Genomics Proteomics Bioinformatics 12 (2014) 120–126

Genomics Proteomics Bioinformatics

www.elsevier.com/locate/gpb
www.sciencedirect.com

ORIGINAL RESEARCH

Effects of Three Commonly-used Diuretics on the Urinary Proteome

Xundou Li, Mindi Zhao, Menglin Li, Lulu Jia, Youhe Gao *

National Key Laboratory of Medical Molecular Biology, Department of Physiology and Pathophysiology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100005, China

Received 11 October 2013; revised 8 December 2013; accepted 12 December 2013
Available online 4 February 2014

Handled by Siqi Liu

KEYWORDS
Urinary proteome;
Diuretics;
Biomarkers

Abstract Biomarker is the measurable change associated with a physiological or pathophysiological process. Unlike blood which has mechanisms to keep the internal environment homeostatic, urine is more likely to reflect changes of the body. As a result, urine is likely to be a better biomarker source than blood. However, since the urinary proteome is affected by many factors, including diuretics, careful evaluation of those effects is necessary if urinary proteomics is used for biomarker discovery. Here, we evaluated the effects of three commonly-used diuretics (furosemide, F; hydrochlorothiazide, H; and spirolactone, S) on the urinary proteome in rats. Urine samples were collected before and after intragastric administration of diuretics at therapeutic doses and the proteomes were analyzed using label-free liquid chromatography–tandem mass spectrometry (LC–MS/MS). Based on the criteria of $P \leqslant 0.05$, a fold change $\geqslant 2$, a spectral count $\geqslant 5$, and false positive rate (FDR) $\leqslant 1\%$, 14 proteins (seven for F, five for H, and two for S) were identified by Progenesis LC–MS. The human orthologs of most of these 14 proteins are stable in the healthy human urinary proteome, and ten of them are reported as disease biomarkers. Thus, our results suggest that the effects of diuretics deserve more attention in future urinary protein biomarker studies. Moreover, the distinct effects of diuretics on the urinary proteome may provide clues to the mechanisms of diuretics.

Introduction

Biomarker is the measurable change associated with a physiological or pathophysiological process. Unlike blood is homeostatic, urine is more likely to reflect changes of the body. In other words, urine is likely to be a better biomarker source than blood [1]. Saving urinary protein samples on the membrane can facilitate storage of samples in large numbers and help to speed up the biomarker research in urine proteome [2]. Furthermore, compared to plasma, urine can be collected

* Corresponding author.
E-mail: gaoyouhe@pumc.edu.cn (Gao Y).

Peer review under responsibility of Beijing Institute of Genomics, Chinese Academy of Sciences and Genetics Society of China.

Production and hosting by Elsevier

1672-0229/$ - see front matter © 2014 Beijing Institute of Genomics, Chinese Academy of Sciences and Genetics Society of China. Production and hosting by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gpb.2013.12.002

continuously and noninvasively. Second, the urinary proteome directly reflects the conditions of the urinary system. Third, it can also reflect the physiological status of the whole human body [3]. These advantages make the urinary proteome a suitable source for disease biomarker discovery.

To date, many urinary biomarkers have been reported in a variety of diseases [3], such as various chronic and acute renal injuries [4], bladder cancer [5], prostate cancer [6] and coronary artery disease [7]. However, studies focusing on the urinary protein biomarker discovery still face certain challenges. A major issue is that the urinary proteomic pattern of an individual may be affected by multiple factors, such as gender, age, diet [8], medication, daily activities, exercises [9,10], smoking [11], stress, menstrual cycle and other physiological variations. Environmental factors including temperature and humidity may also affect the urinary proteome. Therefore, these factors should be taken into consideration in the urinary biomarker research.

Effects of some factors, such as gender, age, daily activity and environmental conditions, have been investigated previously [12–14]. However, effects of some other factors, especially medication, are difficult to examine, since the regular therapeutic process of patients should not be disturbed during urine collection. Therefore, influences of medications on the urinary proteome should be taken into account during data analysis and interpretation.

Diuretics are among the most commonly used medications. They are used to induce negative fluid and sodium balances in a variety of clinical situations, including hypertension, heart failure, renal failure, nephritic syndrome, and cirrhosis [15]. However, it remains unclear whether and how diuretics affect the urinary proteome, which hampers the urinary biomarker discovery for those diseases.

