Utilizing Purified β-Glucuronidase and Arylsulfatase to Accurately Quantitate Metabolites in Human Urine

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ABSTRACT

Several metabolites are known to be glucuronidated and sulfated in the human body. Monitoring the glycosides with the glucuronidated and sulfated metabolites creates six additional messengers to monitor on tandem mass spectrometry, which can limit the total number of metabolites being monitored. In addition, the current standards are expensive and often not available. The purpose of this study was to report the use of purified β-glucuronidase (IMCSzyme®) and reconstituent arylsulfatase that effectively cleaves glucuronides and sulfates at neutral pH with no detectable CVF activity or enzyme activity. The results demonstrated significant increase in detection of individual cortisol metabolites on tandem mass spectrometry before and after cleaving glucuronides and sulfates with the purified enzymes. Among cortisol metabolites, glucuronidated and sulfated α,β-tetrahydrocortisol (αβTHF) and cortisol were reported to be the most abundant metabolites on tandem mass spectrometry before and after cleaving glucuronides and sulfates with the purified enzymes. Among cortisol metabolites, glucuronidated and sulfated α,β-tetrahydrocortisol (αβTHF) and cortisone were reported to be the most abundant metabolites on tandem mass spectrometry before and after cleaving glucuronides and sulfates with the purified enzymes.

INTRODUCTION

The study and quantitation of metabolites can give insights to health and disease. This poster focuses on cortisol metabolites as an example. Cortisol is quantitatively the major glucocorticoid product of the adrenal cortex. The deficiency of adrenocortical secretion is found in Addison’s disease leading to hypoglycemia. On the other hand, the overproduction can give rise to hypercortisolism. Quantitative measurement of cortisol in both serum and urine are widely done by immunoassays; however, the limitations are cross-reactivity and low sensitivity. Recently, gas chromatography or liquid chromatography coupled to tandem mass spectrometry, abbreviated GC or LC-MS/MS, respectively, have proven to have specificity and sensitivity (1). This feasibility relates reliable methods and enables the detection of individual cortisol metabolite.

Metabolites of cortisol are illustrated in Figure 1. In human and cow, 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) inactivates cortisol (C) to cortisone (E). While in human urine, 11β-HSD1 hydrolyzes the dehydrogenase to cortisone and 11β-glucuronidase to glucuronide (1). Moreover, acting hydrolysis reactions are performed with the enzymes of the former (C, E) and latter two (C, E) and glucuronidated metabolites. Medical diagnosis is generally performed by metabolite monitoring. It is noted that these compounds are excreted in urine as conjugates and reports mention the use of β-glucuronidase and arylsulfatase to deconjugate prior to MS analysis (2, 5–7).

Some of the metabolites are absent or appear at low concentrations as well. Hence, we have used a combination of non-commercial human urine samples to commercially available human urine samples to test different systems. Several different metabolites in the route solution (11) (Figure 2). Therefore, the generally modified β-glucuronidase and arylsulfatase enzymes were used to test different products. The last test was using trastuzumab (trastuzumab) in urine since the conjugated analytes were not commercially available.

RESULTS

Since cortisol (C), tetrahydrocortisol, and allo-α,β-tetrahydrocortisol have the same major transition, it is important to separate them on LC gradient. Figure 3 illustrates ion chromatograms for cortisol analysis and their corresponding retention times.

Table 1. Multiple Reaction Monitoring Transition

Table 2. Negative Ion Multiple Reaction Monitoring Transition

Figure 4. Hypodensity in urine urine sample containing the highest amount of αβTHF for determining hydrolysis efficiency versus incubation time. The hydrolysis percentage is calculated in the maximum amount as 100%, (p) cortisol, (c) cortisone, (a) α,β-tetrahydrocortisol, (h) with tetrahydrocortisol, and (p) pentoterhydrocortisol. A complete hydrolysis indicating a plateau recovery was reached after 10 minutes of incubation using both enzymes. IMCSzyme and IMCS arylsulfatase. (αβTHF-Metabolites with ICMSzyme and IMCS arylsulfatase).

Figure 5. Average calculated amount of cortisol, cortisone, allo-tetrahydrocortisol, tetrahydrocortisol, and tetrahydrocortisol in five different urine samples with and without αβ-thrombin hydrolysis using IMCSzyme and IMCS arylsulfatase. Error bars indicate ± 1 standard deviation.

CONCLUSION

The sample hydrolysis step allows the detection of total cortisol metabolites in urine, which include both free and conjugated forms. Several reports pointed to the ratio between αβTHF and THF in the amounts (αβTHF+THF) as an indication to monitor for both clinical and biological relevance of human diseases (1, 2). Moreover, IMCSzyme or IMCS arylsulfatase allows to deconjugate before analysis. As mentioned earlier, 11β-HSD2 inactivates the αβTHF in the conversion between the biological active form and inactive form. It is important to mention that the conjugated metabolites to accurately determine this ratio. Figure 4 shows that after hydrolyzing using IMCSzyme and IMCS arylsulfatase in m/z 411.213, the ratio is 0.97 using IMCSzyme or IMCS mix, while it is only 0.98 using crude extract from H. pomatia. The ratio calculation could vastly affect the interpretation of αβTHF quantitation, leading to incorrect conclusions to disease pathologically. In Figure 5, the measured F and P were improved 2.4-fold. THF and αβTHF were improved by 60–70 fold. Finally, αβTHF was undetectable before hydrolysis but this level went up to 4,700 at 90 minutes after hydrolysis. The total metabolite detection result from sample hydrolysis elim- inates the need of costly conjugated standards for hormone-monitoring.

REFERENCES


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