

Abemaciclib Inhibits Renal Tubular Secretion Without Changing Glomerular Filtration Rate

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Abemaciclib, an inhibitor of cyclin dependent kinases 4 and 6, is indicated for metastatic breast cancer treatment. Reversible increases in serum creatinine levels of ~15–40% over baseline have been observed following abemaciclib dosing. This study assessed the *in vitro* and clinical inhibition of renal transporters by abemaciclib and its metabolites using metformin (a clinically relevant transporter substrate), in a clinical study that quantified glomerular filtration and iohexol clearance. *In vitro*, abemaciclib inhibited metformin uptake by organic cation transporter 2, multidrug and toxin extrusion (MATE)1, and MATE2-K transporters with a half-maximal inhibitory concentration of 0.4–3.8 μ M. Clinically, abemaciclib significantly increased metformin exposure but did not significantly affect measured glomerular filtration rate, serum neutrophil gelatinase-associated lipocalin (NGAL), serum cystatin-C, or the urinary markers of kidney tubular injury, NGAL and kidney injury molecule-1.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Multiple drugs decrease estimated glomerular filtration rate (eGFR) via inhibition of renal transporters without directly affecting renal function. Clinical studies of abemaciclib revealed treatment-associated increases in serum creatinine (SCr) and decreases in eGFR.

WHAT QUESTION DID THIS STUDY ADDRESS?

Does abemaciclib cause increases in SCr due to inhibition of renal transporters or because of alterations to GFR?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Abemaciclib inhibits organic cation transporter 2, multidrug and toxin extrusion (MATE) 1, and MATE2-K

transporters *in vitro* and significantly decreases renal clearance of metformin, a transporter substrate. Abemaciclib did not significantly increase measured GFR or acute renal damage biomarkers, suggesting that observed increases in SCr are due to inhibition of proximal tubule secretory transporters.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

These findings indicate that abemaciclib does not seem to affect renal function: alternative renal function assessment methods may be required in patients taking abemaciclib when increases in creatinine are reported and clinicians are concerned about impairment of renal function. The design allows transporter inhibition and effects upon GFR to be evaluated in the same study.

Abemaciclib is an orally administered small molecule that is a potent and selective inhibitor of cyclin-dependent kinases (CDKs) 4 and 6.¹ Inhibition of CDK4 and CDK6 prevents cell cycle progression through the G1 restriction point, which controls entry into S phase, thus arresting tumor growth.² Abemaciclib is indicated for the treatment of metastatic breast cancer.

In clinical studies of patients with cancer and healthy subjects, reversible increases in serum creatinine (SCr) levels of ~15–40% over baseline were measured following dosing with abemaciclib.^{2,3} Creatinine, an endogenous product of creatine phosphate metabolism in skeletal muscle, is the most widely used marker to estimate glomerular filtration rate (eGFR) in the clinical setting. Although creatinine is primarily filtered in the kidneys,^{4,5} active tubular secretion accounts for ~10–40% of creatinine clearance.^{6–9} Thus,

the glomerular filtration rate (GFR) calculated using SCr levels could be an overestimate.

Active tubular secretion of creatinine is mediated by multiple solute carrier (SLC) transporters in the kidney, including organic cation transporter 2 (OCT2: SLC22A2), multidrug and toxin extrusion protein (MATE) 1 (SLC47A1), and MATE2-K (SLC47A2).^{5,10,11} OCT2 is expressed on the basolateral membrane of proximal tubule cells and mediates uptake of organic cations, such as creatinine and metformin, from blood into the cells by facilitated diffusion;¹² however, MATE1 and MATE2-K are expressed on the apical membrane of proximal tubule cells and are responsible for proton-coupled efflux of drugs and endogenous compounds from cells to urine.¹³ Inhibition of OCT2 and/or MATEs can, therefore, lead to a decrease in creatinine clearance,

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and a corresponding increase in SCr due to inhibition of its tubular secretion. These changes can occur without clinically meaningful alterations in renal function or impact on measured GFR (mGFR).^{9,10,13}

The *in vitro* assessment of OCT2 and/or MATE inhibition is important in evaluating potential for drug–drug interactions (DDIs) at these transporters and for many drugs recognized as having inhibitory potential.¹⁴ A number of marketed drugs cause elevations in SCr by inhibition of these renal transporters.^{15,16} Early assessment of the clinical inhibition of renal transporters may be prudent if transporter inhibition has been identified *in vitro* or if increases in SCr concentrations have been identified during preclinical development, or first-in-human clinical trials.

A widely used drug, metformin, is excreted unchanged in urine, principally via active tubular secretion in the kidneys^{17,18} by OCT2-mediated uptake followed by efflux via MATEs. It is well documented that concomitant administration of metformin with MATE and/or OCT2 inhibitors, such as pyrimethamine, cimetidine, or dolutegravir, changes the pharmacokinetics (PKs) of metformin.^{18–21}

In the past decade, alternative markers for assessing GFR and renal function have been identified, including other endogenous substances, such as cystatin-C, which unlike SCr, is not subject to active secretion and not affected by changes in diet or muscle mass.²² Techniques using clearance rates of freely filtered and non-metabolized exogenous compounds, such as iohexol, are also used to calculate absolute GFR and better estimate the actual rate of glomerular filtration.²³ A range of biomarkers, including kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL), have been shown to be promising markers of acute kidney injury compared to SCr.^{16,24–26}

This study aimed to assess the *in vitro* and clinical inhibition of renal transporters by abemaciclib and its active metabolites, M2 and M20, using metformin as a clinically relevant competing transporter substrate. The effect of abemaciclib administration on metformin PK was examined using a clinical study design incorporating monitoring of renal filtration, by measuring iohexol clearance, to demonstrate that observed increases in creatinine concentrations and subsequent decreases in calculated creatinine clearance and eGFR were due to transporter inhibition and not changes in renal function.

