Steroids 123 (2017) 43–49

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Absolute quantification of cholesteryl esters using liquid chromatography-tandem mass spectrometry uncovers novel diagnostic potential of urinary sediment

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ARTICLE INFO

Keywords:
Cholesteryl ester
LC–MS/MS
Stable isotope labeling
Quantitative analysis
Urinary sediment
Renal disease

ABSTRACT

Background: Urine has been utilized as a source of biomarkers in renal disease. However, urinary lipids have not attracted much attention so far. Here we studied urinary cholesteryl ester (CE) and its relevance in renal disease.
Methods: Quantitative analysis of CE molecular species in serum, urinary supernatant, and urinary sediment from patients with renal disease ($N = 64$) and non-renal disease ($N = 23$) was carried out using liquid chromatography-tandem mass spectrometry (LC–MS/MS) and deuterated CEs as internal standards.
Results: Validation study showed good precision and accuracy of LC–MS/MS. Many CE species were detected in the urinary sediment and supernatant in the renal disease group, whereas only a few CE species were detected in the other group. In the renal disease group, the sum of the concentrations of all CE species showed a significant correlation between the sediment and the supernatant from urinary samples ($r = 0.876$, $p < 0.001$); however, the composition of CEs was significantly different between them. Further, the composition of CEs of the supernatant was similar to that of the serum.
Conclusions: Our LC–MS/MS analysis uncovered a distinct CE profile in urinary sediment from patients with renal disease, suggesting a possible contribution of CEs in urothelial cells to the development of renal disease.

1. Introduction

Urine has major advantages as a source of biomarkers, not only because it can be obtained in large volumes noninvasively but also contains much information about the plasma [1]. Chronic kidney disease (CKD) is a worldwide public health concern, and urine is generally employed for a diagnosis of CKD as per international criteria [2]. As proteinuria alone has limitations as a biomarker of CKD, other markers, such as Cystatin C, NAG, NGAL, and KIM-1, are also used in clinical tests [3]. However, these markers are less specific because CKD shares several biomarkers with acute kidney injury, and patients with CKD usually have comorbidities [4]. Therefore, a single biomarker is insufficient for a diagnosis of CKD, and an evaluation based on multiple biomarkers can reveal a specific clinical condition with sufficient accuracy [4,5]. Combining information from various sources, we could understand the difference in patients with complex renal disease.

Despite many studies on urinary proteomics, peptidomics, and metabolomics having already documented the discovery of biomarker candidates for CKD [5–7], there is strikingly limited information about urinary lipids. Rockwell et al. noted that there is no literature on the molecular species composition of urinary lipids [8]. Recently, much attention has been focused on renal lipid metabolism, for example, lipid accumulation in glomerular and tubular cells from patients with diabetic nephropathy was reported [9]. In urinary sediment of patients with nephrotic syndrome, diabetic nephropathy, and Fabry disease, lipid components such as oval fat bodies and fatty casts were occasionally observed [10–12]. Although examination of urinary sediment might provide useful information that assists with a disease diagnosis, an analysis of urinary sediment has not been developed intensively [13]. It is not surprising, that there is no information about lipid composition in urinary sediment because most urinalysis was performed using whole urine or urinary supernatant. We consider that novel biomarker candidates could be identified by comparing the lipid composition in urinary sediment and supernatant. Here, we report a

Abbreviations: CE, cholesteryl ester; CKD, chronic kidney disease; NAG, *N*-acetyl-β-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; KIM-1, kidney injury molecule-1; MS, mass spectrometry; SRM, selected reaction monitoring; LOQ, limit of quantification

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http://dx.doi.org/10.1016/j.steroids.2017.05.003
Received 15 March 2017; Received in revised form 20 April 2017; Accepted 3 May 2017
Available online 11 May 2017
0039-128X/

