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Determination of creatinine-related molecules in saliva by reversed-phase liquid chromatography with tandem mass spectrometry and the evaluation of hemodialysis in chronic kidney disease patients

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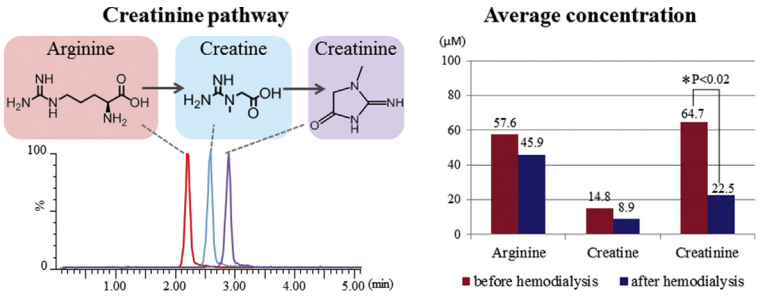
HIGHLIGHTS

- Creatinine and related molecules were determined by UPLC-MS/MS.
- Fast separation (<4 min) was performed using an HS-F5 column.
- The proposed method was used for creatinine determination in saliva.
- The concentration of creatinine in the saliva and serum correlated.
- The creatinine level in saliva of CKD patients was decreased after hemodialysis.

GRAPHICAL ABSTRACT



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ABSTRACT

The serum concentrations of creatinine (Cre) and urea are used for the determination of the renal function. However, the use of blood is not always suitable due to the invasive, hygienic and infection problems during its sample collection and handling. In contrast, saliva is relatively clean and the samples can be quickly and noninvasively collected and easily stored. Therefore, the simultaneous determination of Arginine (Arg), creatine (Cr) and Cre in the saliva of chronic kidney disease (CKD) patients was performed by UPLC-ESI-MS/MS together with the saliva of healthy volunteers. The evaluation of hemodialysis of CKD patients was also carried out by the determinations before and after the dialysis. An HS-F5 column was used for the simultaneous determination of Arg, Cr and Cre in the saliva. These molecules were rapidly separated within 4 min and sensitively determined by the multiple reaction monitoring (MRM) of the precursor ion $[M+H]^+$ → product ions (*m/z* 175.1 → 70.1 for Arg; *m/z* 132.0 → 44.1 for Cr; *m/z* 114.0 → 44.1 for Cre). The concentration of Cre in the CKD patients was higher than that in the healthy persons. The concentrations of Cre in the saliva of the patients before hemodialysis were moderately correlated with the serum Cre concentrations (R^2 = 0.661). Furthermore, the concentration in the saliva obviously decreased after hemodialysis (before 0.73 mg/dL, after 0.25 mg/dL; $p < 0.02$). Thus, the proposed detection method using saliva by

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UPLC-MS/MS is useful for the evaluation of the renal function in CKD patients. The present method offers a new option for monitoring the hemodialysis of CKD patients.

1. Introduction

The kidneys play a critical role in maintaining metabolic homeostasis in a living body. The renal hypofunction causes various diseases such as cardiovascular onset [1–3]. Therefore, the detection of renal hypofunction is highly desired. A few subjective symptoms are observed during the deterioration of the renal function. For instance, the serum concentrations of creatine (Cr) and creatinine (Cre) are used for the determination of the renal function. The concentration of blood urea nitrogen (BUN) and Cre generally increase after a serious kidney problem. Therefore, a more accurate glomerular filtration rate (GFR) or approximation of creatinine clearance (Ccr) is measured when a renal disease is suspected [4,5]. Consequently, the determination of Cr and Cre is a key step for the evaluation of the renal function.

Chronic kidney disease (CKD) refers to conditions in which the renal function is irreversibly reduced by a variety of chronic diseases such as diabetes and hypertension. Since deterioration of the kidney function is a high risk factor for cardiovascular disease, the condition has been attracting attention in recent years. The diagnosis of CKD is commonly done by creatinine-based estimated GFR (eGFR), which is calculated from the patient's serum Cre. However, the use of blood is not always suitable due to the invasive, hygienic and infection problems during its sample collection and handling. The sampling is usually performed by the medical staff, such as a doctor and nurse, while self-sampling is difficult. In contrast, saliva is relatively clean and the samples can be quickly and noninvasively collected and easily stored [6,7]. Therefore, we focused on saliva as an alternative to blood and as a new diagnostic material. Because saliva is derived from plasma, the components are believed to reflect the blood levels [8–10].

