2008 International Conference on BioMedical Engineering and Informatics

Toxicity Study of Oral Vanadyl Sulfate by NMR-based Metabonomic

Xiaoxia Dai [a], Jiyang Dong [b*], Zhong Chen [a,b] and Qiqing Zhang [a]
[a] *Research Center of Biomedical Engineering, Xiamen University, Xiamen 361005, P R China;*
[b] *Department of Physics, Xiamen University, Xiamen, 361005, P. R. China;*
**E-mail: jydong@xmu.edu.cn*

Abstract

Vanadium compounds have been believed to be ideal drugs for diabetes biological therapy in future, but they suffer setback for the potential toxicity now. Toxicity study is necessary for vanadyl drugs development. This paper investigated the toxicity effects of vanadyl sulfate ($VOSO_4$) oral administration in male Wistar rats using ^1H NMR-based metabonomic analysis and clinical biochemical analysis. Rat urine were collected and their ^1H NMR spectra were acquired, and then subjected to multi-variable statistical analysis. Compared to control groups, urinary excretion of lactate, TMAO, creatinine, taurine and hippurate increased following $VOSO_4$ dosing, with concomitant decrease in the level of acetate and succinate. The dosed groups can be readily discriminated from the control groups by principle component analysis. The results showed that $VOSO_4$ can affect energy metabolism process, interrupted intestinal microfloral metabolism, and induced liver and kidney injury. NMR-based metabonomic can offer additional information to traditional clinical chemistry in the sensitivity and specificity of results obtained.

1. Introduction

The anti-diabetic effects of vanadium compounds, such as vanadyl sulfate ($VOSO_4$), are well-documented in both type 1 and type 2 diabetes in the past decades [1]. Unfortunately, the potential toxicity has been the major problem of vanadium treatment of diabetic animals. Vanadium salts may cause the decrease of food intaking, diarrhea and weight loss. Hematological and biochemical changes, reproductive and developmental toxicity, hepatotoxicity, nephrotoxicity, as well as a number of miscellaneous toxic effects have been also found in healthy rats and mice following vanadium exposure[1]. However, their toxic mechanism, especially the changes in metabolic profiling, is unclear.

Biochemical items detection is a conventional method for the toxicological analysis. But the conventional biochemical method is limited since only a small number of endogenous markers or key enzymes can be monitored routinely. Recently, NMR-based metabonomics has brought a new systematic analysis method to identify the site and possible mechanism of toxicity and offered much information of endogenous metabolites and their variation in pathological states [2]. The biochemical composition of biofluids, such as urine and serum, reflects the toxin- or disease-induced changes of organism. NMR spectroscopy of biofluids can present comprehensive biochemical profiles of low-molecular-weight metabolites reflecting the biochemical elects caused by xenobiotics. And then, through pattern recognition to classify NMR-derived data and multivariate statistical analysis methods, much information of endogenous metabolites and their variation in pathological states can be obtained. NMR-based metabonomics technology in biofluids has shown strong potential to the identification of biomarkers of toxicity and disease, monitoring sequential metabolic perturbations in biofluids and tissues following toxic insult [3,4,5,6].

In this study, $VOSO_4$, as an example of inorganic vanadium compounds, was investigated for the acute biochemical profiles in urine to further understand the biochemical effects of $VOSO_4$ using NMR-based metabonomic approaches. Significant changes of urinary metabolites were observed, which indicated that $VOSO_4$ treatment affected energy metabolism process, interrupted intestinal microfloral metabolism, and induced liver and kidney injury.

