effects of exposure to ^{56}Fe particles

B.M. Rabin [a,*], J.A. Joseph [b], B. Shukitt-Hale [b], A.N. Carey [b]

[a] *Department of Psychology, University of Maryland Baltimore County, Baltimore, MD 21250, USA*
[b] *Human Nutrition Research Center on Aging, USDA-ARS, Tufts University, Boston, MA 02111, USA*

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Abstract

On exploratory missions to other planets, astronauts will be exposed to galactic cosmic rays composed of protons and heavy particles, such as ^{56}Fe. Long-term exposure to these particles can cause cancer. However, there are significant uncertainties in the risk estimates for the probability of developing heavy particle-induced cancer, and in the amount of shielding needed to provide an adequate level of radiation protection. The results of this preliminary study, using a ground-based model for exposure to cosmic rays, show reduced tumorigenesis in rats maintained on diets containing blueberry or strawberry extract prior to exposure to ^{56}Fe particles. Because the study was not initially designed to evaluate tumorigenesis following exposure to ^{56}Fe particles, additional research is needed to evaluate the effectiveness of strawberry and blueberry supplementation. However, the preliminary results presented in this study suggest that diets containing antioxidant phytochemicals can provide additional radiation protection on interplanetary voyages.

Keywords: Diet; Antioxidant; Tumorigenesis; Iron-56; Survival

1. Introduction

As the manned exploration of space moves beyond the protection provided by the magnetic shield of the earth to include interplanetary travel, astronauts will encounter types of radiation that are not routinely encountered in low earth orbit (Badhwar, 1998; Letaw et al., 1989; Townsend et al., 1992). The radiation environment outside the magnetosphere is composed of galactic cosmic rays (GCR) which consist of protons and of particles of high energy and charge (HZE particles), such as ^{56}Fe. There is a general consensus that a significant risk facing astronauts on exploratory missions to other planets is the possibility of developing cancer (Ball and Evans, 2001; National Academy of Sciences, 1996; Edwards, 2001). However, the level of the risk of carcinogenesis as a result of exposure to GCR involves significant uncertainties (Cucinotta et al., 2001). One way to reduce the risk of heavy particle-induced carcinogenesis is to increase the amount of shielding. However, the degree of uncertainty in the attempt to determine the risk of developing cancer means that unnecessarily large amounts of shielding may be used, which might make a mission to Mars economically infeasible (Wilson et al., 2001). A complementary approach to radiation protection, in addition to shielding, would be to manipulate other factors that might influence tumor development.

Among other effects on the health and functioning of the organism, oxidative stress and the production of reactive oxygen species have been linked to carcinogenesis (Kovacic and Jacintho, 2001; Oberley, 2002). Exposing organisms to ionizing radiation leads to oxidative stress and the production of free radicals. It is possible that the development of cancer following exposure to heavy particles similarly results from radiation-produced oxidative stress (Riley, 1994; Choudhury et al., 1998). If this is the case, then it would be possible to reduce the risk of carcinogenesis on missions outside the magnetosphere by treatments that reduce oxidative stress.

One treatment that has been reported to be effective in reducing oxidative stress and the production of free

* Corresponding author. Tel.: +1 410 455 2430; fax: +1 410 455 1055.
E-mail address: rabin@umbc.edu (B.M. Rabin).

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radicals has been the use of antioxidant diets. Previous research (Wang et al., 1996) has shown that strawberries and blueberries are very high in antioxidant activity, as assessed by analyzing their oxygen radical absorbance capacity. In addition to reducing the physiological and cognitive effects of aging (Joseph et al., 1998, 1999; Youdim et al., 2000), diets containing blueberry or strawberry extract are effective in attenuating some of the effects of exposure to ^{56}Fe particles, including the disruption of amphetamine-induced taste aversion learning (Rabin et al., 2002) operant responding (Rabin et al., 2005a,b) and spatial learning and memory (Shukitt-Hale et al., 2006). It is possible that diets containing antioxidant phytochemicals might also be effective in preventing the development of tumors following exposure to heavy particles. As part of another study, rats that had been given either antioxidant or control diets prior to ^{56}Fe exposure were maintained in the labs at UMBC and evaluated for tumor development for up to one year after irradiation.

