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Orally Administered Fructose Increases the Numbers of Peripheral Lymphocytes Reduced by Exposure of Mice to Gamma or SPE-like Proton Radiation

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Abstract

Exposure of the whole body or a major portion of the body to ionizing radiation can result in Acute Radiation Sickness (ARS), which can cause symptoms that range from mild to severe, and include death. One of the syndromes that can occur during ARS is the hematopoietic syndrome, which is characterized by a reduction in bone marrow cells as well as the number of circulating blood cells. Doses capable of causing this syndrome can result from conventional radiation therapy and accidental exposure to ionizing radiation. It is of concern that this syndrome could also occur during space exploration class missions in which astronauts could be exposed to significant doses of solar particle event (SPE) radiation. Of particular concern is the reduction of lymphocytes and granulocytes, which are major components of the immune system. A significant reduction in their numbers can compromise the immune system, causing a higher risk for the development of infections which could jeopardize the success of the mission. Although there are no specific countermeasures utilized for the ARS resulting from exposure to space radiation(s), granulocyte colony-stimulating factor (G-CSF) has been proposed as a countermeasure for the low number of neutrophils caused by SPE radiation, but so far no countermeasure exists for a reduced number of circulating lymphocytes. The present study demonstrates that orally administered fructose significantly increases the number of peripheral lymphocytes reduced by exposure of mice to 2 Gy of gamma- or SPE-like proton radiation, making it a potential countermeasure for this biological end-point.

Keywords

ionizing radiation; acute radiation sickness; countermeasures

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1. Introduction

Exposure of the whole body or a large portion of the body to ionizing radiation can result in the development of Acute Radiation Sickness (ARS), which is characterized by the expression of specific signs and symptoms that occur according to the absorbed dose, the rate of cell proliferation and the duration of the exposure (Heslet et al, 2012). Three ARS syndromes have been recognized: the hematopoietic syndrome, also known as the bone marrow syndrome, the gastrointestinal syndrome and the cardiovascular/neurovascular syndrome (Epelman and Hamilton, 2006). The present study focuses on the hematopoietic syndrome; therefore, the other syndromes are not discussed here. The bone marrow cells have a high proliferation rate and are among the most sensitive cells to acute radiation exposure; thus, the hematopoietic syndrome (HS) occurs at the lowest doses of radiation that are known to have lethal effects. The HS occurs at doses between 0.7 to 10 Gy (Epelman and Hamilton, 2006), and it is characterized by a reduction in the numbers of bone marrow cells and circulating blood cells, which can result in immune suppression, with a consequent increase in susceptibility to the development of infections (Waselenko et al, 2004). The HS is a common side effect of conventional cancer therapy using ionizing radiation (Testa et al, 1985). Levels of radiation exposure capable of causing the hematopoietic syndrome can also result from exposure to solar particle event (SPE) radiation during space exploration missions (Epelman and Hamilton, 2006). SPEs are sporadic bursts of energetic charged particles emitted from solar flares and coronal mass ejections which are composed predominantly of low energy protons (Harra, 2002; Hellweg and Baumstark-Khan, 2007). The highest total internal dose that an astronaut can receive from SPE radiation exposure has been calculated to be 2 Gy (Kim et al, 2009; Townsend, 2005). We have reported that a significant decrease in all blood cells occurs within 30 days of the exposure of mice to 2 Gy of SPE-like, low energy protons (Romero-Weaver et al, 2013a). A significant number of infections has been observed in astronauts during prolonged space flights (Taylor and Sommer, 2005). The treatment of these infections is complicated since the efficacy of antibiotics is reduced in a microgravity environment (Klaus and Howard, 2006), which increases the risk of infections in astronauts. We have also shown that a pegylated form of granulocyte colony-stimulating factor (G-CSF), known as pegfilgrastim, which is commonly used to increase the number of circulating neutrophils after chemotherapy and radiotherapy, can potentially be used to increase the neutrophil numbers in mice exposed to SPE-like proton radiation (Romero-Weaver et al, 2013b). However, there are essentially no countermeasures for other biological end-points of the hematopoietic syndrome. Lymphocytes are the most radio-sensitive of all white blood cells (Dainiak, 2002); they are important components of the adaptive immune response; thus, a reduction in their circulating numbers can increase the risk for infections in astronauts. The purpose of the present work was to evaluate a potential countermeasure for the HS that could potentially be utilized as a countermeasure for astronaut exposure to SPE radiation during prolonged space flights.