2. Materials and methods

2.1. Animal handling and sample collecting

$VOSO_4$ were purchased from Sigma company. Male Wistar rats were bred in the animal room of the Xiamen University Medical College Laboratory Animal Center (temperature: 25 ± 3°C; humidity: 50 ± 10%, 12 h light/12 h dark cycle). Fifteen Male Wistar

978-0-7695-3118-2/08 $25.00 © 2008 IEEE
DOI 10.1109/BMEI.2008.132

rats (weighing 200 ~ 250g) were divided into three groups (nD5) and housed individually in metabolism cages which allowed free access to food and water under controlled conditions. Each rat received an oral dose of either $VOSO_4$ (15mg/kg body weight), $VOSO_4$ (30 mg/kg body weight), or saline (0.9%, 10ml/ kg body weight). After 16 days, urine samples were collected overnight (24 h) in metabolism cages at ambient temperature. Then samples collected were stored frozen at -20°C until NMR analysis. For each blood sample, serum was obtained by centrifugation (3000 rpm, 15 min), and was retained frozen at −80°C until clinical chemistry analysis. One part of each liver and kidney samples was fixed in 10% formalin for histopathological assessment.

2.2. Clinical chemistry and histopathology

Serum samples were characterized on HITACHI 7170 analyzer to obtain clinical chemical parameters: blood urea nitrogen (Urea), creatinine (CRE), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), and uric acid (UA). Histological sections of $VOSO_4$-treated and control rats were prepared and stained with hematoxylin and eosin (H and E) and examined by light microscopy.

2.3. ^{1}H NMR spectroscopy of urine samples

200 microlitres of phosphate buffer (0.2M Na_2HPO_4/0.2M NaH_2PO_4, pH7.0, 100% D_2O) was added into 400 microlitres urine samples to minimize variations in the pH of the urine samples. 0.3mM DSS was used as an internal reference standard (d 0.00). The mixture was pipetted into 5 mm NMR tubes. ^{1}H NMR spectra were recorded on Varian Unity plus 500 MHz at 300K. Water signals were suppressed by presaturation pulse sequence. 128 FIDs were collected into 8K data points with relaxation delay 5s and flip angle 90 degree. The spectral width was 5 KHz.

2.4. Data preprocessing

All NMR spectra were phased, baseline corrected and integrated into region of 0.04 ppm width in the region of 0.5 ~ 9.5 ppm. The region of 4.5 ~ 5.0 ppm in the urine and serum spectra for all groups was excluded prior to pattern recognition analysis to refrain from the variation in water suppression efficiency. For urine spectra, the region containing urea (5.2 ~ 6.0 ppm) was also excluded to eliminate any cross-relaxation effects on the urea signal. The remaining spectral segments were scaled to total integrated area of each spectrum. The data set was mean-centered prior to principal components analysis (PCA). The NMR spectral processing, PCA of NMR data were performed using in house software.

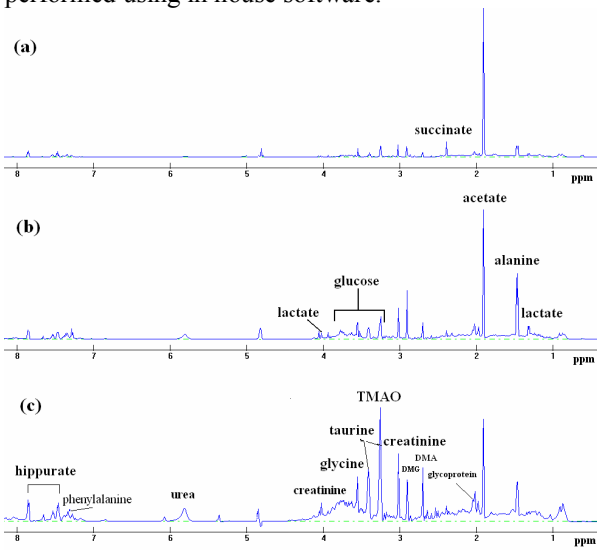


Fig.1 ^{1}H NMR spectra of urine of control and $VOSO_4$-dosed rats. (a) control rat; (b) dosage of 15 mg/kg; (c) dosage of 45 mg/kg.