2. Methods

2.1. Subjects

The subjects were 60 male Sprague–Dawley rats weighing 175–200 g at the start of the experiment. They were housed in AAALAC-accredited animal facilities at Brookhaven National Laboratory (BNL) and maintained on a 12:12 light:dark cycle with food and water continuously available. Two months prior to irradiation, rats were placed on diets containing either 2% blueberry or strawberry extract or a control diet. Twenty rats were fed each of the three diets. Half the rats in each diet condition were irradiated while the remaining rats served as non-irradiated controls.

2.2. Diets

Details of the diet have been previously published (Youdim et al., 2000). Briefly, blueberries or strawberries were homogenized in water (1:1 or 2:1 w/v, respectively) for 3 min. The recovered homogenate was centrifuged at 13,000*g* for 15 min at 4 °C and the supernatant collected and lyophilized. Freeze dried extracts were shipped to Harlan Teklad (Madison, WI), where they were combined with the control diet (2 g extract/100 g diet). The control diet was a modification of the NIH-31 diet. The amount of corn in the control diet was adjusted to compensate for the addition of strawberry or blueberry extract.

2.3. Radiation

Rats were irradiated with ^{56}Fe particles using the Alternating Gradient Synchrotron at BNL. For irradiation, rats were placed in a well-ventilated plastic restraining tube which was placed perpendicular to the beam. An X-ray film indicated that the head of the rat was located near the center of the 7 cm. diameter beam such that the area that was irradiated included the neck and shoulders. The rats were exposed to 1.5 Gy of 1 GeV/n ^{56}Fe particles at a nominal dose rate of 1.0–1.5 Gy/min. Control rats were not exposed to the beam. The details of the beam and dosimetry have been provided by Zeitlin et al. (1998).

2.4. Procedure

Following irradiation the rats were shipped to UMBC for testing. Except for the conditioning periods during which the animals were food deprived to 85–90% of their base weight (1 week for acquisition of a food-rewarded operant response and two 2-week periods in which the rats were tested on an ascending fixed-ratio schedule), food and water were continuously available. While at UMBC the rats were fed a standard laboratory rat chow (Purina 5001). Rats were examined weekly for the development of a tumor. If a tumor or other health-related problem was noted, the rat was sacrificed with an overdose of sodium pentobarbital (50 mg, to effect). All remaining rats were euthanized 54–56 weeks following irradiation. Some rats, selected randomly, were sent to the Veterinary Pathology Department of the University of Maryland at Baltimore for analysis of the tumor.

3. Results

The effects of exposure to 1.5 Gy of ^{56}Fe particles and diet on tumorigenesis and survival are summarized in Fig. 1 and Table 1. Fig. 1 presents the number of days between irradiation and time of sacrifice as a function of diet. Rats were sacrificed when a tumor of 3–5 cm developed or when there was some obvious health problem (*e.g.*, the animal appeared to be apathetic and was losing hair). Analysis of the survival data was performed using analysis of variance, which showed that the main effect for the comparison between radiated and non-irradiated animals was significant ($F[1,54] = 5.85$, $p < 0.02$), with the radiated rats surviving for a significantly shorter time than the controls. The comparison between the diets did not achieve significance ($F[2,54] = 2.59$, $p < 0.10$). However, a significant interaction ($F[2,54] = 4.39$, $p < 0.02$) indicated that the survival time of rats on the three diets varied as a function of radiation condition. *Post-hoc* comparisons using the Neuman–Keuls test showed that the survival time of the radiated rats fed the control diet prior to irradiation was significantly less than that of the non-irradiated rats fed the control diet ($t = 13.65$, $p < 0.01$). The differences in survival time between the radiated and non-irradiated rats fed either the blueberry or strawberry diets were not significant. The survival times of the radiated rats maintained on either the blueberry ($t = 7.81$, $p < 0.01$) or strawberry ($t = 9.03$, $p < 0.05$) were significantly greater than those of the radiated rats maintained on the control diet.