Traditional Chinese medicine, in combination with other compounds, has been used successfully for the treatment of victims of Chinese radiation accidents (Coggle, 1989). Analyses of the effects of the Chinese formula Si-Wu-Tang (SWT) and four of its major

constituents: Paenoflorin (Pae), ferulic acid (FA), tetramethyl pyrazine (TP) and fructose, in mice exposed to 3.5 Gy gamma radiation indicated that SWT and fructose were the most effective at increasing the numbers of circulating white blood cells (WBCs) reduced by exposure to gamma radiation (Liang et al, 2006). However, the specific blood cell(s) affected by these treatments were not determined. In a preliminary study, we evaluated the effects of the four major components of SWT to determine their abilities to affect blood cell counts in mice exposed to a single whole body dose of 2 Gy gamma radiation, which is a lower dose than that used by Liang et al. (2006). The results indicated that Pae and FA had no significant effects in blood cells of irradiated mice between 4 and 18 days post-irradiation. TP showed significant increases in total WBCs, lymphocytes and granulocytes reduced by radiation exposure at 4 days after exposure to 2 Gy γ -rays; however, the cost of this agent did not allow further experimentation. Of the four SWT components analyzed, fructose was the most effective at increasing the numbers of circulating WBCs at various time points after γ -radiation exposure in mice. In the present investigations, the effects of oral administration of fructose were evaluated in mice exposed to 2 Gy gamma radiation or 2 Gy SPE-like proton radiation, simulated with low energy protons, to determine which blood cell(s) is (are) affected by this treatment. The results indicate that peripheral lymphocytes and, to a lesser extent, granulocytes, are increased by fructose treatment of irradiated mice.

2. Materials and Methods

2.1 Mice

Female ICR mice at the age of 6 weeks were purchased from Taconic Farms Inc. (Germantown, NY, USA). Animals were acclimated for one week in the University of Pennsylvania animal facility. Five animals were housed per cage with *ad libitum* access to water and food pellets. The animal care and treatment procedures were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

2.2 Irradiation

Mice were restrained in custom designed Plexiglass chambers and exposed to total body irradiation with ^{137}Cs gamma or SPE-like proton radiation at a dose of 2 Gy, administered as previously described (Romero-Weaver et al, 2013b). Un-irradiated control mice were restrained in plexiglass chambers but were not irradiated. Both un-irradiated and irradiated mice were in the plexiglass chambers for the same period of time.

2.3 Treatments

Gamma irradiated mice were treated daily with either 7 or 21 g of fructose per mouse starting seven days before irradiation until one day before irradiation or daily for seven days starting before irradiation and continuing post-irradiation up until one day before euthanasia and blood sample collection. Proton irradiated mice were treated daily with 21 g of fructose per mouse starting seven days before irradiation and continuing post-irradiation up until one day before sample collection or starting after irradiation and continuing at post-irradiation times until one day before sample collection. Fructose was dissolved in 200 μl PBS. Un-

irradiated control mice and irradiated mice without fructose treatment received 200 μ l of PBS. All administrations were given by oral gavage.

The effects of fructose treatment in the gamma irradiated mice (7 or 21 g per mouse) were determined in independent experiments. Thus, un-irradiated and irradiated mice were included in each experiment at all time-points analyzed. The experiments involving two treatment groups/regimens for the proton irradiated mice (before and after irradiation or after irradiation) were performed simultaneously. Thus, just one group of un-irradiated and irradiated mice treated with PBS was included at each time point analyzed for the two regimens.

