Toxicology Letters 248 (2016) 52–60

Contents lists available at ScienceDirect

Toxicology Letters

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

Characterization of urinary metabolites as biomarkers of colistin-induced nephrotoxicity in rats by a liquid chromatography/mass spectrometry-based metabolomics approach

Eun Sook Jeong[a], Gabin Kim[a], Kyoung-Sik Moon[b], Yong-Bum Kim[b], Jung-Hwa Oh[b], Ho-Sook Kim[a], Jayoung Jeong[c], Jae-Gook Shin[a], Dong Hyun Kim[a,*]

[a] Department of Pharmacology and Pharmacogenomics Research center, Inje University, College of Medicine, Bokjiro 75, Busanjin-Gu, Busan 614-735 South Korea
[b] Korea Institute of Toxicology, 141 Gajeong-ro, Yuseong-gu, Daejeon, 305-343, South Korea
[c] Ministry of Food and Drug Safety, Osong-eup, Heungdeok-gu, Cheongju-si, Chungcheongbuk-do 361-951, South Korea

HIGHLIGHTS

- Urinary metabolic profiles were assessed in rat after treatment of colistin methane sulfonate.
- Urinary amino acids and creatine levels were significantly increased whereas levels of purine metabolites and metabolites related to the tricarboxylic acid cycle were reduced.
- Concentrations of colistin in kidney were higher than those in liver.
- Colistin may cause tubular damage, leading to increased urinary levels of metabolites.

ARTICLE INFO

Article history:
Received 24 December 2015
Received in revised form 24 February 2016
Accepted 29 February 2016
Available online 3 March 2016

Keywords:
Colistin
Nephrotoxicity
Metabolomics
Urinary amino acid
Creatine

ABSTRACT

Colistin is a polypeptide antibiotic that effectively treats infections caused by multidrug-resistant Gram-negative bacteria, but its clinical use is limited due to nephrotoxicity. The purpose of the present study was to identify biomarkers of colistin-induced nephrotoxicity and to further characterize the mechanisms underlying this process by analyzing urinary metabolites using untargeted metabolomic approach. Rats receiving intraperitoneal administration of colistin sodium methanesulfonate (CMS) (25 or 50 mg/kg) exhibited histopathological changes in the kidney and increased blood urea nitrogen levels. Additionally, the levels of phenylalanine, tryptophan, and tyrosine in the urine of the CMS-treated group were significantly higher than those of the control group, suggesting that colistin caused proximal tubular damage. Urinary acetylcarnitine and butyrylcarnitine levels also increased after CMS treatment, but the levels of purine metabolites and metabolites related to the tricarboxylic acid cycle were reduced. The most significant increase in the CMS-treated groups was observed in creatine levels. CMS-induced selective nephrotoxicity may be attributed to relatively high tissue concentrations of colistin in the kidney. Taken together, our results indicate that high levels of colistin in the kidney caused perturbations in the tricarboxylic acid cycle, amino acid metabolism, creatine metabolism, and purine metabolism and ultimately led to kidney injury.

1. Introduction

Colistin, also known as polymyxin E, is a peptide antibiotic that was developed several decades ago, but its clinical use has been limited because it can cause nephrotoxicity. However, the use of colistin has recently increased due to its utilization as a last-line therapy for the treatment of infections caused by multidrug-resistant Gram-negative bacteria such as *Pseudomonas aeruginosa,*

* Corresponding author at: Department of Pharmacology and Pharmacogenomics Research center, Inje University, College of Medicine, Bokjiro 75, Busanjin-Gu, Busan 614-735 Korea.
E-mail address: dhkim@inje.ac.kr (D.H. Kim).

http://dx.doi.org/10.1016/j.toxlet.2016.02.018
0378-4274/

Acinetobacter baumannii, and *Klebsiella pneumoniae* (Landman et al., 2008; Li et al., 2006). The nephrotoxic effects of colistin are dose-dependent, and a reversal of symptoms is observed when use of the drug is discontinued (Wallace et al., 2008). The reported rates of colistin-induced nephrotoxicity in clinical use vary widely and range from 10% to 45% (Falagas et al., 2010; Pogue et al., 2011).

Although the clinical predictors of colistin-induced nephrotoxicity have been identified (Kim et al., 2009; Kwon et al., 2010), the physiological mechanisms underlying this toxic process are not yet fully understood. Serum clinical chemistry analyses and histopathological examinations in experimental animals have shown that colistin induces renal toxicity but not hepatotoxicity in a dose-dependent manner (Ghlissi et al., 2013). Recently, colistin-induced nephropathy has been more extensively investigated, and several possible mechanisms for this organ-specific toxicity have been suggested. For example, increased cell membrane permeability, cell swelling, and lysis are associated with an increased influx of cations, anions, and water and may underlie colistin-induced nephrotoxicity and apoptosis (Yousef et al., 2012). Rhabdomyolysis may be another nephrotoxic mechanism (Ozkan et al., 2012), and the role of oxidative stress has also been studied (Dai et al., 2014; Dezoti Fonseca et al., 2012; Eadon et al., 2013; Ozyilmaz et al., 2011).

