Changes in Liver Metabolic Gene Expression after Radiation Exposure

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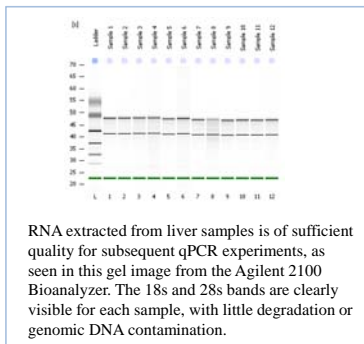[2]Pharmacology Discipline, NASA Johnson Space Center, [3]Universities Space Research Association , Houston TX

INTRODUCTION

The health of the liver, especially the rate of its metabolic enzymes, 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. Exposure to cosmic radiation is one aspect of spaceflight that can be modeled in ground experiments.
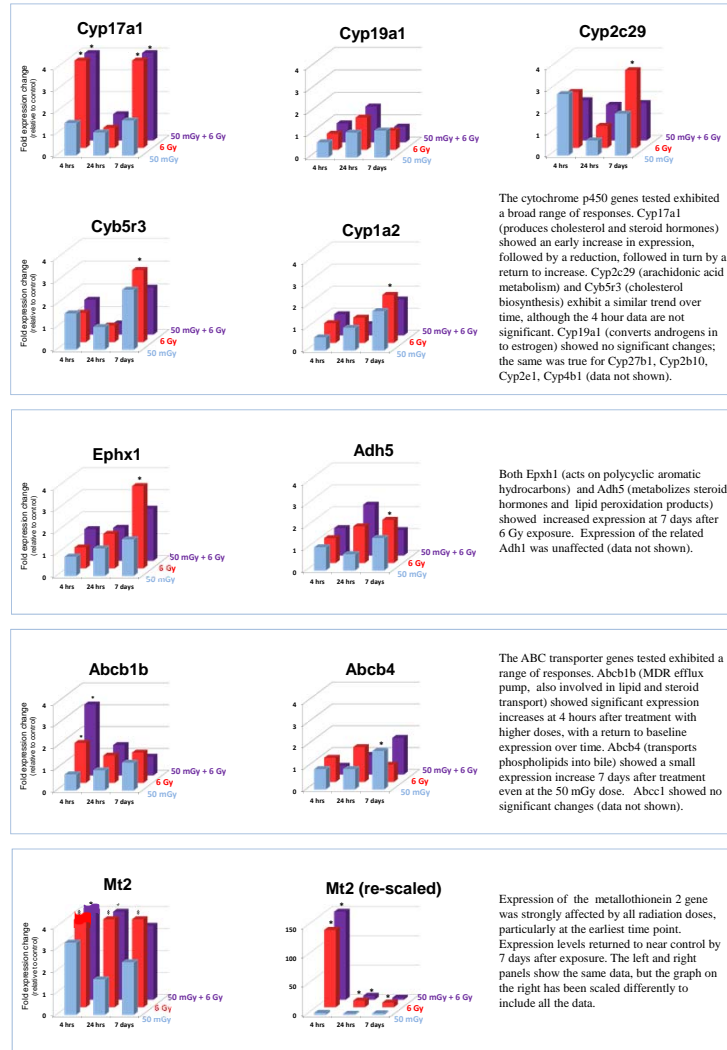
METHODS

Using procedures approved by the JSC Animal Care and Use Committee, male C57 mice were exposed to ^{137}Cs in groups: controls (no radiation exposure, but handled similarly to the other groups), low dose (50 mGy), high dose (6 Gy) and a fourth group that received both radiation doses separated by 24 hours. Animals were anesthetized and sacrificed at varying time points after their last radiation exposure (4 hours, 24 hours and 7 days). Livers were removed immediately and flash-frozen in liquid nitrogen. Tissue was homogenized, RNA extracted (Absolutely RNA, Agilent), purified and quality-tested (Agilent 2100 Bioanalyzer). Complementary DNA was prepared from high-quality RNA samples (RIN > 8; RT^2 First Strand, Qiagen/SABiosciences), and used to run RT-qPCR screening arrays for DNA Repair and Drug Metabolism (RT^2 Profiler Arrays, Qiagen/SABiosciences). This study used unwanted tissue from another study, and is limited by the sample number from the parent study. This study is *not* powered to determine differences among the radiation doses or among the time points; all comparisons are to the unexpected control group at the corresponding time point.