3. Results and discussion

3.1. Spectral analysis of rat urine samples

A typical ^{1}H NMR spectra of urine sample of three groups were illustrated in Fig.1. The assignments of important metabolites in urine samples are presented in Table1. A number of alterations were identified in urine samples of $VOSO_4$-fed groups compared to control group, with a dose-dependent variability. Following $VOSO_4$ dosing, there were increases in alanine, TMAO, taurine, creatinine, DMA, DMG, glycine, urea, hippurate, phenylalanine and lactate, together with decreases in succinate, acetate. ^{1}H NMR urinalysis identified a rise in creatine and taurine. These are well-known urinary biomarkers of liver injury [7], and this pattern of changes in creatine and taurine is indicative of hepatic necrosis. Creatine is synthesized and metabolized in the liver, with the kidney providing the necessary synthetic precursor. Guanidinoacetate, formed in the kidney, is transported through the blood, undergoing methylation in the liver to form creatine, which enters the blood for use in peripheral tissues [8]. Therefore, the observed increases in liver, kidney, plasma, and urine suggest leakage from necrotic cells or upregulated creatine biosynthesis.

Damage to the proximal tubule is characterized by marked increases in the relative concentrations of

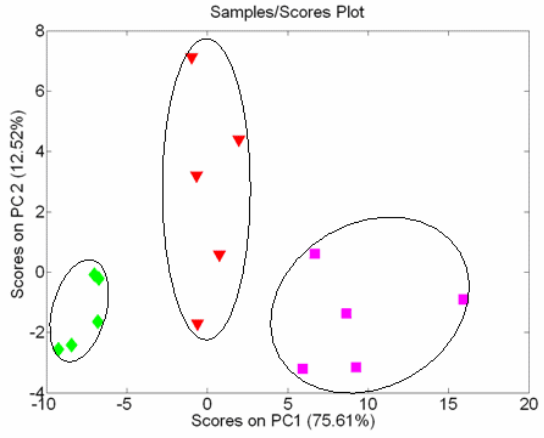
highly abundant metabolites in serum, due to a lack of reabsorption: glucose, lactate, alanine, and several other amino acids such as lysine, glutamine, glutamate, and valine. Glomerular toxicity hinders the filtration process and thus also produces the appearance of serum constituents in urine. Considerable increase of the concentration of amino acids (glycine and alanine) and glucose in the urine from VOSO4-treated rats indicated the decline of reabsorption ability of the renal tubule. The elevated urinary TMAO, DMG and DMA are the known markers of renal papillary lesion [9]. These findings implied the $VOSO_4$-induced renal papillary lesion which was also confirmed by the alterations of biochemical parameters, the elevated creatinine level in serum.

Table 1. Characteristic urinary metabolites of oral $VOSO_4$ treatment in Wistar rats.

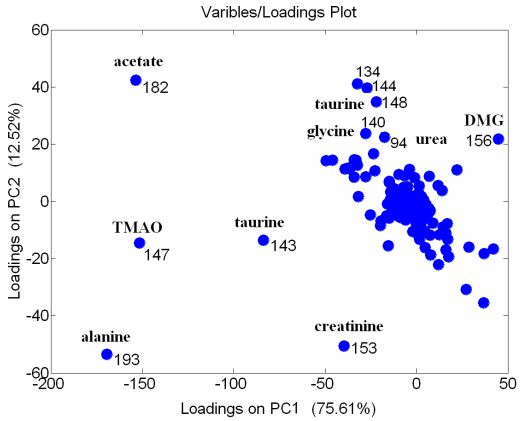
Metabolites	NMR chemical shift or *loading region(s)[a]	$VOSO_4$ dosed	
		15mg/kg	45mg/kg
lactate	1.33 (d)	↑	—
alanine	1.48(d)	↑↑	↑
acetate	1.93(s),4.08(q)	↓	↓↓
succinate	2.42 (s,)	↓	↓
glycoprotein	2.06	↑	↑↑
DMA	2.70 (s)	↑	↑↑
DMG	2.98 (s)	↑	↑
creatinine	4.05 (s), 3.05 (s)	↑	↑↑
TMAO	3.26 (s)	↑	↑↑
taurine	3.43 (t,), 3.26 (t)	↑	↑↑
glycine	3.54 (s)	↑	↑↑
glucose	3.5~4.0	↑	↑↑
urea	5.80(br)	↑	↑↑
phenylalanine	7.35(m),7.42(m)	—	↑
hippurate	7.73 (d), 7.64 (t), 7.55 (t)	—	↑

