Evaluation of Fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl Ether ("compound A") Effects on Urine Protein Excretion in Rats Using Mass Spectrometry

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ABSTRACT
Fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether (FDVE or "compound A"), a haloketone degradant of the volatile anesthetic sevoflurane, is nephrotoxic in rats. FDVE bioactivation mediates the toxicity, but the molecular and cellular mechanisms of toxification are unknown. FDVE caused rapid and brisk changes in kidney gene expression, providing potential insights into mechanisms of toxicity, and potential biomarkers for nephrotoxicity[1]. Nevertheless, it is unknown whether gene expression changes are reflected in protein expression, or whether such tissue changes would be reflected in excreted urine proteins. This investigation was to evaluate FDVE effects on urine protein excretion using mass spectrometry and 8-plex iTRAQ reagents for relative quantitation. Results demonstrate that FDVE causes certain alterations in urine protein/peptide excretion. Multiple components were differentially expressed in a time-dependent manner.

INTRODUCTION
Fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether (FDVE or "compound A"), a haloketone degradant of the volatile anesthetic sevoflurane, is nephrotoxic in rats. FDVE bioactivation mediates the toxicity, but the molecular and cellular mechanisms of toxification are unknown. FDVE caused rapid and brisk changes in kidney gene expression, providing potential insights into mechanisms of toxicity, and potential biomarkers for nephrotoxicity[1]. Nevertheless, it is unknown whether gene expression changes are reflected in protein expression, or whether such tissue changes would be reflected in excreted urine proteins. This investigation was to evaluate FDVE effects on urine protein excretion using mass spectrometry and 8-plex iTRAQ Reagents for relative quantitation.

MATERIALS AND METHODS
After Animal Use Committee approval, Male Fisher 344 rats (250-300g) housed in individual metabolic cages received a single intraperitoneal injection of 0.25 mmol/kg FDVE, and all urine was collected daily for one week, as described previously[2]. Equal volumes of 6 replicate time points were pooled to create assay time point samples. 150 uL of each pool was brought to 2mls PBS. Each sample was filtered through a 0.22µm filter. 300 µL of each sample was diluted 100X with 0.1% acetic acid. The flow-through (albumin depleted) was desalted on a POROS R150 column. The protein was eluted and dried. Samples were then reconstituted in 1M TEAB. 50µg of each sample (from day 0 to day 7) was processed with the 8-plex iTRAQ® reagents according to manufacturer’s instructions. The iTRAQ® reagent labeled sample was then subjected to strong cation exchange chromatography separation and nine fractions were collected. Six of the fractions were analyzed using LC-MALDI on the 4800 MALDI TOF/TOF™ Analyzer (AB/MDS SCIEX). MS and MS/MS data were processed and searched against the IPI rat database (ipi.RAT.v3.26.fasta) using ProteinPilot™ Software (ABSciex).

RESULTS

Figure 1. Ion exchange chromatography fractionation

![Figure 1](image1)

Figure 2. Example of MSMS spectrum.

![Figure 2](image2)

iTRAQ labeled peptide fragmentation spectrum is shown.

ADLSGITEDAPLIT[78]
Alpha-1-antiproteinase precursor

Figure 3. Protein expression pie chart

![Figure 3](image3)

In this experiment there are 223 proteins identified from the FDVE treated rat urine samples. As would be expected, the majority of the identified proteins (79%) show no change in protein expression levels after FDVE treatment. The proteins which do show expression level changes are categorized into 4 groups: There are 11% showing downward trend, 1% upward, 4% up then down, and 1% down first and then up (see Figure 4 for examples).

Figure 4. Examples of rat urine protein expression level changes after FDVE treatment.

![Figure 4](image4)

There are generally 4 types of trends in the protein level changes: 1) protein level decreases after initial FDVE treatment and slowly levels off, 2) protein level increases after initial FDVE treatment and then reaches maximum at the end of sampling, 3) protein level increases initially after FDVE treatment then slowly decreases after reaches maximum, 4) protein level decreases initially after FDVE treatment and then slowly increases after then turns upward.

CONCLUSIONS
The results obtained demonstrate that FDVE causes certain alterations in urine protein/peptide excretion.

REFERENCES

ACKNOWLEDGEMENTS
This project was supported by NIH DK53765

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