In this study, we examined the effects of furosemide, hydrochlorothiazide, and spirolactone on the urinary proteome using label-free quantitative proteomics [16]. These drugs represent thiazide diuretics, loop diuretics, and potassium-sparing diuretics, respectively, which are the three types of commonly used diuretics with different modes of action [17]. The rat urine samples were collected before and after the diuretics were administered, digested using the filter aided proteome preparation (FASP) method [16], and analyzed with a high-speed TripleTOF 5600 system. Progenesis LC–MS was used to quantify the urinary proteins.

Results and discussion

The effects of diuretics on rat urine volumes

In order to evaluate the direct effects of diuretics on rats, urine samples were collected before and 1, 3, or 5 days after the diuretics were administered intragastrically. As shown in Table S1, the rat urinary volumes increased significantly (~2–3 folds, $P < 0.05$) after the administration of furosemide (F) and hydrochlorothiazide (H), especially within the first 8 h after lavage. This period is the effective time of the diuretics. However, there is no significant increase in urine output ($P > 0.05$), after the rats were administered with spirolactone (S), probably due to the fact that spirolactone is not an efficient diuretic on its own and usually is applied in combination with other diuretics.

SDS–PAGE analysis of the urine samples

As a first step of the sample analysis, the urine samples collected on different days were separated by SDS–PAGE. As shown in **Figure 1A**, the protein patterns of the urine samples in the H group changed only modestly among those obtained before and 1, 3 and 5 days after the diuretic administration. However, for the F and S groups, there were some significant changes among samples obtained at different time points, especially those on Day 3 after gavage for the F group (**Figure 1B**) and Day 1 for the S group (**Figure 1C**). Therefore, normal urine samples, Day 3 for the F and H group and Day 1 for the S group were further analyzed using 1D-LC–MS/MS.

The changes of the rat urine proteome after diuretic administration

To investigate the changes of the urine proteome after diuretic administration, a total of 18 LC–MS/MS runs of urine samples from three different rats in each diuretic group were analyzed. The 18 datasets were analyzed using Progenesis LC–MS and Mascot Daemon. The false discovery rate (FDR) was adjusted to be less than 1%. As a result, we identified 331, 302, and 325 proteins in the F, S and H group, respectively (Tables S2–S4). The identified peptides are listed in Table S5. All the Supplementary materials can be found in the urinary protein biomarker database [3] (http://122.70.220.102/biomarker).

The coefficients of variation (CVs) for each of the three levels of sample variation—before gavage, after gavage, and between these two conditions—were calculated. As shown in **Figure 2**, the CV values of the samples after gavage were slightly higher than those before gavage (median CV values: 0.25 *vs*. 0.34 for F group; 0.35 *vs*. 0.39 for S group and 0.28 *vs*. 0.31 for H group), probably because rats respond differentially to the diuretics. In contrast, the CV values of the samples for between before and after gavage and for after gavage (median CV of F group is 0.45; median CV of S group is 0.55) are significantly higher ($P < 0.05$), suggesting that furosemide and spirolactone can change the urine proteome. However, the CV values of H-Between (median CV is 0.33) were not changed significantly, indicating that hydrochlorothiazide has no discernable effects on the rat urine proteome at this dosage.

The effects of different diuretics on the urinary proteome

Using the label-free quantification by the Progenesis LC–MS software, we identified seven (five up-regulated and two down-regulated) (**Table 1**), five (one up-regulated and four down-regulated) (**Table 2**) and two (one up-regulated and one down-regulated) proteins with significantly changed expression in all three rats in the F, S and H groups, respectively, according to the criteria: $P \leqslant 0.05$, a fold change $\geqslant 2$ and a spectral count $\geqslant 5$. Five of the seven proteins in the F group and all of the five proteins in the S group have been reported to be disease biomarkers. For example, haptoglobin is a candidate biomarker for patients with bladder cancers, acute

Download English Version:

https://daneshyari.com/en/article/2822418

Download Persian Version:

https://daneshyari.com/article/2822418

Daneshyari.com