RESULTS

In vitro analysis of transporter interactions

The inhibitory effects of abemaciclib and its two active metabolites, M2 and M20, on renal transport of metformin were assessed *in vitro* using transfected human embryonic kidney (HEK) cells expressing OCT2, MATE1, and MATE2-K. Abemaciclib, M2, and M20 inhibited OCT2, MATE1, or MATE2-K-mediated metformin uptake with half-maximal inhibitory concentration (IC₅₀) values ranging from 0.4–3.8 μM (Table 1 and Figure 1). When the unbound peak plasma concentration (C_{max}) to *in vitro* IC₅₀ ratio (DDI index) for abemaciclib was combined with DDI indices of M2 and M20, the DDI indices of OCT2, MATE1, and MATE2-K were 0.03, 0.13,

Table 1 Subject demographics and baseline characteristics

		Overall (N = 40)
Age (years)	Mean (SD)	50.4 (10.6)
	Range	23–69
Age group (%)	21–30 years	1 (2.5)
	31–40 years	6 (15.0)
	41–50 years	13 (32.5)
	51–60 years	12 (30.0)
	61–70 years	8 (20.0)
Sex (%)	Male	4 (10.0)
	Female	36 (90.0)
Ethnicity (%)	Hispanic or Latino	8 (20.0)
	Not Hispanic or Latino	32 (80.0)
Race (%)	Asian	3 (7.5)
	Black or African American	18 (45.0)
	White	19 (47.5)
Weight (kg)	Mean (SD)	73.56 (11.22)
	Range	54.7–99.2
BMI (kg/m ²)	Mean (SD)	27.25 (3.06)
	Range	20.4–31.8
eGFR (mL/minute/1.73 m ²)	Mean (SD)	99.84 (15.63)
	Range	62.6–128.4

BMI, body mass index; eGFR, estimated glomerular filtration rate, as calculated with the Chronic Kidney Disease Epidemiology Collaboration equation²⁸; N, number of subjects.

0.06, respectively (Figure 1). The DDI index for MATE1 and MATE2-K exceeded the regulatory guidance threshold of 0.02 indicating the potential for a DDI at these transporters at clinically relevant concentrations.^{27,28}

Clinical study

Demographics. Forty healthy subjects, 4 men and 36 women, between the ages of 23 and 69 years were enrolled in the study. The demographic and baseline characteristics of the subjects are shown in Table 1.

All subjects were randomly assigned to a dosing sequence and received at least one dose of study drug. Twenty-five subjects completed the study through to the follow-up visit (Figure S1).

One subject discontinued due to adverse events of elevated alkaline phosphatase, elevated alanine aminotransferase, elevated aspartate aminotransferase, and elevated gamma glutamyltransferase enzymes, considered to be related to study treatment beginning on day 15 of period 2 (14 days after receiving abemaciclib and metformin). Five subjects were lost to follow-up, and five subjects withdrew their consent for personal reasons. For the PK analysis, data were available from 30 subjects who received placebo and metformin, 32 subjects who received abemaciclib and metformin, 32 subjects who received placebo and iohexol, and 33 subjects who received abemaciclib and iohexol. Figure S1 summarizes subject disposition and data evaluability.