2.4 Blood sample collection

Blood samples were collected by cardiac puncture from un-irradiated control mice treated with PBS and gamma or proton irradiated mice treated with either PBS or fructose, on days 4, 10, 13, 16 and 18 post-irradiation. Blood was placed into lavender top blood BD microtainer collection tubes containing EDTA (BD, Franklin Lakes, NJ, USA) and sent to Antech Diagnostics facility (Lake Success, NY, USA) for complete automated blood cell count analyses. Three to five mice were used per treatment group.

2.5 Statistical analyses

Statistical significance was determined using GraphPad Prism 5.

3. Results

In the first experiment, two regimens of fructose administration were evaluated (see Materials and Methods) for their effects on blood cells after exposure of mice to 2 Gy gamma radiation. Table 1 shows the results of daily administration of fructose (7g/mouse) starting at seven days before irradiation until one day before irradiation. The dose of fructose chosen for this study was based on previously reported studies (Liang et al, 2006). Irradiated mice treated with PBS showed significant decreases in WBC and lymphocyte counts at all analyzed times and in granulocytes at all times except 13 days post-irradiation, compared with un-irradiated mice treated with PBS (*). Irradiated mice treated with fructose showed an increased number of total WBCs and lymphocytes compared to irradiated mice treated with PBS; the increases were observed at all analyzed times, but were statistically significant at 13 and 16 days post-irradiation (#, bolded numbers) and were of borderline statistical significance at 18 days-post-irradiation (¥, $p=0.07$ and 0.06 , respectively). At the 18 day time point, the differences between the number of WBCs and lymphocytes in irradiated mice treated with fructose and those from un-irradiated control mice treated with PBS were not statistically significant. Increased granulocyte numbers were observed in fructose treated irradiated mice from day 10 to day 18 post-irradiation compared to irradiated mice treated with PBS; however, the increase was statistically significant only on day 16 post-irradiation (#, bolded numbers). The results of the second regimen of fructose administration, 7 g/mouse of fructose daily starting seven days before irradiation and continuing post-irradiation until one day before sample collection, are shown in Table II. Irradiated mice treated with PBS showed statistically significant decreases in WBC,

lymphocyte and granulocyte counts at all analyzed times, except at 10 days post-irradiation, compared with un-irradiated mice treated with PBS (*). Increases in the number of total WBC, lymphocyte and granulocyte counts were observed at all analyzed times in fructose treated mice compared to irradiated mice treated with PBS, but were statistically significant only on days 4 and 10 post-irradiation (#, bolded numbers) and were of borderline statistical significance at 18 days postirradiation (¥. $p=0.07$, 0.06 and 0.06 , respectively)

A small experiment was conducted to determine whether an increase in fructose dose to 21 g/mouse could result in a greater increase in the numbers of lymphocytes and granulocytes in irradiated mice than those obtained with the dose of 7 g of fructose per mouse. Since intake of higher concentrations of fructose are associated with metabolic disturbances (Johnson et al, 2007), the dose of 21 g/mouse was translated to the human equivalent dose (HED), using the equation reported by Reagan-Shaw et al. (2008), to determine whether this dose would be safe for use in humans. Thus, the proposed equation for HED is as follows:

$$\text{HED} = \text{mouse dose (mg/kg)} \times \text{mouse Km} / \text{human Km}, \quad (1)$$

The Km factor reported for mice is 3 and the Km for a 60 kg human is 37, the approximate average weight of the mice in this study is 0.02 kg. If these values are put into Eq. (1), the result is:

$$\text{HED} = (21 \text{ mg}/0.02 \text{ kg}) \times 3/37 = 85 \text{ mg/kg}$$

If the HED is multiplied by the human weight, assuming 60 Kg, then the amount of fructose is $85 \text{ mg/kg} \times 60 \text{ kg} = 5,100 \text{ mg} = 5.1 \text{ g}$ of fructose.