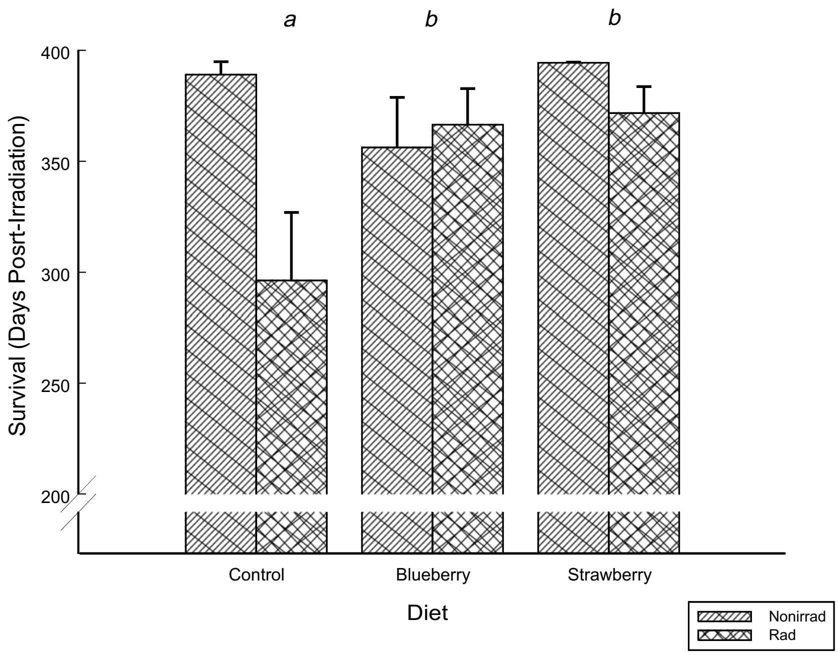

Fig. 1. Mean number of days between exposure to 1.5 Gy of 1 GeV/n ^{56}Fe particles and sacrifice. Rats were euthanized when they showed the development of a tumor or other health problem. The maximum possible survival time was 395 days following irradiation. *a*: survival of radiated rats significantly shorter ($p < 0.05$) than that of the non-irradiated animals; *b*: no differences ($p > 0.05$) in survival between the radiation and non-irradiated rats.

Table 1
Numbers of animals alive in each treatment condition at specific intervals following exposure to ^{56}Fe particles (1.5 Gy, 1 GeV/n)

Time after irradiation	Treatment condition					
	Con/Non	Con/Rad	Blue/Non.	Blue/Rad	Straw/Non.	Straw/Rad
16 Week	10	10	10	10	10	10
26 Week	10	7 (3)	10	10	10	10
51 Week	9 (0)	3 (4)	7 (1)	7 (1)	10	7 (1)
54–56 Week	9	2 (0)	7	5 (2)	8 (2)	7
Total tumors	0	7	1	3	2	1

The numbers in parenthesis give the numbers of animals that developed tumors. The bottom line of the table gives the total number of animals in the treatment group that developed tumors across the span of the experiment.

Specific data on tumorigenesis is shown in Table 1 which presents the data at four discreet time intervals (when the animals were tested at 16, 26 and 51 weeks and when they were euthanized at 54–56 weeks after exposure) and the total number animals developing tumors over the span of the post-irradiation survival time. Differences in the frequency of tumor development between the radiated animals maintained on the control diet and the rats maintained on the antioxidant diets appeared by 26 weeks after exposure to 1.5 Gy of ^{56}Fe particles. Statistical analysis of the frequency of tumors at the specific time intervals using the nonparametric Wilcoxon Signed Rank Test indicated that the differences at the 26-week interval were not significant ($z = -1.000$, $p > 0.10$). However, the group differences at 51- and 54- to 56-week intervals were significant ($z = 2.023$, $p < 0.05$; $z = 2.201$, $p < 0.05$, respectively).