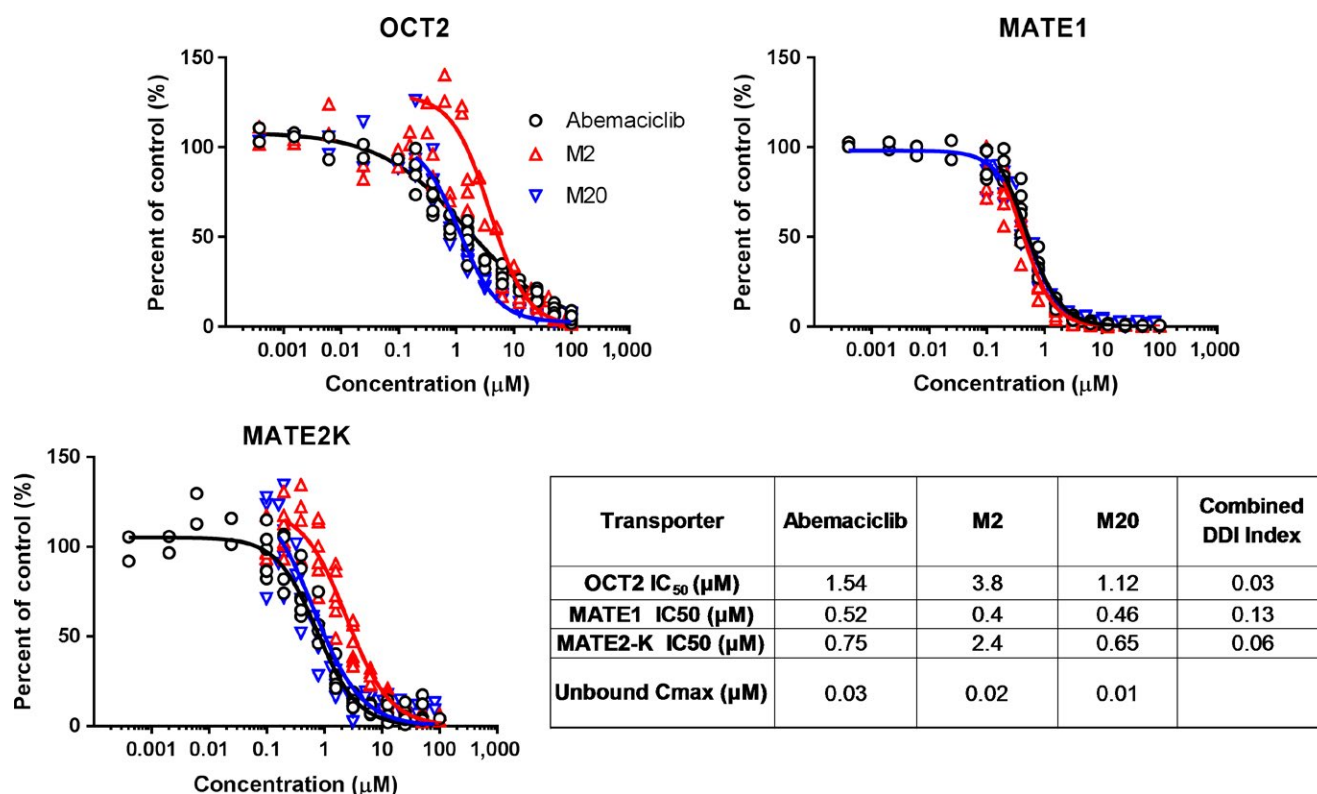


Figure 1 Inhibition of organic cation transporter (OCT2), multidrug and toxin extrusion (MATE1), and MATE2-K-dependent transport of metformin by abemaciclib and metabolites M2 and M20. Combined drug–drug interaction index (DDI) = $(I_u \text{ abemaciclib} / \text{half-maximal inhibitory concentration (IC}_{50}) \text{ abemaciclib}) + (I_u \text{ M2} / \text{IC}_{50} \text{ M2}) + (I_u \text{ M20} / \text{IC}_{50} \text{ M20})$ where I_u = unbound peak plasma concentration (C_{\max}).

Effect of abemaciclib coadministration on metformin PKs. Coadministration of metformin with abemaciclib increased exposure to metformin (Figure 2), causing a statistically significant increase in metformin geometric least squares (LS) mean area under the concentration–time curve from zero to infinity ($AUC_{0-\infty}$) and C_{\max} , and a nonsignificant delay in time of maximum plasma concentration (T_{\max} ; Table 2).

Statistically significant decreases in metformin renal clearance (CL_R) and clearance of renal secretion (CL_{RS}) were observed in the presence of abemaciclib (Figure 2 and Table 2).

Effect of abemaciclib on iohexol clearance and GFR. Coadministration of abemaciclib with iohexol did not affect iohexol PK, and there was notable overlap of the iohexol plasma concentration vs. time profiles in the presence of placebo or 400 mg abemaciclib (Figure 3). Iohexol clearance (mGFR) was equivalent following coadministration of iohexol with placebo or abemaciclib, with a ratio of geometric LS means of 0.982 (90% confidence interval (CI) 0.958–1.01, mean mGFR of 89.0 and 85.6 mL/minute for placebo and abemaciclib periods, respectively; Figure 3b).

Following both placebo and abemaciclib administration, SCr concentrations increased between 10 and 12 hours postdose (mean increases of 25–29 and 35.7–44.6 µmol/L, following placebo and abemaciclib administration, respectively), then decreased over time, with the maximum increase being higher following abemaciclib administration. Based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation using SCr, eGFR decreased

in the presence of abemaciclib compared to placebo for the combination with metformin (65.4–57.2 mL/minute/1.73 m²) and iohexol (62.2 and 53.6 mL/minute/1.73 m²), respectively.

eGFR calculated using serum cystatin-C concentrations did not change between abemaciclib and placebo periods. At the time of maximal postdose creatinine concentrations, eGFR calculated using CKD-EPI showed decreases corresponding to higher creatinine values, whereas the eGFR calculated using cystatin-C at the same time point did not change. There were no trends in serum concentrations of cystatin-C following administration of placebo or the study drugs.

There were no trends in serum cystatin-C or NGAL indicative of renal injury following administration of placebo or abemaciclib with either metformin or iohexol (Figure 4). When the ratio of urine concentrations of KIM-1 and NGAL to urine creatinine concentrations were calculated, there seemed to be higher variability following dosing of abemaciclib with metformin but no other notable changes following dosing in any of the groups (Figure 5).