According to a study of the content of fructose in several beverages (Ventura et al, 2011), this amount is similar to the amount of fructose present in 100 ml of Hawaiian Fruit Punch (128 Fluid Ounce: FL, bottle), Kroger Apple Juice (cocktail (64 FO bottle) and Tampico Citrus Punch (128 FO bottle), which is an intermediary amount between the highest reported fructose dose (11.1g) found in 100 ml of Coca-Cola (14 FO bottle) and the lowest reported fructose dose (1.9 g) found in 100 ml of Red Bull (8.4 FO can). Therefore, 21g fructose per mouse (equivalent to 5.1g of fructose in humans) can be considered a safe dose that is comparable to the amount of fructose found in the widely used beverages named above.

Thus, mice were treated daily with either 7 or 21 g of fructose per mouse for seven days before gamma irradiation and post-irradiation until one day before sample collection. The results indicated higher numbers of WBCs, lymphocytes and granulocytes in irradiated mice treated with 21 g/mouse of fructose compared to the numbers obtained using 7 g/mouse of fructose; however, the changes were not statistically significant (data not shown). The following studies were conducted with 21 g/mouse of fructose.

The next experiment was performed to determine whether administration of 21 g of fructose/ mouse could also increase the numbers of circulating lymphocytes and granulocytes in mice exposed to SPE-like proton radiation. Since SPEs are difficult to predict, it is more likely that, in the event of exposure to SPE radiation, astronauts would receive treatment after exposure. Thus, the schedules of fructose administration that were analyzed in this study

were daily administration of 21g/mouse of fructose starting at seven days before and continuing after 2 Gy SPE-like proton radiation, until one day before blood sample collection, or daily administration starting immediately after 2 Gy SPE-like proton radiation and continuing until one day before blood sample collection.

The results are shown in Table III, where it can be observed that SPE-like proton radiation caused statistically significant decreases in WBC, lymphocyte and granulocyte counts at all analyzed times, except for granulocytes at 10 days post-irradiation (*). Treatment with fructose before and after irradiation caused a significant increase in WBC counts on days 4 and 13 post-irradiation, compared with irradiated mice treated with PBS (#, bolded numbers), and were of borderline statistical significance on day 16 post-irradiation. Also, a significant increase was observed in lymphocyte counts on days 4, 13 and 16 post-irradiation (#, bolded numbers) and granulocyte counts were of borderline statistical significance on day 4 post-irradiation (¥, $p=0.07$).

Treatment with fructose after SPE-like proton irradiation caused a statistically significant increase in WBC counts on day 13 post-irradiation, in lymphocytes on days 13 and 16 post irradiation and in granulocytes on day 18 post-irradiation compared with irradiated mice treated with PBS (#, bolded numbers). The granulocyte counts at 18 days post-irradiation were not significantly different compared with granulocyte counts of un-irradiated control mice treated with PBS.

4. Discussion

Exposure to conventional radiotherapy, ionizing radiation accidents or radiation from SPE radiation has the potential to cause significant decreases in all blood cells at different times after exposure. Reduction of circulating lymphocyte and granulocyte counts are of special concern since they have the potential to increase the risk of astronauts for the development of infections which can jeopardize the success of the mission. Therefore, countermeasures for these biological endpoints are critical. Granulocyte colony stimulating-factor has been proposed as a countermeasure for low neutrophil counts resulting from radiation exposure (Waselenko, et al, 2004), including SPE radiation exposure (Romero-Weaver et al, 2013b). Currently, no specific countermeasure exists for the reduction in the number of lymphocytes caused by exposure to ionizing radiation. Liang et al. (2006) showed that fructose treatment significantly increased WBC numbers reduced by exposure of mice to 3.5 Gy gamma radiation, but they did not report which specific type of WBC was affected by the treatment. This study shows that fructose, at a dose of 7 to 21 mg/mouse, which corresponds to 1.7 and 5.1g in humans [according to the equation reported by Reagan-Shaw et al. (2008)], respectively, significantly increases the number of lymphocytes reduced by exposure of mice to 2 Gy gamma-rays or 2 Gy SPE-like proton radiation.

