Study on Vanadyl Sulfate Toxicity Using NMR-based Metabonomics

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Introduction

The anti-diabetic effects of vanadium compounds, *e.g.*, vanadyl sulfate ($VOSO_4$), are well-documented in both type 1 and type 2 diabetes in the past decades [1]. But their potential toxicity becomes a major setback being anti-diabetic drugs for human subjects. It is important to know the toxicity and the toxic mechanism of the vanadium treatment of diabetic animals. 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 help identify the site and possible mechanism of toxicity and offered much information of endogenous metabolites and their variation in pathological states [2]. In this study, $VOSO_4$ as an example of inorganic vanadium compounds, was investigated the acute biochemical profiles in urine to further understand the biochemical effects of $VOSO_4$ using NMR-based metabonomic approaches.

Materials and Methods

In this study, $VOSO_4$ were purchased from Sigma company. Male Wistar rats were bred in the animal room (temperature: 25 ± 3 °C; humidity: 50 ± 10%, 12 h light/12 h dark cycle). Fifteen Male Wistar rats (weighing 200 ~ 250 g) 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 $VOSO_4$ (15 mg/kg body weight), $VOSO_4$ (30 mg/kg body weight), or saline (0.9%, 10 ml/kg body weight). After 16 days, urine samples were collected overnight (24 h) in metabolism cages at ambient temperature. Following collection samples were stored frozen at -20 □ until analysis. Blood biochemical examinations were also carried out. ^{1}H NMR spectra of urine samples were acquired on Varian Unity plus 500 MHz at temperature 300 K. All NMR spectra were phased, baseline corrected and integrated into region of 0.04 ppm width in region of 0.5 ~ 9.5 ppm. The region of 4.5 ~ 5.0 ppm was excluded prior to pattern recognition analysis to refrain from the variation in water suppression efficiency. The remaining spectral segments were scaled to total integrated area of each spectrum.

Table 1. Endogenous urinary metabolites responsible for $VOSO_4$ dosed animals.

Metabolites	$VOSO_4$ dose	
	15mg/kg	45mg/kg
Lactate	↑	—
Alanine	↑↑	↑
Acetate	↓	↓↓
Succinate	↓	↓
Glycoprotein	↑	↑↑
DMA	↑	↑↑
DMG	↑	↑
Creatinine	↑	↑↑
TMAO	↑	↑↑
Taurine	↑	↑↑
Glycine	↑	↑↑
Glucose	↑	↑↑
Urea	↑	↑↑
Phenylalanine	—	↑
Hippurate	—	↑

Note: "—" denote that the concentration is the same as the control; "↑" and "↓" denote the amount is higher or lower; "↑↑" and "↓↓" denote the amount is much higher or much lower, respectively, compared with that of the control.

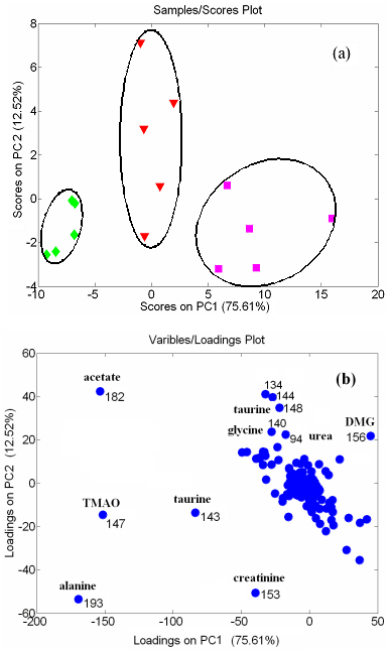

Fig.1 PCA result of control group and dosed group. (a) PC1-PC2 score plot, (b) PC1-PC2 loading plots. (KEY:■:control group;▼:15 mg/kg; □: 45 mg/kg)

Results and Discussion

Compared to control group, the $VOSO_4$ fed groups showed external signs of gastrointestinal 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 $VOSO_4$-fed groups. However, no obvious alterations were observed in biochemical parameters besides the increase of creatine. Several typical ^{1}H NMR spectra of urine samples of three groups were obtained, the endogenous urinary metabolites responsible for the separation of samples from control and $VOSO_4$ dosed animals are presented in Table 1. A number of alterations were identified in urine samples of $VOSO_4$-fed groups with a dose-dependent variability. Changes in creatine and taurine is indicative of hepatic necrosis, and considerable increase of amino acids (glycine and alanine) and glucose in the urine from $VOSO_4$-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. These findings implied the $VOSO_4$ induced liver injury and renal papillary lesion [3]. 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 [4]. The different urinary excretions of hippurate and 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 [5]. The dose-dependent toxicity of $VOSO_4$ was investigated by applying PCA to the data sets containing NMR spectra of urine samples (Fig.1), The scores plots (Fig.1a) 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 (Fig.1b) shows those spectral regions that contribute the most to the separation of samples in scores plot. As potential biomarkers for $VOSO_4$ toxicity response, creatinine, taurine, TMAO, alanine, acetate, and glycine could be identified. Further studies are in progress to identify these metabolites as $VOSO_4$ toxicity biomarker.

Conclusions

The results showed that NMR-based metabonomics has advantages compared to traditional clinical chemistry in the sensitivity and specificity of results obtained. Significant changes of urinary metabolites indicated that $VOSO_4$ 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. NMR-based metabonomics 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.

Acknowledgment

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