The final row in Table 1 presents the total numbers of rats in each condition that developed tumors. Significantly fewer irradiated animals fed the diets containing blueberry or strawberry extract (3 rats and 1 rat, respectively) developed tumors compared to the animals fed the control diet (7 rats) (Wilcoxon Signed Rank Test, $z = 2.023, p < 0.05$).

The tumors of six of the rats were examined by the veterinary pathologist of the University of Maryland, Baltimore. Four of the tumors were characterized as round cell tumors showing high mitosis consistent with histocytoma. Another tumor was characterized as mammary adenocarcinoma of the tubulopapillary type. The final one involved severe hyperplasia of immature benign mammary ducts in a background of severe fibrosis and collage.

4. Discussion

The data presented above suggest that maintaining rats on antioxidant diets can reduce the probability of developing tumors following exposure to ^{56}Fe particles, a ground-based model for exposure to GCR. Specifically, diets containing blueberry or strawberry extract may have the capability to act as physiological countermeasures to reduce the tumorigenic consequences of irradiation with heavy particles. Exposing rats to ^{56}Fe particles produces oxidative stress and reactive oxygen species. In turn, oxidative stress has been linked to carcinogenesis. When oxidative stress is reduced by antioxidant diets then the effects of oxidative stress on tumor development are similarly reduced. In addition, because the rats were maintained on the diet for only 1 week following irradiation the data suggest that enhanced free radical scavenging capacity is critical for reduced tumorigenesis only during the time of increased oxidative stress.

While the data presented in this report suggest that diets containing blueberry or strawberry extracts can function to reduce the development of tumors following exposure to ^{56}Fe particles, this data must be considered preliminary for several reasons. First, only a single dose and energy of ^{56}Fe particles was tested. Second, the sample sizes were relatively small for this type of experiment, although statistically significant differences were observed between the control and antioxidant groups. And third, the rats were followed for only one year following irradiation (a maximum of 395 days for some of the subjects). As a result, it is not clear whether the effect of the antioxidant diets was merely to delay the development of heavy particle-induced tumorigenesis or whether the diet functioned to permanently prevent the development of tumors resulting from exposure to ^{56}Fe particles.

Despite the fact the present results are suggestive only, these results are consistent with the results of other experiments which show that maintaining rats on diets containing strawberry or blueberry extract prevents the development of heavy particle-induced neurobehavioral deficits (Rabin et al., 2002, 2005a,b; Shukitt-Hale et al., 2006). To the extent that the underlying mechanisms leading to the development of tumorigenesis and neurobehavioral deficits following exposure to ^{56}Fe particles result from increased oxidative stress and the formation of reactive oxygen species, it is to be expected that treatment with antioxidant diets would provide a significant degree of radiation protection. In addition, the present results are consistent with epidemiological studies which also note that diets high in fruits and vegetables provide a significant degree of radiation protection from neutrons and low LET sources (see Hayes, 2005, for a recent review).

On exploratory missions to other planets, the protection provided by the magnetic field of the earth will be lacking and astronauts will be exposed to continuous, if low level, GCR which can affect the health of astronauts (Ball and Evans, 2001; National Academy of Sciences, 1996; Edwards, 2001). While the major source of protection against the carcinogenic effects of GCR will be provided by physical shielding, there are significant uncertainties about the risk of carcinogenesis on such missions (Cucinotta et al., 2001) and about the amount of shielding that may be needed to provide adequate protection. Too little shielding may provide an inadequate level of protection for the astronauts; too much shielding may make the mission economically infeasible (Wilson et al., 2001). The present results suggest a complementary approach to radiation protection on long-duration missions outside the magnetosphere: that is, to provide diets containing antioxidant phytochemicals, such as blueberry and/or strawberry extract, to astronauts. While additional research is needed to confirm these results, the preliminary data presented in this report suggest that dietary antioxidants may provide a significant level of long-term radiation protection for astronauts on exploratory missions to other planets.

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