Note. a Letters in parentheses indicate the peak multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, complex multiplet; br, broad peak; "—", the concentration is the same as the control; "↑" and "↓", the amount is higher or lower; "↑↑" and "↓↓", the amount is much higher or much lower respectively, compared with that of the control

Decreases in acetate and an increase in lactate glycoprotein were evident in both low and high dose groups. These observations were suggestive of changes in carbohydrate and energy metabolism. This is supported by the progressive loss in body weight observed after dosing, likely to result from reduced feeding [10]. The different urinary excretion of hippurate, phenylalanine were also observed. These species are present in urine as products of intestinal microfloral metabolism. This implies that $VOSO_4$ affected the symbiotic gut microflora and the enzyme systems induced by diarrhea [11].



(a) PC1-PC2 score plot (■: control group;▼: 15 mg/kg; □: 45 mg/kg)



(b)PC1-PC2 loading plot.

Fig.2 PCA results of control and dosed group.

3.2. Multivariable Statistical Analysis

Principal components analysis (PCA) is a widely used dimension– reduction method, in which the NMR spectra can be reduced to a set of peak intensity descriptors, and used to detect toxicity and disease based on NMR biomarkers as well as identify similarities or differences between the samples from control and that from toxin-treated animals.

The dose-dependent toxicity of $VOSO_4$ was investigated by applying PCA to the data sets containing NMR spectra urine samples (Fig.2), The scores plots of the NMR spectra of urine samples

(Fig.2a) highlighted separation in the control group, low dose group and high dose group. This probably implied that the degree of energy metabolism disturbance dose induced by 15mg/kg body weight was different from that induced by 45mg/kg body weight. The loadings plot of the PCA result for urine samples (Fig.2b) shows those spectral regions that contribute the most to the separation of samples in scores plot. Based on the analysis of this plot, biomarkers could be identified. These included creatinine, taurine, TMAO, alanine, acetate, glycine, *etc.*. And hence, they may provide potential biomarkers for $VOSO_4$ toxicity response. Further studies are in progress to identify these metabolites as $VOSO_4$ toxicity biomarker.

3.3. Biochemical characteristics and histopathology

Compared to control group, the VOSO4-fed group showed external signs of toxicity after 3~5 day $VOSO_4$ administration, including diarrhea, decreased locomotor activity and general weakness. A significant decrease in weight gain was observed in both low dose group (15mg/kg body weight) and high dose group (45mg/kg body weight). These showed gastrointestinal toxic effects were main toxicity of $VOSO_4$. $VOSO_4$ entered digestive tract and induced diarrhea by restraining involved enzymic activity and promoting gastrointestinal movement. Reduction in group mean body weight following dosing with $VOSO_4$ may be mainly induced by diarrhea which resulted in serious dehydration.

However, no substantial variations were observed in the biochemical parameter besides the elevation of creatine in $VOSO_4$-fed group. Liver and kidney histopathology examinations were taken to check the changes in tissues. No microscopic abnormalities were observed in any of the animals killed after 16-day dosing (data not shown). These may imply $VOSO_4$ only induce slight alterations of liver and kidney function in clinical chemistry and histopathology. However, metabonomic approach may offer subtler information prior to conventional biochemical methods, which is more meaningful in early toxicological assessment.