RNA extracted from liver samples is of sufficient quality for subsequent qPCR experiments, as seen in this gel image from the Agilent 2100 Bioanalyzer. The 18s and 28s bands are clearly visible for each sample, with little degradation or genomic DNA contamination.

RESULTS

Of 86 drug metabolism genes examined, expression of 52 were unchanged by any treatment condition (determined by a relative expression change of less than 2-fold). Expression of some genes was changed in an apparently dose-dependent fashion, for example Abcb1b and Mt2, while in other cases, there is little correlation of expression with dose (Cyp17a1, Cyp19a1). Some genes exhibited a post-exposure temporal pattern that is consistent regardless of dose (Cyp17a1, Cyb5r3, Adh5).

The cytochrome p450 genes tested exhibited a broad range of responses. Cyp17a1 (produces cholesterol and steroid hormones) showed an early increase in expression, followed by a reduction, followed in turn by a return to increase. Cyp2c29 (arachidonic acid metabolism) and Cyb5r3 (cholesterol biosynthesis) exhibit a similar trend over time, although the 4 hour data are not significant. Cyp19a1 (converts androgens in to estrogen) showed no significant changes; the same was true for Cyp27b1, Cyp2b10, Cyp2e1, Cyp4b1 (data not shown).

Both Epxh1 (acts on polycyclic aromatic hydrocarbons) and Adh5 (metabolizes steroid hormones and lipid peroxidation products) showed increased expression at 7 days after 6 Gy exposure. Expression of the related Adh1 was unaffected (data not shown).

The ABC transporter genes tested exhibited a range of responses. Abcb1b (MDR efflux pump, also involved in lipid and steroid transport) showed significant expression increases at 4 hours after treatment with higher doses, with a return to baseline expression over time. Abcb4 (transports phospholipids into bile) showed a small expression increase 7 days after treatment even at the 50 mGy dose. Abcc1 showed no significant changes (data not shown).

Expression of the metallothionein 2 gene was strongly affected by all radiation doses, particularly at the earliest time point. Expression levels returned to near control by 7 days after exposure. The left and right panels show the same data, but the graph on the right has been scaled differently to include all the data.

Data are normalized gene expression relative to a set of reference genes whose expression was not significantly by any treatment at any time point (Adh1, Blvrb, Gstm4, Gstm5, Marcks, and Snn). No change in expression is at y=1 on all graphs above; deviations from 1 (either above or below) indicate changes in expression relative to the housekeeping genes compared to control animals. * Indicates significance with p < 0.05 (compared to control) . Sample sizes: n= 6, with the following exceptions: 50 mGy, 4 hour n=3; 50 mGy, 42 hour n=5; 6 Gy + 50 mGy, 7 day n=5.

CONCLUSION

Although this was a preliminary study and the gene expression results have yet to be verified at the protein level, some interesting trends are evident. It has previously been shown that gamma radiation causes physiological oxidation (Ding, et.al., 2005). Many of the affected genes in this study are involved in reduction or removal of oxidized compounds. The greatest expression changes were in Mt2 (metallothionein) and Cyp17a1, one of the cytochrome p450 enzymes. In these two cases, large expression increases were seen in response to high and low + high exposures. 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 and glucocorticoids. Increases in metallothionein expression have also been reported in livers of fish exposed to 75 mGy γ radiation (Olsvik et al., 2010). Cyp17a1 encodes an enzyme that adds an hydroxyl group to progesterone, which can then be converted to testosterone, estrogen or glucocorticoids. It can also contribute to the metabolism of administered medications that have complex ring structures, like hormones or promethazine. It is interesting to note that expression of the related Cyp19a was not significantly by all treatments, like 52 other genes that were examined.

It seems likely that radiation exposure triggers a variety of homeostatic mechanisms, which could include alterations of gene expression. Better understanding of these pathways could aid in development of new countermeasures to ameliorate or prevent radiation-induced damage to cells and tissues.

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