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

Poster No. P27-T

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ABSTRACT

Fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether (FDVE or "compound A"), a haloalkene 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 quantitiation. Results demonstrate that FDVE causes certain alterations in urine protein/peptide excretion. Multiple components were differentially expressed in a time-dependent manner.

INTRODUCTION

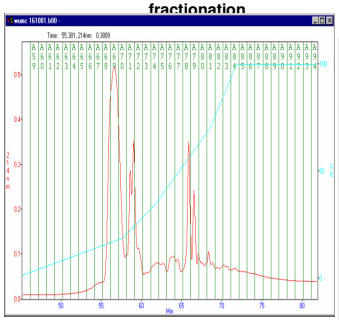
Fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl ether (FDVE or "compound A"), a haloalkene degradant of the volatile anesthetic sevoflurane, is nephrotoxic in rats. FDVE bioactivation mediates the toxicity, but the molecular and cellular mechanisms of toxification are unknown. FDVE caused rapid and 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 quantitiation.

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 applied to an anti-HSA column (POROS® Affinity Depletion Cartridges). 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. 50ug 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 chromatoghapy 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).

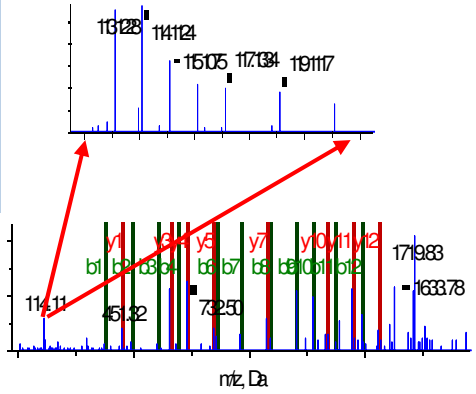
RESULTS

Figure 1. Ion exchange chromatography fractionation



fraction 1	A67-68
fraction 2	A69-70
fraction 3	A71-72
fraction 4	A73-75
fraction 5	A76-77
fraction 6	A78
fraction 7	A79
fraction 8	A80-81
fraction 9	A82-85

Figure 2. Example of MSMS spectrum.

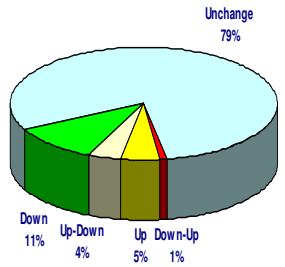


iTRAQ labeled peptide fragmentation spectrum is shown.

ADLSGITEDAPLK[IT8]

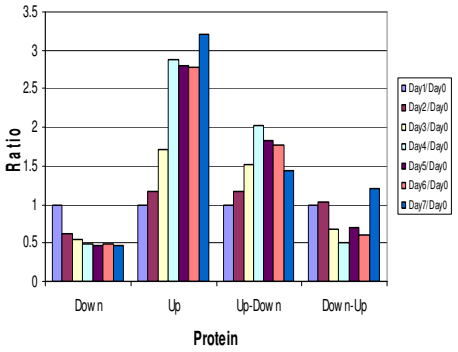
Alpha-1-antiproteinase precursor

Figure 3. Protein expression pie chart



In this experiment there are 231 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; 5% upward; 4% up first 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.



Example	Protein
Down	Haptoglobin precursor
Up	Protein-tyrosine phosphatase LC-PTP
Up-Down	Neuroligin-3 precursor
Down-Up	Alpha-2-macroglobulin precursor

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 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 slowly levels off then turns upward.

Table 1. List of identified proteins with expression level change after FDVE treatment

