Radiation Exposure Alters Expression of Metabolic Enzyme Genes in Mice

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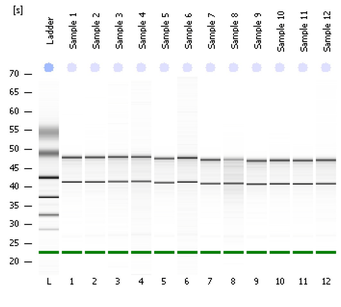
INTRODUCTION

Most administered pharmaceuticals are metabolized by the liver. 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 the effects of spaceflight on the enzymes of the liver and exposure to cosmic radiation is one aspect of spaceflight that can be modeled in ground experiments.
Additionally, it has been previous noted that pre-exposure to small radiation doses seems to confer protection against later and larger radiation doses (reviewed by Tapio, 2007). This protective power of pre-exposure has been called a priming effect or radioadaptation. This study is an effort to examine the drug metabolizing effects of radioadaptation mechanisms that may be triggered by early exposure to low radiation doses.

METHODS

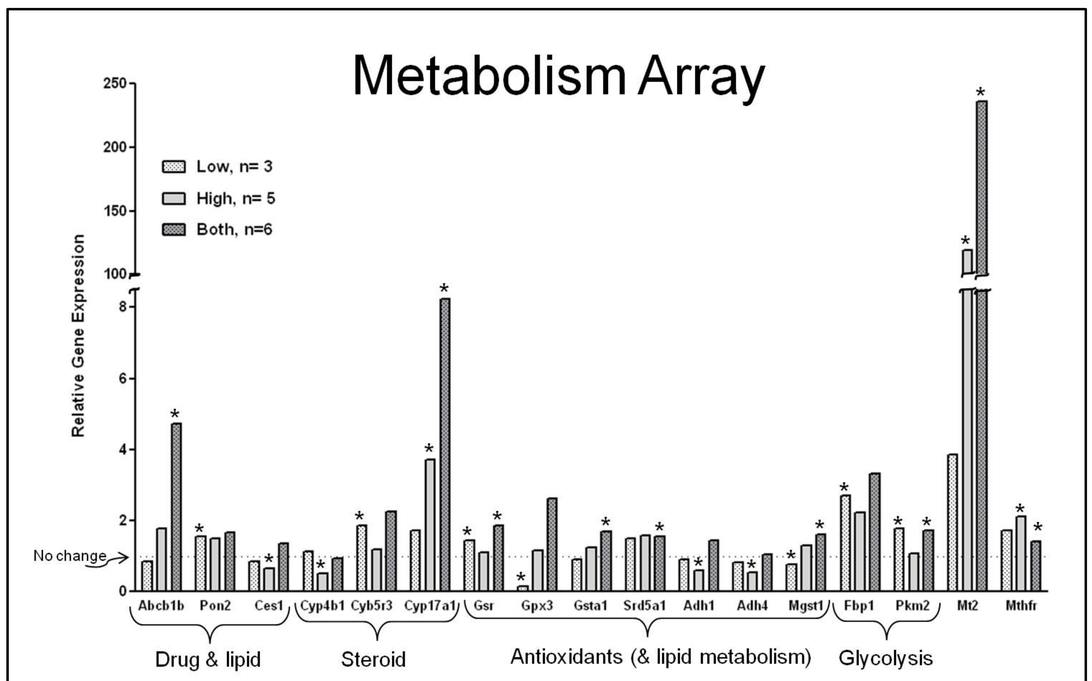
Using procedures approved by the JSC Animal Care and Use Committee, male C57 mice were exposed to ^{137}Cs in groups: controls (no radiation exposure, but handled similarly to the other groups), low dose (50 mGy), high dose (6 Gy) and a fourth group that received both radiation doses separated by 24 hours. Animals were anesthetized and sacrificed 4 hours after their last radiation exposure. 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, SABiosciences), and used to run RT-qPCR screening arrays for DNA Repair and Drug Metabolism (RT^2 Profiler Arrays, SABiosciences). The data shown here are preliminary, in that they only show changes in gene expression. Additional experiments to corroborate these findings at the protein level are planned.



