INTRODUCTION

Liver function, especially the rate of its metabolic enzyme activities, determines the concentration of circulating drugs as well as the duration of their efficacy. Most pharmaceuticals are metabolized by the liver, and clinically-used medication doses are given with normal liver function in mind. A drug overdose can result in the case of a liver that is damaged and removing pharmaceuticals from the circulation at a rate slower than normal. Alternatively, if liver function is elevated and removing drugs from the system more quickly than usual, it would be as if too little drug had been given for effective treatment. Because of the importance of the liver in drug metabolism, we want to understand any effects of spaceflight on the enzymes of the liver.

Crewmembers on spaceflight missions are exposed to several unusual environmental stressors, including microgravity and chronic low dose radiation, as well as a diet of preserved food. Dietary factors and exposure to radiation are aspects of spaceflight that can be modeled in ground experiments. In this experiment, we examine the effects of high dietary iron and low dose gamma radiation, individually and combined, on the gene expression of key metabolic enzymes.

METHODS

All procedures were approved by the JSC Animal Care and Use Committee. Male Sprague-Dawley rats were divided into control and 3 treatment groups of 8 animals each: high iron diet, radiation treated, and both high iron diet + radiation treatment. “Chronic” radiation treatment: The irradiated animals were exposed to gamma radiation (0.375 Gy/exposure) from a Cs-137 source (energy = 662 KeV) every other day for 16 days (8 doses) for a total whole body dose of 3 Gy. Non-irradiated animals (sham) were handled similarly, but not exposed to radiation.

High Fe: The high iron diet consisted of standard AIN93G chow (45 mg iron/kg; Research Diets, New Brunswick, NJ) supplemented with ferric citrate to 650 mg iron/kg. Both: Animals received the high Fe diet and radiation treatments.

After treatments were completed, animals were anesthetized and sacrificed. Livers were removed immediately and flash-frozen in liquid nitrogen. Tissue was homogenized, RNA extracted (Absolutely RNA, Agilent), purified and quality-tested (Agilent liquid nitrogen). There were 8 animals in each treatment group, and one liver sample from each was analyzed with qRTPCR.

RESULTS

In a preliminary analysis, 13 of 25 genes demonstrated no significant expression changes with any treatment condition, determined by a relative expression change of at least 2-fold with p <0.05. Selected results are shown below.

Drug metabolizing genes were differentially affected by treatments

The cytochrome p450 gene test exhibited a range of responses. Cyp2e1 (metabolizes anesthetics and acetaminophen) was doubled in the animals that received both treatments. Expression of Cyp3a11 (converts andropregna to estradiol) was highly variable among the samples in each group; although expression tended higher in radiation treatment groups, the changes were not significant. Cyp3a4 (produces cholesterol and steroid hormones) and Cyp1a2 (metabolizes many administered medications) showed no significant changes.

Antioxidant genes were differentially affected by treatments

Metallothioneins (Mts) are involved in regulation of metals in the body, and may act as antioxidants. Expression of the metallothionein 2 gene decreased by almost 4-fold in the radiation group, but was unchanged by high Fe. Both treatments together resulted in a smaller, and insignificant decrease in expression. Methyltransferase/didehydroepiandrosterone reductase (Mthfr), involved in amino acid and antioxidant synthesis, expression was significantly increased by both treatments together. Expression of Gpx1 (glutathione peroxidase), a very important antioxidant gene, showed modest but significant increases in both radiation treatments, but was unaffected by the high Fe. Mts and Nfkb showed no significant changes.

Drug transporter genes were differentially affected by treatments

The ABC transporter genes tested exhibited very different responses. Abcb1b (MDR efflux pump, also involved in lipid and steroid transport) showed significant expression increases upon radiation treatment, but high Fe had no effect. Note the difference in scale from the other figures; these expression changes are roughly 13-fold higher than controls. Abcb1b showed no changes under any of the treatments.

Iron storage genes were differentially affected by treatments

The light chain of iron storage protein ferritin (Fth) showed elevated expression in the high Fe group and the Rad group, with a trend toward higher expression in the group that received both treatments. A very similar pattern was seen in gene expression of the ferritin heavy chain, (Fth1), but expression changes were much smaller in magnitude.

CONCLUSION

Although this was a preliminary study and the gene expression results have yet to be examined at the protein level, some interesting trends are evident.

It has previously been shown that gamma radiation causes physiological oxidation (Ding, et al., 2005). Some of the affected genes in this study are involved in reduction or removal of oxidized compounds. Increased in expression of Gpx is likely explained by higher physiological oxidation. Mt2 (metallothionein) is usually thought to remove heavy metals from the body, but may also play a role in inflammation and oxygen free radical regulation (Sato, et al., 2002). Expression of this gene is regulated by redox state (which can be affected by radiation exposure) in addition to metal concentrations. Increases in metallothionein expression have previously been reported in livers of fish exposed to 75 mGy radiation (Olsvik et al., 2010).

An 11-to 13-fold increase in expression of transporter Abcb1b was seen in both radiation-treated groups. Another member of the same gene family Abcb4 was not affected by any treatment. Both the magnitude and specificity of this result merit additional examination. This result also carries the potential for clinically-relevant alterations in pharmacokinetics of administered medications, as does the increased expression of Cyp2e1.

It seems likely that radiation exposure triggers a variety of homoeostatic mechanisms, which could include alterations of gene expression. Whether high dietary iron exacerbarates oxidative stress is not clear at the gene expression level in the liver; future examinations of protein expression should inform this question. Better understanding of these pathways could aid in development of new countermeasures to ameliorate damage or maintain health of cells and tissues during spaceflight.

REFERENCES


ACKNOWLEDGEMENTS

The authors thank Dr. Sara Zwart for animal tissues from her study (Evaluation of the combined effects of gamma radiation and high dietary iron on oxidative damage and antioxidant status in rats). Dr. Kami Faust and Mr. Andrew Hood for technical assistance (Wyle Enterprises and NASA Career Exploration Program, respectively). The JSC Human Health and Countermeasures Division Core Laboratories provided some necessary instrumentation. Funding was provided to V. Wotring by NASA JSC Human Research Program.