DISCUSSION

Abemaciclib administration has been associated with mild and variable but consistent elevations in SCr.^{14,15,29} Increased SCr can arise from a reduction in glomerular filtration, indicating damage to the nephron and associated reduction in renal function, or from inhibition of the active tubular secretion of creatinine. The active tubular secretion of creatinine, and other organic cations, is mediated at least in part by OCT2 at the

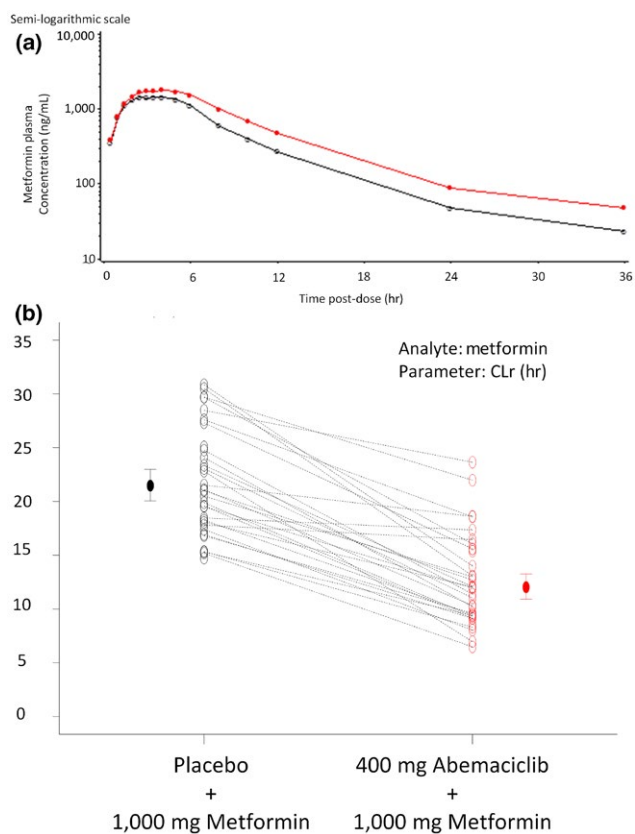


Figure 2 Metformin pharmacokinetics following placebo or abemaciclib administration. **(a)** Mean metformin plasma concentration-time curves after administration of metformin with placebo (open circle) or abemaciclib (red circle) to 30 or 28 healthy subjects, respectively. **(b)** Individual (open circles) and geometric mean values (\pm 90% confidence interval, closed circles) of metformin renal clearance (CLR) following a single oral dose of 1,000 mg metformin with a single oral dose of placebo (black circle) or 400 mg abemaciclib (red circle).

basolateral membrane and MATE1/2-K at the apical membrane of the proximal tubule.⁶

Inhibition constants (IC_{50}) determined *in vitro* predicted that at therapeutic concentrations, abemaciclib and its major circulating metabolites would inhibit OCT2 and MATE1/2-K clinically.

This study was designed to clinically evaluate the hypothesis that mild, abemaciclib-induced elevations in SCr are a result of inhibition of active tubular secretion of creatinine and not a reduction in kidney function (as determined by mGFR).

In this study, healthy subjects were included from a broad age range and multiple races. The majority (90%) of subjects were women. Abemaciclib is approved for the treatment of breast cancer, which primarily affects women. Limited evidence exists regarding the influence of sex upon renal transporter expression in humans; however, evidence from rodent studies suggests that sex has an indeterminate effect on transporter expression, with evidence for increased and decreased expression of renal transporter genes in female rodents compared to males.^{30,31}

A single oral dose of 400 mg abemaciclib, which approximated the steady-state concentration following dosing with 200 mg abemaciclib

twice-daily, caused a statistically significant increase in metformin exposure of \sim 30% (a 37% and 22% increase in metformin $AUC_{0-\infty}$ and C_{max} , respectively) compared to placebo. A statistically significant decrease in metformin clearance was also observed. However, despite this reduction, metformin terminal half-life did not change, which may be due to a concurrent reduction in apparent volume of distribution mediated through inhibition of transporters that move metformin into tissues, such as the liver. Metformin clearance is primarily due to active secretion (approximately three times GFR) via secretory transporters, and the secretion clearance is reduced by 62% in the presence of abemaciclib. Metformin is not metabolized *in vivo* and is excreted unchanged in urine. The transport of metformin from the circulation into the renal epithelia is mediated by OCT2, and the renal excretion is mediated primarily by MATE1 and MATE2-K. In contrast, the overall CL_R of metformin is reduced by only 45%, which is consistent with the observation with iohexol that abemaciclib does not affect GFR. Furthermore, the 62% reduction of metformin secretion clearance suggests that abemaciclib may not completely inhibit the transporters that mediate metformin secretion. These results are consistent with the *in vitro* findings that abemaciclib and its major metabolites inhibit the transporters OCT2, MATE1, and MATE2-K, which are involved in the active secretion of substrates, such as metformin and endogenous creatinine. This analysis assumes that tubular reabsorption of metformin is negligible (Eq. 3). However, if the CL_{RS} were to approximate renal plasma flow (\sim 38 L/hour in a 70-kg human³²), then a fraction reabsorbed of \sim 50% would be estimated from the metformin CL_R we observed (Eq. 2), and the estimated reduction in metformin CL_{RS} would be modestly lower at \sim 50%.

Elevations in SCr post abemaciclib dose generally followed the expected course: Notable rises occurred with a peak at 12 hours postdose, and concentrations gradually declined over the 120-hour collection period, with return to baseline or near-baseline levels by the end of the washout period or follow-up. This peak in SCr occurred \sim 3–5 hours following abemaciclib T_{max} . Both placebo groups seemed also to have transient elevations in SCr at 12 hours postdose; however, the SCr elevations were not as great as those observed following abemaciclib dosing and may be related to the meal administered at \sim 10 hours postdose. The statistical significance of this change was, however, not calculated.

