Proximal Tubular Secretion of Creatinine by Organic Cation Transporter OCT2 in Cancer Patients

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Abstract

Purpose: Knowledge of transporters responsible for the renal secretion of creatinine is key to a proper interpretation of serum creatinine and/or creatinine clearance as markers of renal function in cancer patients receiving chemotherapeutic agents.

Experimental Design: Creatinine transport was studied in transfected HEK293 cells in vitro and in wild-type mice and age-matched organic cation transporter 1 and 2–deficient [Oct1/2(–/–)] mice ex vivo and in vivo. Clinical pharmacogenetic and transport inhibition studies were done in two separate cohorts of cancer patients.

Results: Compared with wild-type mice, creatinine clearance was significantly impaired in Oct1/2(–/–) mice. Furthermore, creatinine inhibited organic cation transport in freshly isolated proximal tubules from wild-type mice and humans, but not in those from Oct1/2(–/–) mice. In a genetic association analysis (n = 590), several polymorphisms around the OCT2/SLC22A2 gene locus, including rs2504954 (P = 0.000873), were significantly associated with age-adjusted creatinine levels. Furthermore, in cancer patients (n = 68), the OCT2 substrate cisplatin caused an acute elevation of serum creatinine (P = 0.0083), consistent with inhibition of an elimination pathway.

Conclusions: Collectively, this study shows that OCT2 plays a decisive role in the renal secretion of creatinine. This process can be inhibited by OCT2 substrates, which impair the usefulness of creatinine as a marker of renal function. Clin Cancer Res; 18(4); 1101–8. ©2012 AACR.

Introduction

The estimation of kidney function is a central requirement in the diagnosis of renal diseases in medical practice. Normally, kidney function is assessed by measuring glomerular filtration rate (GFR). For this reason, an exact determination of GFR and its changes over time are mandatory to make opportune decisions about diagnosis, prognosis, and treatment of renal diseases. In clinical practice, GFR is routinely estimated by the determination of serum creatinine, and, if more exact data are needed, by the measurement of creatinine clearance. Because serum levels of creatinine are determined by its metabolic generation, renal excretion (glomerular filtration, tubular secretion, and reabsorption), and extrarenal elimination (1), a detailed knowledge of these factors is of extreme importance for the appropriate clinical interpretation of creatinine serum concentration and clearance measurements.

It is well known that creatinine is freely filtered by the glomerulus, but also actively secreted by the proximal tubule from the peritubular capillaries in small amounts such that creatinine clearance overestimates actual GFR by 10% to 20% (2). The histamine receptor antagonist cimetidine has been used successfully to increase the accuracy of GFR estimation by suppressing creatinine secretion (3). This effect of cimetidine on creatinine secretion has been attributed to competition with specific transporters expressed on the basolateral membrane of renal proximal tubule cells. These transporters are supposed to mediate the first step in renal creatinine secretion, which is basolateral uptake in proximal tubule cells. Even though identification of the transporters involved in the renal secretion of creatinine is highly relevant both for clinical practice in humans and also for experimental analysis in animal models, knowledge in this area is still scant. In a previous study (4), we observed in mice treated with cisplatin an acute
Accumulation of creatinine in cell lines

HEK293 cell lines stably transfected with human OAT1, OAT2, OAT3, OCT1, OCT2, OCT3, mOat3, mOct1, mOct2, or an empty pcDNA vector were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen) supplemented with 10% FBS, penicillin (100 U/mL), streptomycin (100 µg/mL), and G418 sulfate (400–800 µg/mL) at 37°C under 5% CO2 and 95% humidity. Uptake studies and cell transfections were done as described in detail elsewhere (11). Functional overexpression of transporters was confirmed by assessing the uptake of p-aminohippuric acid (5 µmol/L at 30 minutes) for hOAT1 (7.3-fold increased vs. cells transfected with an empty vector) and hOAT2 (2.4-fold), methotrexate (10 µmol/L at 30 minutes) for hOAT3 (4.2-fold) and mOat3 (5.1-fold), and tetraethylammonium (5 µmol/L at 5 minutes) for hOCT1 (4.6-fold), hOCT2 (62-fold), mOct1 (28-fold), mOct2 (30-fold), and hOCT3 (2.7-fold).