The Cre is produced by the non-enzymatic dehydration of Cr which is derived from the enzyme reaction of arginine (Arg) and glycine (Fig. 1). The determination of these molecules is usually performed by HPLC after derivatization with a suitable reagent such as 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) [11]. The separation efficiency and detection sensitivity increase due to the introduction of a highly sensitive fluorescence moiety and the increased hydrophobicity based on the derivatization. Although the labeling method is excellent for the determination of Arg and Cr, the procedure is time-consuming. Furthermore, the labeling of Cre, which has no reactive functional group in its structure, is generally difficult. The Cre in blood and urine has been successfully determined by several methods such as ELISA [12], colorimetry using a molecularly imprinted polymer (MIP) membrane [13], LC-UV [14], LC-MS and GC–MS [15]. A review paper related to a Cre biosensor has also been published by Mohabbati-Kalejahi et al. [16]. With respect to the saliva analysis, Xing et al. [17] reported the determination of Cre by capillary electrophoresis with electrochemical detection (CE-ED). However, the simultaneous determination of Arg, Cr and Cre in saliva has not been reported till now. Based on these backgrounds, the simultaneous determination of Arg, Cr and Cre in the saliva of CKD patients was performed by UPLC-ESI-MS/MS together with the saliva of healthy volunteers. Furthermore, the evaluation of the hemodialysis of CKD patients was carried out by the determination of Arg, Cr and Cre before and after the dialysis.

2. Experimental

2.1. Materials and chemicals

Creatine (Cr), creatinine (Cre), and arginine (Arg) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Arginine-2,3,3,4,4,5,5-d_7 (Arg-d_7), creatine-methyl-d_3 (Cr-d_3), and creatinine-methyl-d_3 (Cre-d_3), used as the internal standard (I.S.), were purchased from C/D/N Isotopes (Quebec, Canada). Ammonium acetate (AA) of analytical grade was from Wako Pure Chemicals (Osaka, Japan). Acetonitrile (ACN), methanol (MeOH) and formic acid (FA) of LC-MS grade were obtained from Kanto Chemicals (Tokyo, Japan). Deionized and distilled water (PURELAB flex 3, ERUGA, Tokyo, Japan) was used throughout the study. All other reagents and solvents were of analytical reagent grade.

One-mM solutions of Cre and the related compounds were prepared in water as the stock solutions. The working solutions were prepared by the sequential dilutions with water.

2.2. UPLC-ESI-MS/MS

The LC-ESI-MS/MS analysis was performed using an ACQUITY ultraperformance liquid chromatograph (UPLC I-class) connected to a Xevo™ TQ-S triple quadrupole mass spectrometer (Waters, Milford, MA). Six different columns, i.e., ACQUITY UPLC BEH C18 (1.7 μm, 100 × 2.1 mm i.d.; Waters), TCI Dual ODS-CX15 (3.0 μm, 100 × 2.0 mm i.d.; Tokyo Kasei, Tokyo, Japan), Discovery HS-F5 (3.0 μm, 150 × 2.1 mm i.d.; Spelco), Amide-80 (3.0 μm, 150 × 2.0 mm i.d.; Tosoh), ZIC-HILIC (3.5 μm, 150 × 2.1 mm i.d.; Merck) and ZIC-cHILIC (3.0 μm, 150 × 2.1 mm i.d.; Merck), were used at the flow rate of 0.2 mL/min and at 40 °C. The separation conditions are shown in Table 1. The Arg, Cr and Cre were analyzed by UPLC-ESI-MS/MS in the positive-ion mode. The MS detection

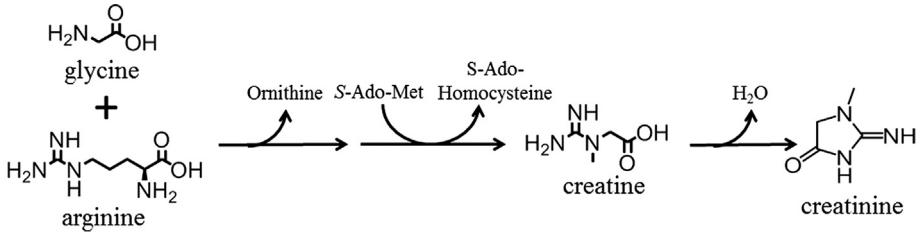

Fig. 1. Pathway of Cre and related molecules.