The eGFR (as assessed by CKD-EPI) showed decreases following abemaciclib dosing, which were not reflected in mGFR (as determined by iohexol clearance), or GFR calculated from serum cystatin-C concentrations. No notable change in creatinine-normalized urinary concentrations of NGAL and KIM-1 (both biomarkers of renal injury^{24,33}) were observed. These data, combined with the observed changes in metformin PK, indicate that the changes in SCr observed in clinical studies of abemaciclib are due to reversible inhibition of renal tubular secretion of creatinine, and are not the result of acute kidney injury.

The majority of commonly used estimates of GFR, such as those recommended by the Modification of Diet in Renal Disease study³⁴ and CKD-EPI, rely on accurate steady-state measurement of SCr, which can introduce errors in estimates of renal function. With a crossover design, this study was able to combine a measurement of “true” GFR (as assessed by iohexol clearance) alongside renal transporter inhibition (as assessed by metformin PK) to both quantify the inhibitory effect of an investigational drug and its impact on renal function *in vivo*.

Table 2 Summary of metformin pharmacokinetics

Parameter	Treatment	N	Geometric LS means	Ratio of means	90% CI	P value
AUC _{0-∞} (ng.hour/mL)	Placebo + 1,000 mg metformin (reference)	30	12,245	1.37	1.28–1.46	NA
	400 mg abemaciclib + 1,000 mg metformin (test)	28	16,718			
C _{max} (ng/mL)	Placebo + 1,000 mg metformin (reference)	30	1,586	1.22	1.13–1.30	NA
	400 mg abemaciclib + 1,000 mg metformin (test)	28	1,930			
CL _R (L/hour)	Placebo + 1,000 mg metformin (reference)	30	21.5	0.550	0.504–0.600	NA
	400 mg abemaciclib + 1,000 mg metformin (test)	28	11.8			
CL _{RS} (L/hour)	Placebo + 1,000 mg metformin (reference)	29	16.0	0.381	0.323–0.450	NA
	400 mg abemaciclib + 1,000 mg metformin (test)	25	6.08			
T _{max} (hour)	Placebo + 1,000 mg metformin (reference)	24	3.03 ^a	0.500 ^b	0–0.983 ^c	0.070
	400 mg abemaciclib + 1,000 mg metformin (test)	24	3.76 ^a			

CL_{RS} was calculated using Eq. 3 and assumes no renal tubular reabsorption.

AUC_{0-∞}, area under the concentration-time curve from zero to infinity; CI, confidence interval; CL_R, renal clearance; CL_{RS}, clearance of renal secretion; C_{max}, peak plasma concentration; LS, least squares; NA, not applicable; T_{max}, time of maximum plasma concentration.

^aMedian. ^bMedian of differences. ^cApproximate 90% CI.

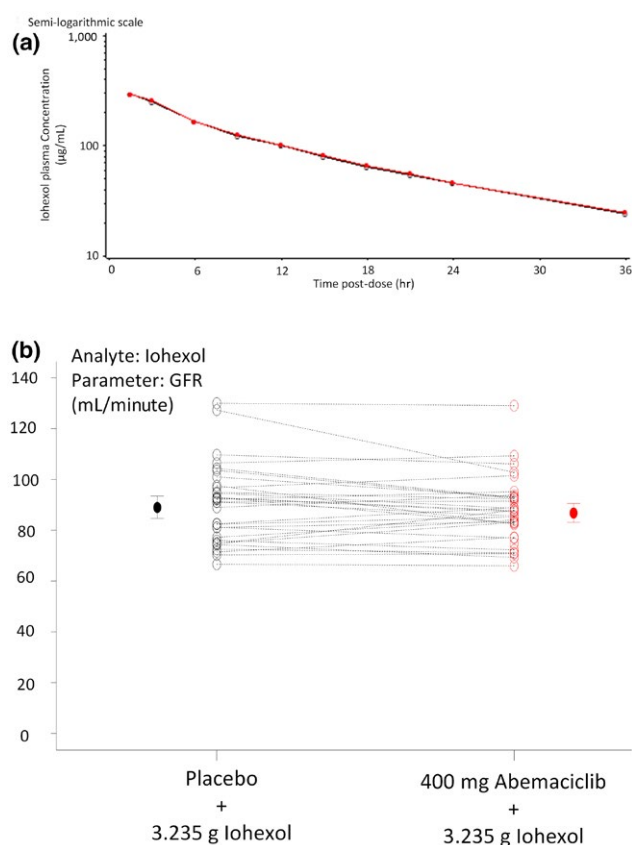


Figure 3 Iohexol pharmacokinetics following placebo or abemaciclib administration. (a) Mean iohexol plasma concentration-time curves after administration of iohexol with placebo (open circle) or abemaciclib (red circle) to 32 or 30 healthy subjects, respectively. (b) Individual (open circles) and geometric mean values (\pm 90% confidence interval, solid circles) of growth factor receptor (GFR) following a single infusion of 3.235 g iohexol with a single oral dose of placebo (black circles) or 400 mg abemaciclib (red circles).