Transport of creatinine in freshly isolated proximal tubules

Human kidney samples were obtained from patients undergoing tumor nephrectomy. Pieces of normal kidney tissue surrounding the tumor were used. The procedure was approved by the ethics commission of the Universität Klinikum Münster, and written consent was obtained from each patient. Renal tissue was transferred into chilled HCO3− free phosphate buffer immediately after nephrectomy. Proximal tubules from mice or humans were isolated for functional analyses using the procedure customary in our laboratory (12). Briefly, thin corticomedullary slices were cut from small human kidney pieces (approximately 5–7 mm3 each) or mouse kidneys using a scalpel and immediately transferred into a dissection dish containing minimum Eagle’s culture medium and 5 mmol/L glycine at 4°C. Single proximal tubules were mechanically isolated using fine forceps under stereomicroscopic observation and transferred to a perfusion chamber and fixed by 2 holding pipettes closing the lumina at the ends of the tubule segment for microfluorimetric measurements. In this way, only the basolateral side of the tubules, where OCTs are located, could be reached by the experimental solutions.

Fluorescence measurements

As substrate for OCTs, the fluorescent organic cation 4-(4-(dimethylamino)styryl)-N-methylpyridinium (ASP+; Molecular Probes) at a concentration of 1 µmol/L was used. First, the influence of creatinine on ASP+ transport has been evaluated in HEK293 cells stably transfected with hOCT2. These measurements were carried out at 37°C using a fluorescent plate reader (Infinity M200; Tecan), according to the method described by us before (13). Briefly, hOCT2-expressing cells confluently grown on 96-well microplates (Nunclon 96 Flat bottom; Nunc) were excited with monochromatic light of 450 nm and fluorescence emission, filtered by a second monochromator at 590 nm, was finally measured by a fluorescence detector. Fluorescence was measured dynamically in each well before and after ASP+ injection. ASP+ uptake was quantified by linear regression of the initial fluorescence increase. For the ASP+ uptake experiments, HEK293 cells were stably transfected with hOCT2 and selected as described earlier (14), and cultivated at 37°C in DMEM containing 5.7 g/L NaHCO3, 1.0 g/L d-glucose, and
Renal accumulation of creatinine was investigated in Oct1/2(−/−) animals by administration of [14C]creatinine (American Radiolabeled Chemicals Inc.; specific activity, 50 mCi/mmol) as intravenous bolus at a dose of 1 μCi (approximately 20 nmol) in 100 μL saline. Kidney (homogenized in 1N sodium hydroxide solution at 1:10 wt/vol) and serum samples were obtained at 30 minutes after dosing (n = 6 per group) and aliquots analyzed by liquid scintillation counting.

Clinical samples
Variant genotypes in or around the region of interest were determined in germline DNA samples from 590 pediatric patients treated for acute lymphoblastic leukemia at St. Jude Children’s Research Hospital (Memphis, TN). Samples were amplified, labeled, and hybridized to their respective chips and analyzed as described (16, 17). The analysis was restricted to the region spanning 160,500 to 160,900 (kb) on chromosome 6, the locus which includes SLC22A2 and SLC22A3, the genes encoding OCT2 and OCT3, respectively. The chips covered approximately 100 different single-nucleotide polymorphisms (SNP) in this region. Of 590 patients, 297 had their serum creatinine levels measured more than 15 hours before receiving their first consolidation dose of methotrexate, whereas 72 had it measured between 18 and 5 hours and 14 were assayed less than 5 hours before treatment. The time of serum collection had no influence on age-adjusted serum creatinine levels (P > 0.05). Patients and patients gave informed consent and assent as appropriate, and the protocols and analyses were approved by the Institutional Review Board of St. Jude Children’s Research Hospital.

Acute effects of a 3-hour intravenous infusion of cisplatin (100 mg/m²) on serum creatinine changes were assessed in samples retrospectively collected and described previously (18). Paired baseline and day 1 data were available from 68 patients, who were treated at the Erasmus MC–Daniel den Hoed Cancer Center (Rotterdam, the Netherlands). The study protocols were reviewed and approved by the Erasmus MC review board, and patients provided written informed consent.

Statistical analysis
Data are presented as mean values ± SEM unless stated otherwise, with n referring to the number of animals, cell monolayers, or isolated tubules used in the experiments. Unpaired 2-sided Student t test and ANOVA (with Tukey posttest) were used to show statistical significance of the effects. P < 0.05 was considered statistically significant. Statistical analyses and determination of IC50 values were carried out using GraphPad Prism 5.0 (GraphPad Software Inc.).