Lymphocytes are the major type of WBC in mice; therefore, it is not surprising that Liang et al. (2006) observed an increase in WBCs with fructose treatment, since it is likely to be due to the increase in lymphocytes observed in the present study. They also determined that fructose increased hematopoietic cell precursors in the bone marrow and enhanced cellular immunity, as determined by increases in spleen and thymus indices. Thus, it is possible that

similar mechanisms are involved in the fructose mediated increase in the number of circulating lymphocytes shown here. Further studies, however, are necessary to confirm this hypothesis.

Results from the regimens analyzed in this study indicate that treatment of mice with fructose before, before and after, or after irradiation, caused significant increases in lymphocyte counts at different times post-irradiation compared with lymphocytes of irradiated mice treated with PBS (Figures 1 and 2). In some cases, such as when fructose was administered after SPE-like proton radiation, the treatment caused significant increases in lymphocyte numbers at all time points analyzed compared with irradiated mice treated with PBS, but due to variations within each group, statistical significance was not always reached (Figure 2). The use of 21 g/mouse of fructose compared to 7 g of fructose/mouse did not result in a statistically significant increase in lymphocyte numbers in irradiated mice. It is noteworthy that there is a considerable increase in WBCs, lymphocytes and granulocytes in the control group treated with PBS at 10 days post-irradiation, as shown in Table 1. This increase is most likely due to normal circadian variations in blood cell numbers (Sowyer et al, 1987) as we have seen similar significant increases in blood cell counts in some, but not all, mice in most of the control groups. These results emphasize the need to include controls at each time point and to collect the blood samples at the same time during the day (as was done in the present study) to compensate for the normal variations in blood cell counts.

Fructose is a monosaccharide naturally contained in fruits, which has been added to several sugar sweetened beverages. The long term consumption of high levels of fructose can have undesirable side effects, such as obesity (Lakhan and Kirchgessner, 2013), but short term consumption is known to have some beneficial effects, such as protection against oxidation of astroglial C6 cells (Spasojevic et al, 2009) or immune-modulation effects when the fructose biphosphate derivative is utilized (Nunes et al, 2003). In summary, the results of this study indicated that orally administered fructose significantly increases the numbers of lymphocytes reduced by exposure of mice to 2 Gy gamma or 2 Gy SPE-like proton radiation when it was administered before, after, or before and after irradiation. These results are important since no mitigators for radiation reduced lymphocytes have been identified so far. This effect, along with the facts that fructose is safe, inexpensive, easily accessible and orally administered, suggests that this monosaccharide should be considered as a potential countermeasure for use in those irradiated in space or in accidental exposures to ionizing radiation.

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Figure 1. Effect of fructose administration on lymphocytes of gamma irradiated mice treated with PBS (straight lines) or fructose (broken lines)

A) Fructose was administered at 7 g/mouse daily starting at seven days before 2 Gy gamma radiation until the day before irradiation; B) Fructose was administered at 7 g/mouse starting at seven days before 2 Gy gamma radiation and after irradiation until one day before sample collection, B&A. Blood was collected at the indicated time points and lymphocyte counts were determined and the fraction of control was plotted as a function of time post-irradiation, (* $p < 0.05$ for the comparison of lymphocytes from gamma irradiated mice treated with either PBS or fructose to the lymphocytes from un-irradiated control mice treated with

PBS, # $p < 0.05$ for the comparison of lymphocytes from gamma irradiated mice treated with fructose to the lymphocytes from gamma irradiated mice treated with PBS, $^{\text{¥}}0.05 \leq p < 0.1$ [borderline statistical significance] for the comparison of lymphocytes from gamma irradiated mice treated with fructose to the lymphocytes from gamma irradiated mice treated with PBS.