The results described confirm that increases in SCr following abemaciclib administration are likely caused by inhibition of renal transporters and that abemaciclib does not affect renal function as assessed by mGFR, or increase in concentrations of urinary biomarkers of renal injury. The findings suggest that patients dosed with abemaciclib will experience a mild (\sim 10–40%) reversible increase in SCr due to renal transport inhibition. However, it is possible that the observed increases in SCr following abemaciclib administration may be further elevated in subjects with already reduced renal function due to reduced renal reserve. When clinically indicated, alternative measurements of renal function, aside from creatinine-derived eGFR, should be used in patients taking abemaciclib to accurately assess renal function.

METHODS

In vitro analysis of transporter interactions

Materials. Abemaciclib and its metabolites M2 (LSN2839567) and M20 (LSN3106726) were synthesized by Eli Lilly (Indianapolis, IN). Radiolabeled (14 C) metformin was purchased from American Radio labeled Chemicals (St. Louis, MO). All other chemicals were of analytical grade and purchased from commercial sources.

Stably transfected HEK cells expressing OCT2 or vector control (VC) were generated using previously described methods.³⁵ HEK cells transiently transfected with MATE1, MATE2-K, or VC were purchased from Corning Life Sciences (Transporto cells, Bedford, MA).

***In vitro* inhibition studies.** All inhibition studies were performed with (14 C) metformin as a substrate for 2 minutes at 37°C in VC, OCT2, MATE1, and MATE2-K cells in 24-well BioCoat Poly-D-Lysine plates (Becton Dickinson, Franklin Lakes, NJ).

OCT2 and VC cells were washed and preincubated for 10 minutes in varying concentrations of inhibitor (0.00038–100 μ M abemaciclib, M2, or M20, along with a positive control inhibitor, 100 μ M imipramine) in duplicate wells per concentration. Experiments were initiated by the addition of 200 μ L (14 C) metformin (10 μ M, 0.1 μ Ci/mL) in the presence of 0.00038–100 μ M abemaciclib, M2, M20, imipramine, or absence of the inhibitor.

MATE1, MATE2-K, and VC cells were washed and preincubated in 40 mM ammonium chloride for 20 minutes. Inhibition studies were

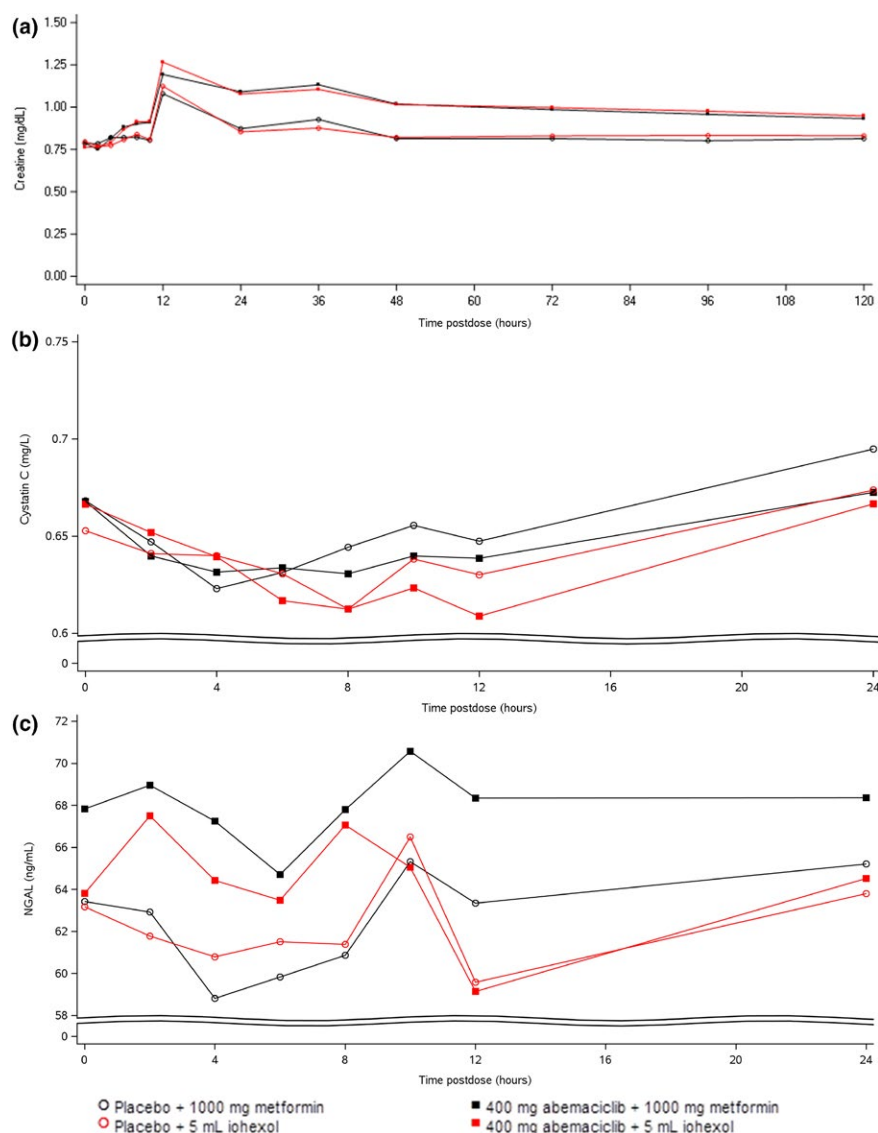


Figure 4 Mean serum creatinine (a), cystatin-C (b), and neutrophil gelatinase-associated lipocalin (NGAL) (c) concentrations following a single oral dose of placebo or 400 mg abemaciclib with a single oral dose of 1,000 mg metformin or a single infusion of 3.235 g iohexol in healthy subjects up to 24 hours postdose. Data is presented \pm SD.

performed in experiment solution containing 200 μ L (14 C) metformin (2 μ M, 0.2 μ Ci/mL) in the presence of 0.00038–100 μ M abemaciclib, M2, M20, 100 μ M pyrimethamine, or absence of inhibitor in duplicate wells per concentration.