Antioxidants such as ascorbic acid, melatonin, natural astaxanthin, and vitamin E are protective against colistin-induced nephrotoxicity (Ghlissi et al., 2014; Yousef et al., 2011, 2012). Additionally, oxidative stress seems to play a key role in the nephrotoxicity caused by many types of drugs, including gentamicin, vancomycin, and cisplatin, and it has been shown that reactive oxygen species that are generated by the mitochondria initiate renal cell apoptosis which, in turn, ultimately leads to renal dysfunction (Elyasi et al., 2012; Maldonado et al., 2003; Santos et al., 2007). Thus, it has been suggested that apoptosis may be involved in the pathogenesis of nephropathy that is secondary to colistin (Lopez-Novoa et al., 2011). For example, the kidneys of rats treated with colistin for 7 days exhibited oxidative damage and caspase-mediated apoptosis (Ozkan et al., 2013). Furthermore, there are significant increases in urinary proteins such as Kim-1, alpha glutathione S-transferase (a-GST), and *N*-acetylgalactosaminidase when rats are intravenously injected with colistin for up to 7 days (Keirstead et al., 2014). Although changes in the metabolites of rats have been examined in established models of gentamicin-, aristolochic acid-, cisplatin-, and puromycin-induced nephrotoxicity, small molecular urinary biomarkers that may be used to predict the development of colistin-induced kidney toxicity have yet to be fully characterized.

Thus, the present study utilized a liquid chromatography/mass spectrometry (LC/MS)-based metabolomics approach to evaluate changes in endogenous metabolites found in rat urine after 1- and 7-day colistin sodium methanesulfonate (CMS) treatment regimens. CMS is a clinically used prodrug of colistin (Bergen et al., 2006). The primary aims of this study were to identify potential biomarkers that may aid in the prediction of colistin-induced nephrotoxicity and to investigate the toxic threshold and toxicity biomarkers associated with this process using conventional clinical chemistry and histopathological techniques.

2. Materials and methods

2.1. Chemicals and reagents

CMS was obtained from Samchundang Pharmaceutical Co. (Korea). Colistin sulfate (100% purity, according to the provider's specification) and polymyxin B sulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cholic acid-d5, as an internal standard was purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). High performance liquid chromatography (HPLC)-grade acetonitrile and methanol were purchased from Burdick & Jackson (Muskegon, MI, USA). All other chemicals used in this study were of the highest grade commercially available.

2.2. Animal experiments and sample collection

All experimental procedures were approved by the Korea Institute of Toxicology Institutional Review Board. Male Sprague–Dawley rats aged 6–7 weeks and with body weights of 200 and 220 g were obtained from Orient Bio (Seongnam, Korea) and acclimated for 1 week prior to the experiment. The animal laboratory was maintained at 23 °C ± 3 °C and 50% ± 10% relative humidity with a 12-h light and dark cycle. The rats had free access to a diet of commercial food pellets and tap water. The experimental groups received intraperitoneal doses of CMS (25 mg/kg [n = 8] or 50 mg/kg [n = 8]) either a single time or once daily for 7 consecutive days, while the control group (n = 8) was administered the same volume of vehicle (saline solution). CMS doses used in this study were determined on the basis of recommended human intravenous CMS dose (2.5–5 mg/kg). The human equivalent doses were calculated to be 15–31 mg/kg for rats. Taking into account the above dose ranges and intraperitoneal route, the administered dose of CMS was determined to be 25 and 50 mg/kg in this study. After receiving the last dose of CMS, the rats were placed in individual metabolic cages and fasted overnight, and urine samples were collected over a 24-hour period. Following this 24-hour period, all rats were anesthetized with inhaled isoflurane. Following collection, the urine samples were frozen and stored at −80 °C until analysis. Blood samples were collected via the posterior vena cava, stored at room temperature for 2 h, and then centrifuged to obtain serum samples. Subsequently, a clinical chemistry analysis was performed on the serum samples using an automated chemistry analyzer (Toshiba Co., Tokyo, Japan). Creatinine clearance rate (CL_{CR}) was determined from the following formula: (urine creatinine [mg/ml] × urine volume collected for 24 h period [ml])/(serum creatinine [mg/ml] × 1440 [min]). Additionally, the liver and kidney of each rat were removed, washed three times with a saline solution, and then stored at −80 °C for the quantitation of colistin. For the histological analysis, small blocks of the left kidney and left lobe of the liver were immediately fixed in 10% formalin after removal. These samples were embedded in paraffin and sliced into 5-μm sections that were stained using hematoxylin and eosin.

2.3. Quantitation of colistin in the serum and tissues

A quantitative analysis of colistin in the serum and tissues was performed using LC/MS/MS. To analyze the serum samples, a 50-μl aliquot of acidic methanol (0.125 N HCl) containing an internal standard (1.0 μg/mL of polymyxin B) was combined with a 50-μl aliquot of rat serum. After vortex-mixing the solution for 5 min and centrifuging it (13,200 rpm for 10 min at 4 °C), a 2-μl aliquot of the supernatant was injected into the LC/MS/MS system. To analyze the tissue samples, approximately 100 mg of tissue was homogenized with three volumes of a cold water/methanol solution (50:50) containing 0.125 N HCl and polymyxin B (1.0 μg/ml) using an ultrasonicator (10 s × three cycles). The solution was centrifuged (13,200 rpm for 10 min at 4 °C) and the supernatant was removed. After 10-fold dilution of the supernatants with HPLC initial buffer, a 2-μl aliquot of the supernatant was then injected into an API 5500 LC/MS/MS system (Applied Biosystems; Foster City, CA, USA) coupled with a 1100 series HPLC system (Agilent). The separation was performed using an Atlantis dC18 column (50 × 2.1 mm, 3 μm) with an isocratic elution of