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# The urinary excretion of metformin, ceftizoxime and ofloxacin in high serum creatinine rats: Can creatinine predict renal tubular elimination?

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ABSTRACT

The renal excretion of creatinine and most drugs are the net result of glomerular filtration and tubular secretion, and their tubular secretions are mediated by individual transporters. Thus, we hypothesized that the increase of serum creatinine (SCr) levels attributing to inhibiting tubular transporters but not glomerular filtration rate (GFR) could be used to evaluate the tubular excretion of drugs mediated by identical or partial overlap transporter with creatinine. In this work, we firstly developed the creatinine excretion inhibition model with normal GFR by competitively inhibiting tubular transporters, and investigated the renal excretion of metformin, ceftizoxime and ofloxacin *in vivo* and *in vitro*. The results showed that the 24-hour urinary excretion of metformin and ceftizoxime in model rats were decreased by 25% and 17% compared to that in control rats, respectively. The uptake amount and urinary excretion of metformin and ceftizoxime could be inhibited by creatinine in renal cortical slices and isolated kidney perfusion. However, the urinary excretion of ofloxacin was not affected by high SCr. These results showed that the inhibition of tubular creatinine transporters by high SCr resulted to the decrease of urinary excretion of metformin and ceftizoxime, but not ofloxacin, which implied that the increase of SCr could also be used to evaluate the tubular excretion of drugs mediated by identical or partial overlap transporter with creatinine in normal GFR rats.

## 1. Introduction

Creatinine is mainly produced from creatine in skeletal muscle, then transported into blood, and finally excreted unchanged by the kidney [1,2]. In clinical practices, Serum creatinine (SCr) level and creatinine clearance ($CL_{CR}$) are routinely used as the primary approach for estimating glomerular function and making dosage adjustments for drugs excreted by the kidney principally [3]. However, increasing evidences suggested that there could be a poor correlation between estimated $CL_{CR}$ and renal clearance of drugs in different clinical settings and $CL_{CR}$ couldn't provide an accurate indication for some drugs in renal clearance [4–9]. For example, renal elimination of fluconazole was markedly decreased in people with HIV infection, despite “normal” calculated $CL_{CR}$ [6,8].

Previous studies suggested that that creatinine was primarily excreted by glomerular filtration apart from negligible tubular secretion. However, in recent years, it was reported that renal elimination of creatinine also underwent active tubular secretion, which could account for 10%–40% of total clearance [3,10]. Shen et al. suggested that the clearance of creatinine also could include reabsorption process [10]. Thus, $CL_{CR}$ could depend on not only glomerular filtration, but also tubular secretion. As described above, researches further found that people with HIV infection could alter renal tubular transport independent of the loss of glomerular filtration, and thus the $CL_{CR}$ of HIV patient was within the ‘normal’ range. Based on this, we have reasons to believe that there are different pathways in the renal tubular clearance of creatinine and fluconazole.

As we all know, the renal tubular transport pathways are performed by two distinctively localized transporters: basolateral uptake transporters and apical efflux transporters. Although a large number of transporters in the renal tubular could mediate a broad spectrum of substrates, they also have substrate specificity. The renal tubular transport of creatinine and drugs could be mediated by the same or different transporters. Therefore, we considered that there could be a lot of secretion pathways which were comprised of transporters in renal tubular. If the renal tubular secretion pathway of drugs could be identically or partially overlapped with creatinine, renal tubular secretion ability of creatinine could be in accordance with that of those

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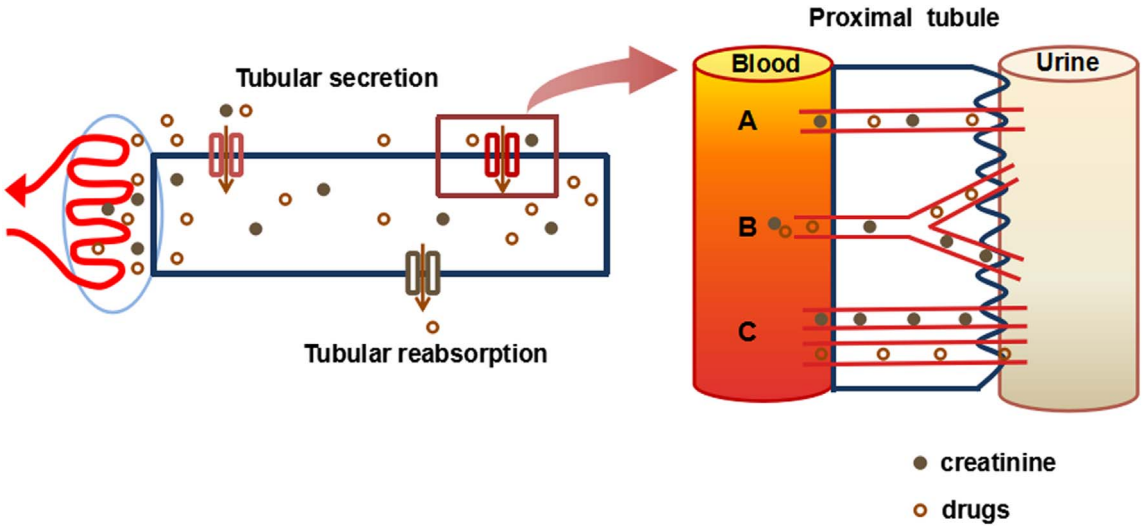