The reaction was stopped by addition of ice cold Hanks balanced salt solution. Each well was then aspirated and washed once with ice-cold phosphate-buffered saline. Following the final aspiration, 400 μ L 1% Triton X-100 in phosphate-buffered saline (v/v) was added per well for radiochemical detection and protein quantification.

Analysis of data for *in vitro* inhibition studies

For all inhibition studies, values were corrected for passive diffusion by subtracting the average velocity of control cells from the velocity at each concentration in transfected cells.

The inhibitor concentration resulting in 50% inhibition (IC_{50} value) was determined by nonlinear regression analysis using WinNonLin

Professional, version 6.4 (Certara L.P., Princeton, NJ), using the following equation:

$$\text{Percent of Control} = Y_{\min} + \frac{(Y_{\max} - Y_{\min})}{\left(1 + \left(\frac{[I]}{IC_{50}}\right)^{\text{slope}}\right)} \quad (1)$$

where [I] is inhibitor concentration, Y_{\min} is minimum percentage activity in relation to control, Y_{\max} is percentage activity when there is no inhibitor present, and slope is the slope of the curve. Four-parameter fitting was used for the IC_{50} estimation for all fittings.

Combined DDI index for each transporter was calculated by adding unbound C_{\max} (I_u) divided by IC_{50} of abemaciclib, M2, and M20, as described by Lutz and Isoherranen³⁶:

$$\text{Combined DDI index} = \frac{I_u \text{ Abemaciclib}}{IC_{50} \text{ abemaciclib}} + \frac{I_u \text{ M2}}{IC_{50} \text{ M2}} + \frac{I_u \text{ M20}}{IC_{50} \text{ M20}} \quad (2)$$