N	Accession #	Name	Trend
#6	sp|P17475|A1AT_RAT	Alpha-1-antiproteinase precursor	Up
#7	sp|P12346|TRFE_RAT	Serotransferrin precursor	Up
#8	sp|P20059|HEMO_RAT	Hemopexin precursor	Up
#12	sp|P24090|FETUA_RAT	Alpha-2-HS-glycoprotein precursor	Up
#13	sp|P08932|KNT2_RAT	T-kininogen 2 precursor	Up
#16	sp|P09006|CPI6_RAT	Contrapsin-like protease inhibitor 6 precursor	Up
#17	sp|Q63KY9|FETUB_RAT	Fetuin-B precursor	Up
#20	sp|P02767|TTHY_RAT	Transthyretin precursor	Up
#27	sp|P08866|HPT_RAT	Haptoglobin precursor	Up
#34	sp|P02625|PRVA_RAT	Parvalbumin alpha	Up
#38	sp|P04276|VTDB_RAT	Vitamin D-binding protein precursor	Up
#41	sp|P02770|ALBU_RAT	Serum albumin precursor	Up
#44	sp|P36953|AFAM_RAT	Afamin precursor	Up
#49	sp|P00502|GSTA1_RAT	Glutathione S-transferase alpha-1	Up
#50	sp|Q63556|SPA3M_RAT	Serine protease inhibitor A3M precursor	Up
#53	sp|P02764|A1AG_RAT	Alpha-1-acid glycoprotein precursor	Up
#60	sp|P20760|GCA_RAT	Ig gamma-2A chain C region	Up
#73	sp|P62898|CYC_RAT	Cytochrome c, somatic	Up
#78	sp|P01048|KNT1_RAT	T-kininogen 1 precursor	Up
#97	sp|P11232|THIO_RAT	Thioredoxin	Up
#105	sp|Q64268|HEP2_RAT	Heparin cofactor 2 precursor	Up
#120	sp|Q62671|EDD1_RAT	Ubiquitin-protein ligase EDD1	Up
#166	sp|Q8R515|ZHX1_RAT	Zinc fingers and homeoboxes protein 1	Up
#169	sp|Q5U2Y9|CF152_RAT	Protein C6orf152 homolog	Up
#66	sp|P13635|CERU_RAT	Ceruloplasmin precursor	Down
#67	sp|P31211|CBG_RAT	Corticosteroid-binding globulin precursor	Down
#71	sp|Q8JU19|NHERF_RAT	Ezrin-radixin-moesin-binding phosphoprotein 50	Down
#82	sp|P42854|REG3G_RAT	Regenerating islet-derived protein 3 gamma precursor	Down
#95	sp|Q8CGJ4|KIF5A_RAT	Kinesin heavy chain isoform 5A	Down
#171	sp|O70535|LIFR_RAT	Leukemia inhibitory factor receptor precursor	Down
#173	sp|O9Rt|J4|MYCC_RAT	Myocilin precursor	Down
#194	sp|P49454|PTN7_RAT	Tyrosine-protein phosphatase non-receptor type 7	Down
#204	sp|Q8K3M6|ERC2_RAT	ERC protein 2	Down
#209	sp|Q09900|ZEP2_RAT	Human immunodeficiency virus type I enhancer-binding protein 2 homolog	Down
#218	sp|Q8R500|MPN2_RAT	Transmembrane GTPase MPN2	Down
#107	sp|P51400|RED1_RAT	Double-stranded RNA-specific editase 1	Up-Down
#124	sp|P23909|SAMP_RAT	Serum amyloid P-component precursor	Up-Down
#140	sp|Q5BK85|TMED1_RAT	Transmembrane emp24 domain-containing protein 1 precursor	Up-Down
#175	sp|Q62886|NLGN3_RAT	Neuroligin-3 precursor	Up-Down
#217	sp|P07263|TRY2_RAT	Anionic trypsin-2 precursor	Up-Down
#226	sp|Q62824|EXOC4_RAT	Exocyst complex component 4	Up-Down
#87	sp|P02783|SVP2_RAT	Seminal vesicle protein 2 precursor	Up-Down
#145	sp|O88420|SODE_RAT	Extracellular superoxide dismutase [Cu-Zn] precursor	Up-Down
#84	sp|P13205|MAP1B_RAT	Microtubule-associated protein 1B	Up-Down
#127	sp|P06238|A2MG_RAT	Alpha-2-macroglobulin precursor	Down-Up
#228	sp|O63863|GBP2_RAT	Interferon-induced guanylate-binding protein 2	Down-Up

CONCLUSIONS

- The results obtained demonstrate/suggest? that FDVE causes certain alterations in urine protein/peptide excretion.
- Multiple components were differentially expressed in a time-dependent manner. Excretion of several endogenously excreted proteins was rapidly decreased by FDVE.
- Other proteins showed increased excretion following FDVE, and then gradually decreased to pre-dose levels.
- Excretion of a third set of proteins, minimally or not detectable in controls, was upregulated following FDVE.
- With the 8-plex iTRAQ® Reagents, relative protein excretion levels can be determined quantitatively, demonstrating that it is an ideal tool for time-course studies.

REFERENCES

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2. P. Sheffels, J. L. Schroeder, T. G. Altuntus, H. D. Liggitt, and E. D. Kharasch, Chem. Res. Toxicol. 17, 1177-1189 (2004)

ACKNOWLEDGEMENTS

This project was supported by NIH DK53765

TRADEMARKS/LICENSING