Figure 2. Effect of fructose administration on lymphocytes from proton irradiated mice treated with PBS (straight lines) or fructose (broken lines)

A) Fructose was administered at 21 g/mouse daily starting seven days before SPE-like proton irradiation and continuing post-irradiation until one day after sample collection; B) Fructose was administered at 21 g/mouse immediately after SPE-like proton irradiation until one day before sample collection, B&A. Blood was collected at the indicated time points and lymphocyte counts were determined and the fraction of control was plotted as a function of time post-irradiation, (* $p < 0.05$ for the comparison of lymphocytes from SPE-like proton irradiated mice treated with either PBS or fructose to the lymphocytes from unirradiated

control mice treated with PBS, #p<0.05 for the comparison of lymphocytes from SPE-like proton irradiated mice treated with fructose to lymphocytes from SPE-like proton irradiated mice treated with PBS.

Table 1

Absolute WBC, lymphocyte and granulocyte numbers in un-irradiated control mice (PBS), 2 Gy gamma irradiated mice treated with PBS (IRR+PBS) or 2 Gy gamma irradiated mice treated with fructose (7 g/mouse, IRR+FRU) starting seven days before irradiation until one day before irradiation plus/minus standard deviations.

CELLS	TREATMENT (BEFORE IRRADIATION)	DAYS AFTER 2 GY GAMMA RADIATION					
		4	10	13	16	18	
WBCs	PBS	7.03±2.19	13.73±2.08	8.74±2.52	7.40±1.81	7.10±2.15	
	IRR+PBS	3.00±0.64 [*]	4.50±1.10 [*]	4.00±0.66 [*]	2.33±0.54 [*]	3.45±1.24 [*]	
	IRR+FRU	3.33±0.68 [*]	5.92±1.56 [*]	6.25±1.46[#]	4.90±1.7[#]	5.04±1.10[¥]	
Lymphocytes	PBS	5.40±1.83	10.81±2.60	6.82±1.77	5.74±1.20	5.60±1.80	
	IRR+PBS	2.00±0.54 [*]	3.45±1.36 [*]	3.10±0.29 [*]	1.92±0.40 [*]	2.63±0.75 [*]	
	IRR+FRU	2.52±0.54 [*]	4.50±1.37 [*]	5.22±0.59[#]	4.20±1.58[#]	3.91±1.08[¥]	
Granulocytes	PBS	1.42±0.41	2.70±0.57	1.74±0.77	1.49±1.05	1.41±0.43	
	IRR+PBS	0.90±0.13 [*]	0.90±0.28 [*]	0.80±0.36	0.39±0.17 [*]	0.68±0.50 [*]	
	IRR+FRU	0.64±0.11 [*]	1.21±0.26 [*]	1.00±0.16 [*]	0.65±0.07[#]	1.06±0.40	

^{*} p<0.05 compared with un-irradiated mice treated with PBS.

[#] p<0.05 compared with irradiated mice treated with PBS.

[¥] borderline statistical significance compared with irradiated mice treated with PBS; i.e. 0.05 p 0.10.

Table 2

Absolute WBC, lymphocyte and granulocyte numbers in un-irradiated control mice (PBS), 2 Gy gamma irradiated mice treated with PBS (IRR+PBS) and 2 Gy gamma irradiated mice treated with fructose (7 g/mouse, IRR+FRU) starting seven days before irradiation and continuing after irradiation until one day before sample collection, plus/minus standard deviations.