Creatinine clearance (Ccr) was calculated according to the following equation:

\[
C_{cr} (\mu L/min) = \frac{\text{Urine creatinine concentration (mg/dL) \times 24h urine volume (μL)}}{\text{Serum creatinine concentration (mg/dL) \times 24 \times 60 min}}
\]
Results

In vitro transport of creatinine

The uptake of creatinine was markedly stimulated in hOCT2- and hOCT3-expressing HEK293 cells compared with cells transfected with the null vector (5.8-fold and 3.7-fold, respectively; Fig. 1A). A significant stimulation of creatinine uptake was also observed in hOAT2- and hOAT3-expressing HEK293 cells (2.3-fold and 1.6-fold, respectively), whereas the uptake by hOCT1- and hOAT1-expressing cells was comparable to that by null vector transfected cells (Fig. 1A). Among the murine transporters investigated (mOat3, mOct1, and mOct2), only mOct2 showed a significantly higher creatinine accumulation compared with null vector transfected cells (1.4-fold; Fig. 1B). These in vitro findings support the possibility that hOCT2 and mOct2 may be the dominant creatinine transporters in the proximal tubule in humans and in mice, respectively.

The uptake of the fluorescent organic cation ASP\(^+\) in hOCT2-expressing HEK293 cells was inhibited by creatinine in a concentration-dependent manner, with an IC\(_{50}\) of 580 \(\mu\)mol/L (Fig. 2A). Because at a concentration of 10 mmol/L creatinine was able to completely inhibit ASP\(^+\) uptake, we used this concentration to investigate the interaction of creatinine with the transport of organic cations in renal proximal tubules freshly isolated from mice or from “normal” renal tissue of humans undergoing tumor nephrectomy. At this concentration, creatinine was able to significantly inhibit the initial ASP\(^+\) uptake across the basolateral membrane from S1 and also S3 segments of proximal tubules from wild-type mice (B). The effects of these concentrations of TEA\(^+\) and creatinine on ASP\(^+\) uptake in S3 segments from Oct1/2(−/−) mice and from humans is also shown. Data are presented as mean values ± SEM. The number of mouse or human tubules for each group is indicated above the column. * indicates a statistical significant difference compared with control experiments in the presence of ASP\(^+\) only (\(P < 0.05\), unpaired 2-tailed t test).

Figure 1. Accumulation of \(^{14}\)C-creatine in HEK293 cells stably transfected with human (A) and murine (B) organic ion transporters. Data are presented as mean values ± SEM. The number of experiments for each group is 6. * indicates a statistical significant difference compared with control experiments with HEK293 cells transfected with null vector (VC; \(P < 0.05\), unpaired 2-tailed t test).

Figure 2. Concentration–response curves for the inhibition of initial ASP\(^+\) uptake by creatinine in HEK 293 cells stably expressing hOCT2 (A). Values are means ± SEM expressed as % ASP\(^+\) uptake in the absence of creatinine with number of observation in parentheses. IC\(_{50}\) value of ASP\(^+\) uptake inhibition by creatinine determined from this curve was 580 \(\mu\)mol/L. Effect of 1 mmol/L TEA\(^+\) (white columns) and 10 mmol/L creatinine (black columns) on the uptake of ASP\(^+\) in freshly isolated S1 and S3 segments of proximal tubules from wild-type mice (B). The effects of these concentrations of TEA\(^+\) and creatinine on ASP\(^+\) uptake in S3 segments from Oct1/2(−/−) mice and from humans is also shown. Data are presented as mean values ± SEM. The number of mouse or human tubules for each group is indicated above the column. * indicates a statistical significant difference compared with control experiments in the presence of ASP\(^+\) only (\(P < 0.05\), unpaired 2-tailed t test).

Figure 3. Schematic representation of the distribution of organic cation transporters in renal proximal tubule segments. The main organic cation transporter expressed in each segment is shown. OCT, organic cation transporter; OAT, organic anion transporter; S1, S2, and S3, segments of the proximal tubule; T1–T4, basolateral domains of the S1–S3 segments; AT1, basolateral domain of the T1 segment (thick black line).
express Oct3 at very low levels (19), neither TEA\(^+\) nor creatinine significantly inhibited ASP\(^+\) transport (5 \pm 16\%, n = 11 and \(-6 \pm 17\%\), n = 12, respectively; Fig. 2B), again confirming an interaction of creatinine with mOct2. In human S3 segments, initial ASP\(^+\) uptake across the basolateral membrane could be significantly inhibited by 1 mmol/L TEA\(^+\) (\(-56 \pm 12\%\), n = 11) and also by 10 mmol/L creatinine (\(-34 \pm 12\%\), n = 12; Fig. 2B), suggesting that also in humans renal creatinine secretion is mediated by OCT2, the most highly expressed OCT isoform in human proximal tubules (20, 21).