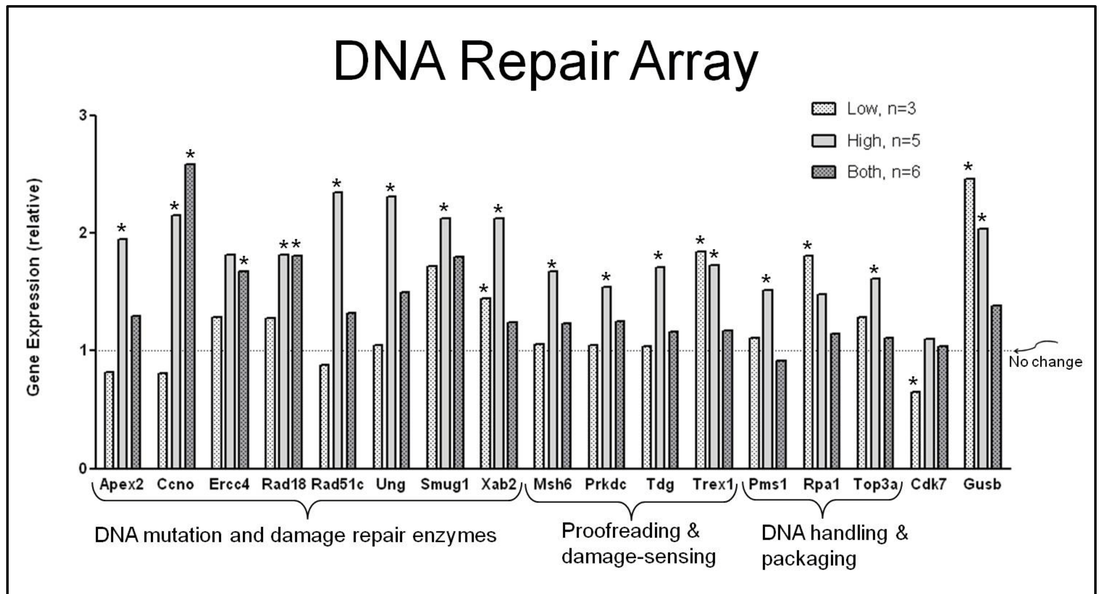
RNA extracted from liver samples is of sufficient quality for subsequent qPCR experiments, as seen in this gel image from the Agilent 2100 Bioanalyzer. The 18s and 28s bands are clearly visible for each sample, with little degradation or genomic DNA contamination.

RESULTS

Of 91 drug metabolism genes examined, expression of 7 was altered by at least one treatment condition. Genes that had elevated expression include those that metabolize promethazine and steroids (4 to 8-fold), many that reduce oxidation products, and one that reduces heavy metal exposure (>200-fold). Of the 91 DNA repair and general metabolism genes examined, expression of 14 was altered by at least one treatment condition. Note that in some cases (Apex2, Ung, Rad51c, etc.) expression was not strictly dose-dependent.



Data are normalized gene expression relative to a set of reference genes (ActB, Hprt1, Gstm1, Gad1, Gapdh); deviations from the dotted line at y=1 indicate changes from gene expression measured in control animals. The data shown here are those whose gene expression changed by > 1.5 fold with $p < 0.05$ (compared to control; indicated by *) in at least one of the treatment groups (low, high or both). Data from 74 genes with no significant expression changes are not shown.



Data are mean normalized gene expression relative to a set of reference genes (Rfc1, Ccnh, Atxn3, Actb); deviations from the dotted line at y=1 indicate changes from gene expression measured in control animals. The data shown here are those whose gene expression changed by > 1.5 fold with $p < 0.05$ (compared to control; indicated by *) in at least one of the treatment groups (low, high or both). Data from 77 genes with no significant expression changes are not shown.

CONCLUSION

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 both 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). Gene expression is regulated by redox state (which can be affected by radiation exposure) in addition to metal concentrations and glucocorticoids. Increases in metallothionein expression (and glutathionine reductase, GSR) 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 unchanged by all treatments, as were dozens of other genes.
The results of the DNA Repair Array showed a similar number of alterations in expression, with up 3-fold expression changes, consistent with other studies (Ding et al., 2005).

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.

FUTURE STUDIES

In the experiment discussed above, animals were sacrificed 4 hours after their last radiation exposure. This experiment has now been repeated to explore the time course of recovery after radiation exposure. We now have samples from animals at three additional time points: 24 hours, 7 days, and 13 days after their last treatment. Analyses of the tissues from the later time points are currently underway.

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