Probing Radiation Biomarkers in Human Urine by 1H NMR

C. Chen[1], D. J. Brenner[2], and T. R. Brown[1]
[1]Department of Radiology, Columbia University, New York, NY, United States, [2]Center for Radiological Research, Columbia University, New York, NY, United States

Introduction

To be prepared for large-scale radiological events, such as a nuclear leak or dirty bomb, a high throughput method to quickly determine who needs immediate medical intervention is crucial for triage support. Current technologies, based on cytogenetic or gene-expression approaches require qualified health professionals to draw peripheral blood through venipuncture. Such a procedure would clearly be a major bottleneck in a mass casualty incident because blood can be acquired from at most 15 to 25 individuals per hour. To address this potential problem we are investigating quantitative metabolomic signatures in urine which are both sensitive and specific to radiation exposure. To identify these urinary signatures, Nuclear Magnetic Resonance (NMR) spectroscopy was employed because it requires minimum sample preparation and a measurement time of only a few minutes, enabling us to analyze a large number of samples in relatively short time. In previous work[1] with a mouse model we have identified a dozen biomarkers in urine from radiation-exposed mice. The mouse model allowed us to understand the effect of key parameters such as dose, time post-exposure on the urinary biomarkers. To validate these biomarkers in humans, here we investigate urinary biomarkers associated with radiation exposure in acute leukemia patients undergoing a series of total body irradiation (TBI) treatments in preparation for a hematopoietic stem cell transplant at Memorial Sloan Kettering Cancer Center.

Materials and Methods

A total of 21 patients with acute leukemia were included in this study. Three urine samples were collected from each patient. During the first 24-hour period of the TBI treatments, patients received 3 fractional doses of 125 cGy. Urine samples were collected prior to the treatments (sample 1), approximately 6 hours after the first TBI (sample 2) and right after the third TBI (sample 3). To make NMR samples, aliquots of 320 μl urine samples were mixed with 320 μl phosphate buffer (0.2 M, pH 7.4) and any precipitate was removed by centrifugation. For each sample, 540 μl of supernatant was transferred to 5-mm NMR tube with 60 μl of sodium 3-trimethylsilyl-(2,3,3,3-D_4)-1-propionate (TSP)/D_2O/sodium azide solution (5 mM TSP and 1% wt/vol sodium azide in 100% D_2O). NMR spectra were acquired on a Bruker 500 MHz spectrometer. The excitation sculpting pulse[2] was used for water suppression. Spectra were analyzed by using standard Principle Component Analysis (PCA) method in a house-developed software *HiRes*.

Results and Discussion

PCA was applied to the whole set of NMR spectra. The first principle component PC1 represents the maximum variation in the dataset with each successive PC representing the next largest variation remaining in the dataset. Preliminary analysis indicated that there are several biomarkers associated with radiation exposure in human urine. The biomarkers include succinate, hippurate and several unassigned NMR peaks. The PC1 scores (essentially the relative peak area) from each urine sample for succinate and an unknown doublet at 2.21 ppm are shown in Figure 1 together with the median and standard error of the pretreatment samples and the two urines acquired subsequent to TBI. A t-test (unequal variances) indicates there is significant difference between the before-TBI and after-TBI samples ($p < 0.002$) but not between the two urines acquired after TBI. The median values of the datasets suggest that most patients initially had very low levels of these metabolites and following TBI the levels of these metabolites significantly increased. Of the five “outliers” among the pretreatment samples, three showed significantly increased levels after TBI while two did not. Both hippurate and succinate were also among the biomarkers identified in the previous mouse model study. Besides the unknown doublet at 2.21 ppm, we have also observed several other NMR peaks showing similar response to radiation (chemical shift in ppm and multiplicity: 1.14(d), 6.23(s), 6.63 (s), 6.8 (m)). To our knowledge, those peaks have not been reported in the literature and the assignments are unknown. We are now using multi-dimensional NMR to assign these peaks. These metabolites could potentially serve as novel radiation signatures in human urine.

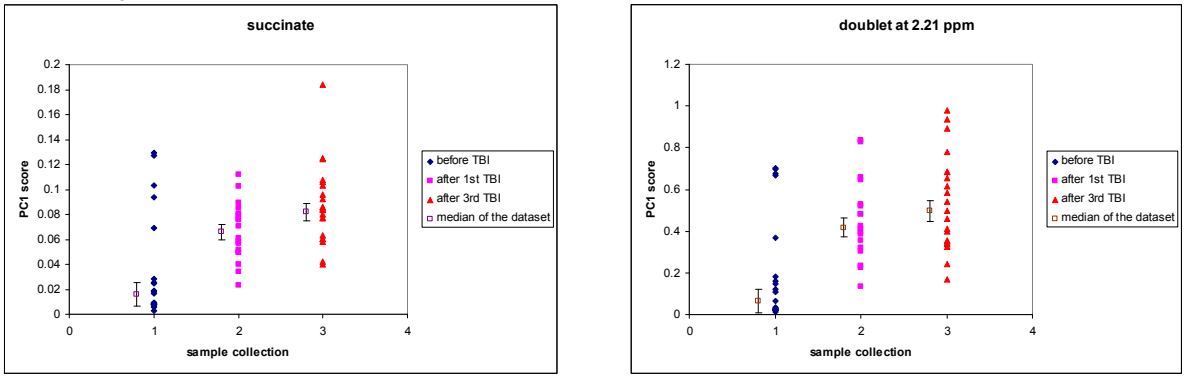

Figure 1 Representative plots of PC1 score representing succinate (left) and unknown doublet at 2.21 ppm (right).

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References

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