**Creatinine clearance in transporter-deficient mice**

The creatinine clearance in male wild-type mice was significantly higher than that observed in male Oct1/2\((-/-)\) mice (566 \pm 88 \muL/min, n = 6 vs. 366 \pm 25 \muL/min, n = 6, respectively; Fig. 3A). Moreover, the renal accumulation of exogenous creatinine in male wild-type mice was significantly higher than that in male Oct1/2\((-/-)\) mice after correction for serum creatinine (kidney/skin ratio of creatinine, 2.05 \pm 0.067, n = 6 vs. 2.65 \pm 0.140, n = 6, respectively; Fig. 3B). These changes occurred without any substantial alterations in the renal expression of other transporter genes [Slc22a6 (Oat1), Slc22a7 (Oat2), Slc22a8 (Oat3), and Slc47a1 (Mate1)] in the Oct1/2\((-/-)\) mice (Supplementary Fig. S1).

**Clinical studies**

An exploratory analysis on samples from 590 children showed that serum creatinine levels were significantly dependent on age (Supplementary Fig. S2), but not on racial ancestry (\(P = 0.42\)) or sex (\(P = 0.41\)). Focusing specifically on chromosome 6 in the region bearing the genetic information for hOC1s and, on the basis of data obtained in the mice, on male subjects (\(n = 214\)), we found one intergenic SNP (rs2504954) that was significantly associated with serum creatinine (\(P = 0.000873\)), and that was in strong linkage with various SNPs within the OCT2 and OCT3 genes SLC22A2 and SLC22A3, respectively (Fig. 4). The rs2504954 exhibits the variation G or T, and the G allele (found in 27% of whites) was associated with higher creatinine levels.

To provide further evidence for the involvement of OCT2 in creatinine secretion, we next assessed the potential...
The exact knowledge of renal creatinine handling is of pivotal importance for an appropriate interpretation of data on serum creatinine concentration and creatinine clearance in the assessment of kidney function in clinical practice. In fact, because of tubular secretion, estimation of GFR by creatinine clearance determination results in an overestimation. Moreover, in renal diseases accompanied by diminished GFR, the contribution of tubular creatinine secretion to creatinine clearance may be dramatically increased and, when equaling creatinine clearance with GFR, this may result in a profound overestimation of GFR (2, 22, 23). In other clinical settings (for example, decompensated heart disease or uncontrolled diabetes mellitus), a primary reabsorption of creatinine by renal tubules was observed (2, 24). Finally, administration of cationic drugs which are substrates of OCTs may interfere competitively with renal creatinine secretion and, consequently, could falsely indicate a reduced GFR.

Because in the attempt to understand the mechanisms and to develop new therapeutic concepts of kidney diseases, preclinical studies with mouse models of renal pathology have been increasingly used, accurate evaluation of the GFR also in these animal models is a critical parameter for translational drug development (2). For this reason, increasing attention has recently been paid to the assessment of the accuracy of renal function determination in mice.

Our in vitro studies showed that the stable expression of hOCT2 is linked to a significant accumulation of creatinine in HEK293 cells. Interestingly, stable expression of hOCT3, hOAT2, and hOAT3, but not of hOAT1 or hOCT1, also allowed HEK293 cells to accumulate creatinine. The IC_{50} of 580 μmol/L for the inhibition of the ASP\(^+\) uptake by creatinine suggests that creatinine is a low affinity substrate for hOCT2. Recently, similar findings have been obtained by Imamura and colleagues (25) who showed that in an in vitro system hOCT2, hOAT3, hOAT4, and hOCT3 mediate a significant uptake of creatinine and that creatinine has a low affinity for hOCT2. Of the murine transporters we investigated in vitro (mOat3, mOct1, and mOct2), only mOct2 was able to mediate a significant transport of creatinine. The interaction of creatinine with the transport by organic cations is also evident in the experiments with freshly isolated proximal tubules: in S1 and also S3 segments from wild-type mice, creatinine significantly inhibited the transport of ASP\(^+\), although this effect was not detectable in Oct1/2(−/−) mice. Because mOct1 seems not to accept creatinine as a substrate and the mOct3-mRNA expression in proximal tubules is only 3% of that of mOct2-mRNA (26), the inhibition of ASP\(^+\) uptake in wild-type mice by creatinine is principally ascribable to an interaction with mOct2. Even more importantly, creatinine showed a significant interaction also with the transport of ASP\(^+\) in freshly isolated human S3-proximal tubules at physiologically relevant levels. Because the principal isoform of OCTs in the human kidney is hOCT2 (20, 21), these findings suggest that in vivo in humans hOCT2 plays an important role in the secretion of creatinine. Such an hypothesis is strongly supported by recent studies with the antibacterial agent des-fluoro(6)-quinolone (25), which at therapeutic dosages can inhibit hOCT2 as well as certain luminal transporters, such as hMATE1 and hMATE2-K, leading to a significant inhibition of tubular secretion of creatinine and, consequently, to elevation of serum creatinine levels.