CELLS	TREATMENT (BEFORE/AND AFTER IRRADIATION)	DAYS AFTER 2 GY GAMMA RADIATION					
		4	10	13	16	18	
WBC _{sc}	PBS	9.85±1.76	8.00±3.61	7.20±0.46	7.30±1.90	8.40±2.59	
	IRR+PBS	2.40±0.57*	4.10±0.76*	3.20±1.38*	3.50±0.54*	3.56±1.09*	
	IRR [‡] +FRU	3.33±0.35[#]	6.20±1.37[#]	4.20±0.39*	3.72±0.88*	5.20±1.36[‡]	
Lymphocytes	PBS	7.32±1.07	5.80±3.22	5.81±0.37	6.00±1.61	6.20±1.65	
	IRR+PBS	1.60±0.30*	2.94±0.58	2.43±1.24*	2.80±0.61*	2.84±0.80*	
	IRR [‡] +FRU	2.30±0.22[#]	4.50±0.95[#]	3.30±0.32*	2.94±0.84*	4.10±1.0[‡]	
Granulocytes	PBS	2.52±0.76	2.0±0.67	1.30±0.32	1.19±0.28	2.10±1.05	
	IRR+PBS	0.61±0.16*	1.0±0.23*	0.63±0.34*	0.67±0.06*	0.68±0.34*	
	IRR+FRU	0.90±0.27[#]	1.6±0.48[#]	0.90±0.19	0.75±0.17*	0.96±0.19	

* p<0.05 compared with un-irradiated mice treated with PBS.

p<0.05 compared with irradiated mice treated with PBS.

‡ borderline statistical significance compared with irradiated mice treated with PBS; i.e. 0.05 p 0.10.

Table 3

Absolute WBC-, lymphocyte and granulocyte numbers in un-irradiated control mice (PBS), 2 Gy SPE-like proton irradiated mice treated with PBS (IRR +PBS) and 2 Gy SPE-like proton irradiated mice treated with fructose (21 g/mouse, IRR+FRU B&A IR) starting seven days before irradiation and continuing after irradiation until one day before sample collection or starting seven days after irradiation until one day before sample collection (IRR +FRU after IR), plus/minus standard deviations.

CELLS	TREATMENT	DAYS AFTER 2 GY PROTON RADIATION					
		4	10	13	16	18	
WBCs	PBS	8.00±1.07	9.60±0.60	10.60±2.14	6.80±0.29	9.00±0.78	
	IRR+PBS	2.70±0.29*	5.80±0.85*	3.70±0.95*	3.20±0.57*	4.60±1.04*	
	IRR+FRU B&A*	3.40±0.63*#	5.90±1.02*#	5.20±0.42*#	4.30±0.94*#	4.50±0.73*	
	IRR+FRU after IR	2.90±0.21*	5.12±1.58*	5.20±1.95*#	4.30±2.08*	5.80±0.92*	
Lymphocytes	PBS	6.40±0.80	7.13±0.50	8.44±1.76	5.20±0.3	7.20±0.88	
	IRR+PBS	1.83±0.28*	3.21±0.87*	2.70±0.64*	2.40±0.43*	3.80±1.02*	
	IRR+FRU B&A IR	2.71±0.63*#	4.20±0.76*	4.18±0.40*#	3.40±0.61*#	3.80±0.51*	
	IRR+FRU after IR	2.10±0.26*	3.81±0.59*	4.86±1.00*#	3.40±1.64	4.50±1.10*	
Granulocytes	PBS	1.43±0.42	2.22±0.21	1.85±0.58	1.30±0.24	1.60±0.18	
	IRR+PBS	0.76±0.09	2.40±0.31	0.83±0.42*	0.67±0.17*	0.77±0.20*	
	IRR+FRU B&A*	0.60±0.16*#	1.63±0.37*	1.14±0.28*	0.89±0.23*	0.57±0.22*	
	IRR+FRU after IR	0.75±0.18*	1.23±0.41*	1.06±0.33*	0.73±0.33*	1.19±0.38#	

* P<0.05 compared with un-irradiated mice treated with PBS.

p<0.05 compared with irradiated mice treated with PBS.

‡ borderline statistical significance compared with irradiated mice treated with PBS; i.e. 0.05 p 0.10.