4. Conclusion

NMR-based metabonomic has been used to assess toxicological effects of $VOSO_4$. Significant changes of creatinine, taurine, TMAO, DMA, DMG, alanine, acetate, glycine and lactate indicated that VOSO4 treatment affected energy metabolism process, interrupted intestinal microfloral metabolism, and induced liver, kidney injury. The greater magnitude of toxic response in the rat was highlighted in the metabolic trajectories of the scores from PCA of the urinary data. But no substantial variations were obtained in blood biochemical analysis and histopathological examination, which showed that NMR-based metabonomic can offer advantages to traditional clinical chemistry in the sensitivity and specificity of results obtained. And it is was proved to be of value in resolving the mechanistic complexity of drug toxicity as well as the benefits of frontloading this approach in drug safety evaluation and biomarker discovery.

5. Acknowledgment

The project was partly supported by the National Natural Science Foundation of China (No.10605019), the Natural Science Foundation of Fujian Province of China (No.T0750015), and the Key Project of Health and Science and Technology of Xiamen City of China (Wkj2005-2-019).

6. References

[1] Srivastava, A.K, Mehdi, M.Z, "Insulino-mimetic and anti-diabetic effects of vanadium compounds", *DIABETIC MEDICINE,* 2005,22 (1),pp. 2-13.

[2] Nicholson, J..K., Connelly, J, and Lindon, J.C., "Metabonomics: aplatform for studying drug toxicity and gene function", *Nat. Rev., Drug Discov*, 2002,1, pp.153–161.

[3] Lindon, J.C, Nicholson, J.K, and Holmes E, "Contemporary issues in toxicology, the role of metabonomics in toxicology and its evaluation by the COMET project", *Toxicology and applied pharcology,* 2003, 187, pp.137-146.

[4] Wen JB, Xiao X, Dai XX, *et al*. "Data Normalization for Diabetes II Metabonomics Analysis", *The 1st International Conference on Bioinformatics and Biomedical Engineering*, Wuhan, China, July 6-8, Vol.2 of 2: 694-697, 2007.

[5] Xu L, Dong JY, Dai XX, *et al.* "Non-negative Matrix Factorization for Diabetes II Metabolic Profiling Analysis", *The 1st International Conference on Bioinformatics and Biomedical Engineering, Wuhan, China,* July 6-8, Vol.1 of 2: 651-653, 2007.

[6] Dong JY, Xu L, Cao HT, Dai XX, *et al.* "A new data processing method for metabonomic and its application in a study of diabetes". *Chinese Journal of Magnetic Resonance,* vol.24(4): 381-393, 2007 (In Chinese).

[7] Holmes, E., Nicholls, A.W., and Lindon, J.C., "Chemometric models for toxicity classification based on

NMR spectra of biofluids", *Chem. Res.Toxico,*. 2000, 13, pp.471-478.

[8] Timbrell, J. A., Waterfield, C. J., and Draper, R. P, "Use of urinary taurine and creatine as biomarkers of organ dysfunction and metabolic perturbations", *Comput. Haematol. Int,* 1995,5, pp.112-119.

[9] Nicholson, J.K., Wilson, I.D, "Understanding Fglobal_ systems biology: metabonomics and the continuum of metabolism", *Nat Rev Drug Discov*, 2003,2(8),pp. 668– 676.

[10] Connor,S.C., Wu, W., and Sweatman, B.C., "Effects of feeding and body weight loss on the 1H-NMR-based urine metabolic profiles of male Wistar Han rats:implications for biomarker discovery". *Biomarkers,*2004,9(2), pp.156– 179.

[11] Williams, R. E., Eyton-Jones, H. W., and Farnworth, M. J., "Effect of intestinal microflora on the urinary metabolic profile of rats: a 1H-nuclear magnetic resonance spectroscopy study", *Xenobiotica,* 2002, 32(9), pp.783– 794.