The in vivo measurements done in wild-type and Oct1/2(−/−) mice further showed the importance of OCTs for creatinine secretion. Indeed, the absence of secretion through OCTs caused a small, but statistically significant, increase in serum creatinine concentration in Oct1/2(−/−) mice compared with wild-type mice at baseline. These knockout mice do not differ with respect to urinary volume, total protein, glucose excretion, GFR (27), blood urea nitrogen, or in the expression of other solute transporters (4). Moreover, the important role of OCTs in the vectorial transport of creatinine in renal proximal tubules is underlined by the significantly lower renal creatinine accumulation in male Oct1/2(−/−) mice compared with wild-type mice upon administration of exogenous creatinine.

Curiously, our current murine data are in contradiction to recent findings observed by Eisner and colleagues (28) using the same animal model. These authors detected no difference in creatinine secretion between wild-type and
OCT2 Involvement in Creatinine Clearance

Oct1/2(+/−) mice and suggested that the mouse Oat1 (mOat1), but not mOct2, is the murine transporter responsible for creatinine secretion (28). Their experiments were conducted under ketamine (100 mg/kg, subcutaneous injection) and xylazin anesthesia. Ketamine is a weak basic compound that has been shown to interact with rat and human OCTs with a Ki for the transport of the organic cation 1-methyl-4-phenylpyridinium of 23 and 207 μmol/L for hOCT2 and rOCT2, respectively (29). There are no data in the literature on plasma ketamine concentration after subcutaneous administration of the agent at a dose of 100 mg/kg. However, intraperitoneal administration of ketamine (200 mg/kg) in mice resulted in plasma ketamine concentrations of 30 and 10 mg/mL after 10 and 60 minutes, respectively (30). Assuming a plasma concentration of 5 mg/mL 60 minutes after a dose of 100 mg/kg, this would correspond to a concentration of 20 mmol/L, which is well above the Ki for interaction with OCTs and also above the concentration producing 80% inhibition of the ASP+ uptake observed in our experiments (Supplementary Fig. S4). Under ketamine anesthesia, secretion by OCTs should be blocked and it can be speculated that for this reason Eisner and colleagues did not detect any difference in creatinine secretion between wild-type and Oct1/2(+/−) mice. However, it cannot be excluded that other transporters such as Oat1 could be involved in the renal secretion of creatinine in mice.

The SNP (rs2504954) in the OCT1-3 gene region identified from our genetic association study further confirms the importance of OCTs for creatinine secretion in humans. Such a link between polymorphisms of hOCT2 and serum creatinine levels has already been observed in studies done in adults that identified rs2279463 (located in the OCT2 gene SLC22A2; ref. 31) and rs3127573 (near SLC22A2; ref. 32) as significantly associated with serum creatinine. These previously identified sites on chromosome 6 were unfortunately not directly interrogated by the chips used in our analysis. Nonetheless, these findings lend further credence to the thesis that OCTs, in particular OCT2, play a role in the renal secretion of creatinine in humans.

The exact identification of the molecular entities responsible for this transport is of prominent importance to furnish a rational basis for the correct interpretation of creatinine as marker of renal glomerular function not only in translational medicine but also in clinical practice. Because OCTs are polysize specific membrane transporters, they can interact with a multitude of substances, among which many are drugs of common use. Drug–creatinine interactions at the transporter level may be the reason for changes of creatinine serum concentration independent from changes of renal glomerular function. Moreover, the expression and activity of these transporters can change under specific pathologic situations. For example, OCT2 seems to be downregulated in chronic renal failure, at least in a rat model (19). Therefore, transporter–drug interaction and regulation of transporter activity in different pathophysiologic situations may be responsible for changes in renal creatinine secretion and thus, in serum creatinine concentrations, without changes in glomerular filtration. From experiments in animal models, it is known that under some pathologic situations the expression of OCTs is up- or downregulated. For example, in kidneys of rats with liver cirrhosis, OCT2 is significantly upregulated (33). Intriguingly, patients with compensated cirrhosis have been shown to have a significant tubular secretion of creatinine (34), which is consistent with an increased expression of OCT2.

In conclusion, our current data show that the organic cation transporter OCT2 plays a decisive role in the tubular secretion of creatinine. This finding should be taken into consideration when using serum creatinine or creatinine clearance to evaluate renal function in rodents and humans.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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