**Fig. 1.** Renal excretion pathway of creatinine and drugs. There are many renal secretion pathways which are comprised of different transporters in renal tubular: (A) Tubular secretion pathway of drugs is identical with that of creatinine. (B) Tubular secretion pathway of drugs is partially overlapped with that of creatinine. (C) Tubular secretion pathway of drugs is different from that of creatinine.

drugs and thus it could be used to predict the tubular secretion of those drugs. Conversely, if the renal tubular secretion pathway of drugs could be different from creatinine, renal tubular secretion ability of creatinine could be unrelated to that of those drugs and it couldn't be used to predict the tubular secretion ability of those drugs (Shown in Fig. 1).

In previous reports, creatinine had been shown to be a substrate for a cation transport pathway comprising with the basolaterally expressed organic cation transporter 2 (OCT2) and the apically expressed multidrug and toxin extrusion (MATE) transporters 1 (MATE1) and 2-K (MATE2-K) [11–13]. Eisner et al. further elucidated that organic anion transport system (OATs) also played an important role in the tubular secretion of creatinine [14]. Vallon et al. confirmed that OAT1 and OAT3 were involved in the renal creatinine secretion in mice [15]. To investigate the ability of creatinine predicting the tubular secretion of drugs, drugs which had the same or different tubular secretion pathways of creatinine were chosen. And the pharmacokinetics of metformin (secreted by transporters rOCT2 and rMATEs), ceftizoxime (secreted by transporters rOAT3 and multidrug resistance protein 4) and organic anion drug ofloxacin (not secreted by OCT2, rOAT1 or rOAT3) [16–19] were investigated in model rats. The protein expression of renal secretion transporters was investigated by Western blot technology, and renal tubular uptake and renal tubular secretion of drugs were evaluated by renal cortical slices uptake and isolated kidney perfusion experiment.

## 2. Materials and methods

### 2.1. Animals

Wistar male rats weighing 180–220 g were obtained from the Laboratory Animal Center of Lanzhou University (Gansu, China). Rats were housed in plastic cages and maintained at 25 °C under 12 h–12 h alternating light-dark cycle with free access to food and water. Rats were fasted overnight (12 h) with free access to water prior to the studies. All studies were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### 2.2. Chemicals and reagents

Metformin (purity 97%), ofloxacin (purity 98%), ceftizoxime (purity 98%), creatinine (purity 98%) and inulin were purchased from Sigma-Aldrich (St Louis, MO, USA). Norethindrone, caffeine, salicylic acid (internal standard) were purchased from the Nation Institute for the Control of pharmaceutical and Biological Products (Beijing, China). Methanol was of HPLC-grade (Fisher Scientific, NJ, USA). All other reagents used were of an analytical grade.

### 2.3. Study design

Study 1 aimed to establish a tubular creatinine transporter inhibition model with normal glomerular filtration. Normal rats were intravenously administered with high dosage creatinine (720 mg/kg) or an equal volume of saline *via* caudal vein. All blood samples (200 μL) were collected at 10, 20, 30, 60, 90, 120, 180, 240, 360, 480 and 600 min post drug administration from femoral artery. The serum concentration of creatinine was determined by LC-MS/MS (Agilent 6460, Agilent Technology Inc., CA, USA). In addition, in order to evaluate effect of high creatinine on glomerular filtration, the urinary excretion of inulin was investigated by isolated kidney perfusion.

Study 2 aimed to investigate the pharmacokinetics of metformin, ceftizoximeand ofloxacinin model rats. Rats in control and model group were intravenously administered with metformin (25 mg/kg), ceftizoxime (300 mg/kg) or ofloxacin (80 mg/kg). Blood samples (250 μL) were collected at 5, 10, 20, 30, 45, 60, 120, 240, 360, 480, and 600 min after drug administration. In addition, rats were treated the same as above, and urine samples were collected during the interval of 0–2 h, 2–4 h, 4–6 h, 6–8 h, 8–10 h and 10–24 h. The concentration of metformin, ofloxacin and ceftizoxime in plasma and urine was determined by HPLC (LC-20A, Shimadzu Inc., Kyoto, Japan), and the pharmacokinetic parameters were calculated using DAS 2.0 program.

Study 3 aimed to test the effect of high SCr level on protein expression of the transporters which were responsible for the tubular transport of metformin and ceftizoxime. The expression of OCT2/MATE1 and OAT3/MRP4 was investigated by Western blotting technology at 12 h after administration of high dosage creatinine (720 mg/kg) or an equal volume of saline.

Study 4 aimed to evaluate the effect of creatinine on the renal tubular transport of metformin, ofloxacin or ceftizoxime. Rat kidney slices uptake and isolated kidney perfusion experiments were performed in this study.

### 2.4. Sample preparation and analysis

After blood samples were centrifuged at 18000*g* for 10 min, and