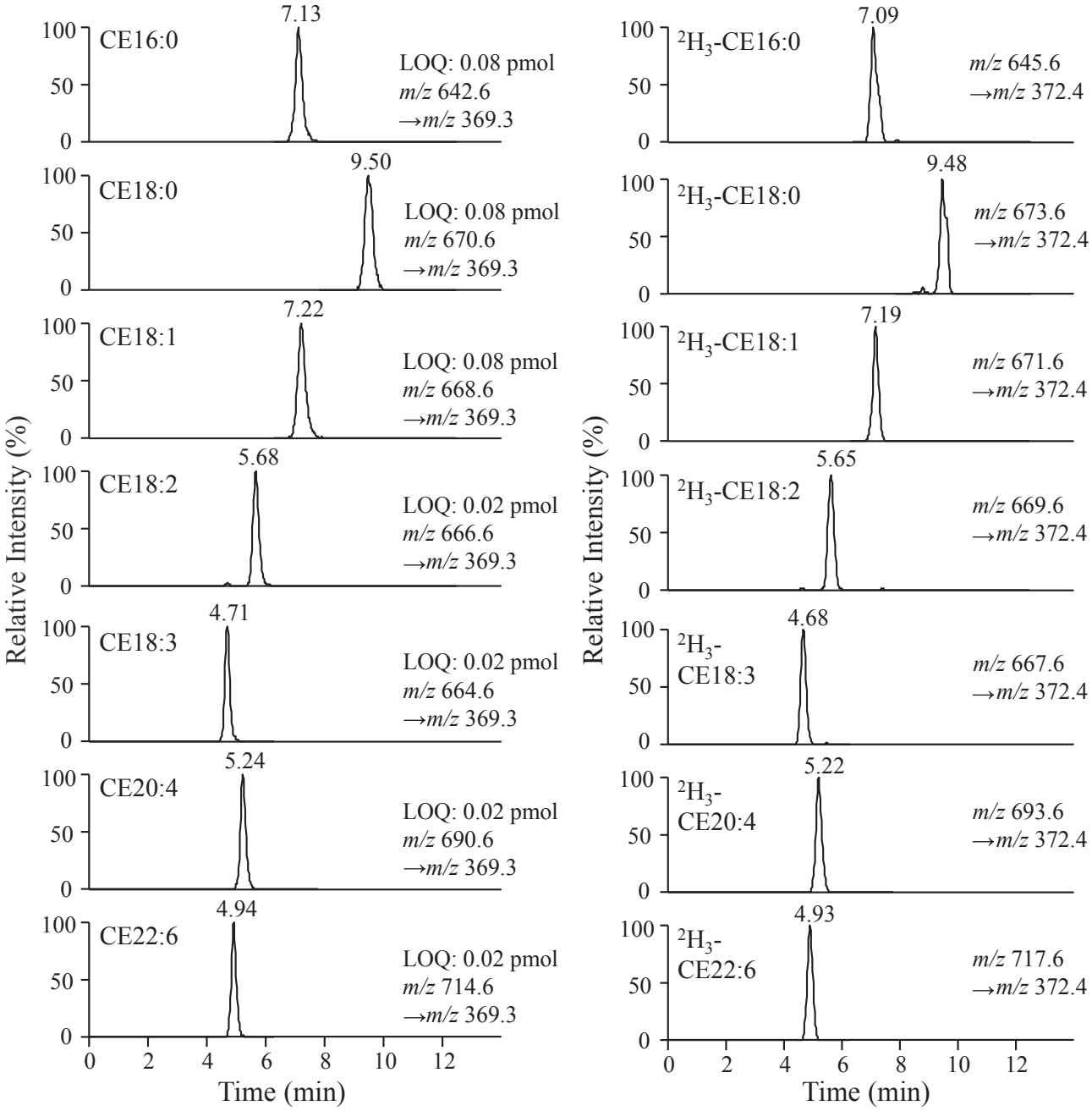

Fig. 1. SRM chromatograms of relative CE species and internal standards.

comparative study on urinary cholesteryl ester (CE) in patients with renal disease.

A primary component of high-density lipoprotein (HDL) and low-density lipoprotein (LDL), CE with its high plasma concentration is known as an important risk factor for cardiovascular disease [14]. Moreover, urinary cholesterol levels in patients with glomerular disease were higher than those in healthy subjects [15], but molecular species information were not available due to the limitations of enzymatic methods. Therefore, we decided to adopt tandem mass spectrometry (MS/MS), whereby CE molecular species were analyzed in various samples [16–20] to quantify CE species in urine.

On MS analysis, ion suppression and enhancement often occurred by matrix components of biological samples, and these problems can affect accuracy and precision of analytical results [21–23]. Therefore, internal standards are essential in quantitative MS analysis, and a stable isotope-labeled analog is an ideal substance for internal standard because it should have identical chemical characteristics with the analyte [21,23]. However, isotopically labeled CEs are not commercially available. Instead, an odd-chain saturated fatty acid ester was often used as an internal standard in previous studies [16,17,19]. A limitation with this concept is that small amounts of odd-chain fatty acids are present in human plasma [24].

Herein, we report absolute quantitation of CE species by using LC–MS/MS and deuterated CEs that we synthesized as described previously [25]. This combination is expected to have higher accuracy and precision than conventional methods. Then, we applied this method to clinical specimens such as serum, urinary supernatant, and urinary sediment from patients with renal disease. This study was undertaken to clarify the relevance of urinary CE in renal disease.