

Changes in Liver Metabolic Gene Expression after Radiation Exposure

C.P. Peters¹ and V. E. Wotring, Ph.D.^{2,3}

¹Bethel University, St. Paul MN

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

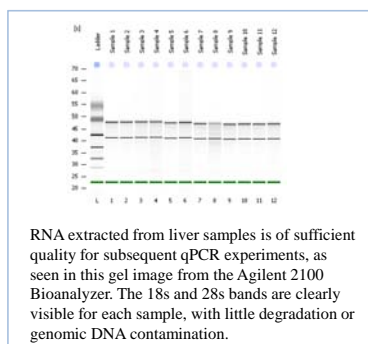


INTRODUCTION

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

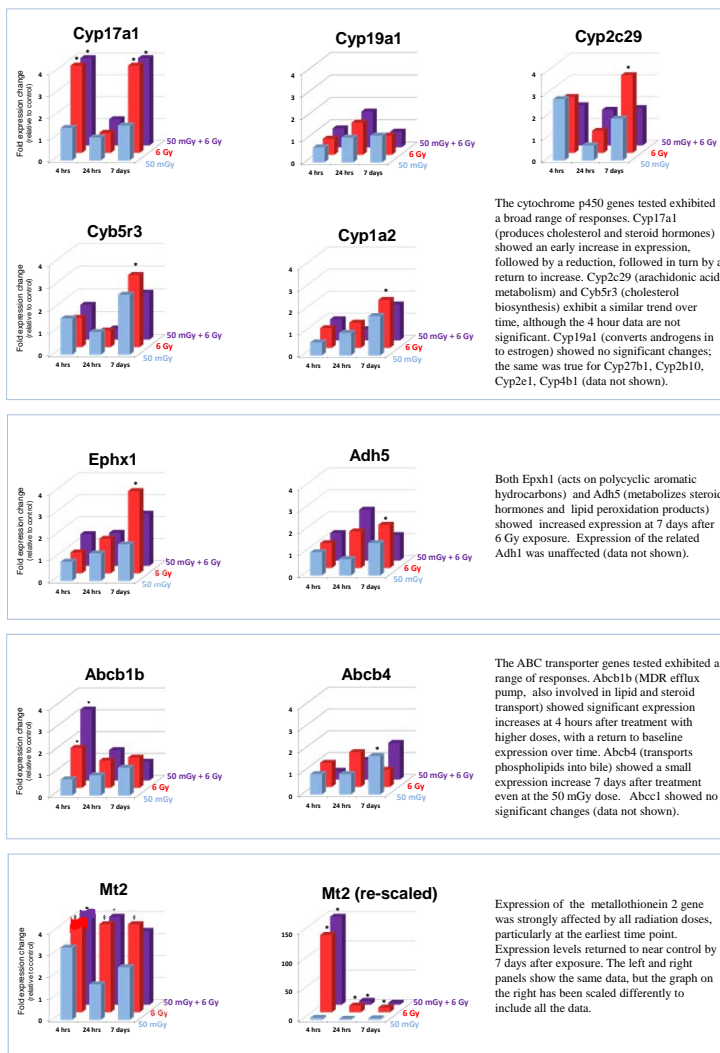
Using procedures approved by the JSC Animal Care and Use Committee, male C57 mice were exposed to ¹³⁷Cs in groups: controls (no radiation exposure, but handled similarly to the other groups), low dose (50 mGy), high dose (6 Gy) and a fourth group that received both radiation doses separated by 24 hours. 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; RT² First Strand, Qiagen/SABiosciences), and used to run RT-qPCR screening arrays for DNA Repair and Drug Metabolism (RT² 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 unexposed control group at the corresponding time point.



www.nasa.gov

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).



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, B1vrb, 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.

REFERENCES

- Ding, L.H, M Shingyoji, F Chen, JJ Hwang, S Burma, C Lee, JF Cheng, and DJ Chen (2005) Gene expression profiles of normal human fibroblasts after exposure to ionizing radiation: a comparative study of low and high doses. *Radiat Res* 164(1): 17-26.
- Olsvik, PA, LS Heier, BO Rosseland, HC Teien, and B Salbu (2010) Effects of combined gamma-irradiation and metal (Al+Cd) exposures in Atlantic salmon (*Salmo salar* L.). *J Environ Radioact* 101(3): 230-6.
- Sato, M, and M Kondoh (2002) Recent studies on metallothionein: protection against toxicity of heavy metals and oxygen free radicals. *Tohoku J Exp Med* 196(1): 9-22.

ACKNOWLEDGEMENTS

The authors would like to thank Ms. Stephanie Bassett for excellent animal care and Dr. Robert Ploutz-Snyder for statistical expertise. Treated tissue courtesy of H. Wu, NASA JSC via DOE grant. The JSC Human Health and Countermeasures Division Core Laboratories provided necessary instrumentation. C. P. Peters was supported by the Minnesota Space Grant. Funds for qPCR experiments were provided to V. Wotring by NASA JSC Human Research Program.