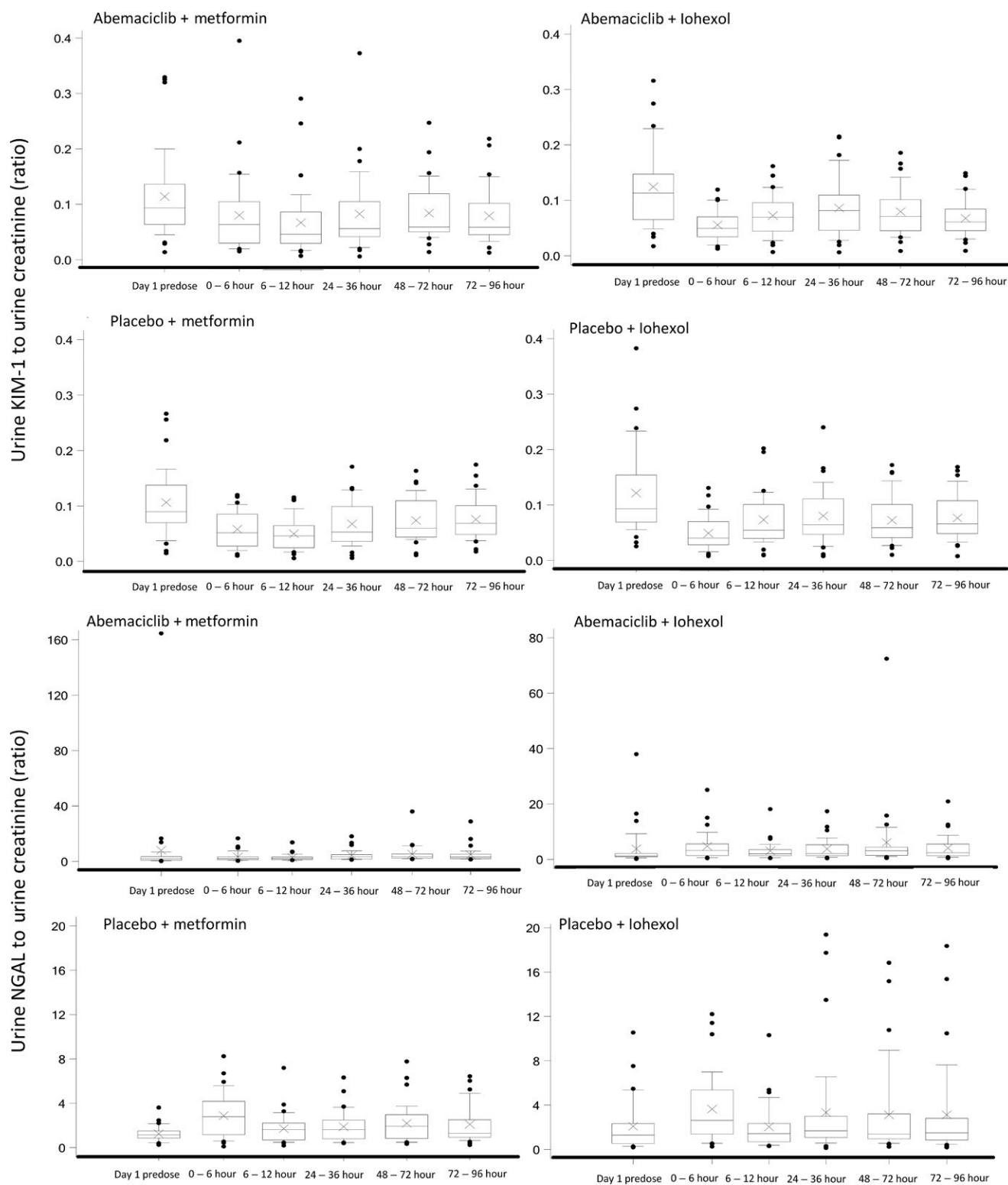


Figure 5 Creatinine-corrected urine kidney injury molecule (KIM)-1 and neutrophil gelatinase-associated lipocalin (NGAL) concentrations following a single oral dose of placebo or 400 mg abemaciclib with a single oral dose of 1,000 mg metformin or a single infusion of 3.235 g iohexol in healthy subjects up to 96 hours postdose. The middle line in each box plot represents the median, the top and bottom margins of the box represent the 75th and 25th percentiles. The whiskers extend to the 90th and 10th percentiles. Data extending beyond the 90th and 10th percentiles are plotted individually.

Bioanalytical methods

Abemaciclib and metformin concentrations were measured in plasma and/or urine (as applicable), at Q² Solutions (Ithaca, NY) and Covance Laboratories (Madison, WI), and iohexol concentrations were measured in plasma at Covance Bioanalytical Services (Indianapolis, IN) using validated liquid chromatograph tandem mass spectrometric methods.

Renal biomarkers were measured in serum and/or urine (as applicable) at Covance Central Laboratory Services (Indianapolis, IN).

Clinical study design

A randomized, single-center, single-blind, four-period, placebo-controlled crossover study was conducted in healthy subjects ($n = 40$) at Covance Clinical Research Unit, Dallas, TX. An independent ethical review board (Midlands Institutional Review Board, Kansas) reviewed and approved the trial protocol. The trial was registered at ClinicalTrials.gov (NCT02884089). Enrolled subjects received each of four dosing regimens (400 mg oral abemaciclib or placebo followed 5 hours later by 1,000 mg oral metformin and 400 mg abemaciclib or placebo followed 8 hours later by 3,235 mg iohexol administered as a 15-minute i.v. infusion) according to a randomized schedule. A 400-mg dose of abemaciclib was administered, as this was expected to achieve plasma concentrations approximating steady state following twice-daily dosing with 200 mg, and doses were timed such that the peak plasma concentrations of the coadministered drugs coincided. Subjects underwent a meal stabilization period of 3 days prior to dosing in order to minimize variation in SCr from diet.

Following study drug administration, timed blood samples were collected up to 120 hours post abemaciclib dose, 36 hours post metformin dose and 6 hours post iohexol dose for assessment of the respective analyte concentrations in plasma. Urine metformin concentrations were also assessed. Blood and urine were also collected up to 120 hours postdose for analysis of renal biomarkers serum cystatin-C, NGAL, and creatinine and urine NGAL, KIM-1, and creatinine, respectively. Study periods were separated by at least 16 days, except the placebo/metformin dosing period after which there was at least 5 days of separation.

Statistical analysis

PK parameter estimates for metformin and abemaciclib were calculated using standard noncompartmental methods using Phoenix WinNonlin software version 6.4 (Certara USA, Princeton, NJ). Iohexol PK parameters were calculated using a two-compartment i.v. infusion model.²³ Metformin CL_R was calculated by dividing the cumulative amount excreted in urine collected for 36 hours by the AUC_{0-36} .³⁷ In general, CL_R is given by:

$$CL_R = (GFR \cdot fu_p + CL_{RS}) - F \cdot (GFR \cdot fu_p + CL_{RS}) \quad (3)$$

where fu_p is the fraction unbound in plasma for metformin, which was assumed to be unity (reference), CL_{RS} is the clearance of renal secretion, and F is the fraction of metformin entering the renal tubule that is reabsorbed. In this analysis, we assumed that renal tubular reabsorption was negligible and F was set to zero. Metformin CL_{RS} was calculated for each subject as follows (Eq. 4):

$$CL_{RS} (\text{reference}) = CL_{R, \text{placebo}} - GFR_{\text{placebo}} \quad (4)$$

$$CL_{RS} (\text{test}) = CL_{R, \text{abemaciclib}} - GFR_{\text{abemaciclib}}$$

PK parameter estimates for plasma and urine were evaluated to delineate effects of abemaciclib on metformin PK. Log-transformed C_{\max} , $AUC_{0-\infty}$, CL_R , and CL_{RS} estimates were evaluated in a linear mixed-effects analysis of variance model with fixed effects for period, sequence, and treatment (metformin with abemaciclib (test) and metformin with

placebo (reference)), and a random effect for subject. The differences were back-transformed to present the ratios of geometric LS means of metformin with abemaciclib vs. metformin with placebo and the corresponding 90% CIs. Metformin T_{\max} was analyzed using a Wilcoxon signed rank test. Medians of differences between the abemaciclib and placebo dosing periods, the 90% CI for the medians of differences, and P values were calculated.

The GFR was analyzed using a similar method to that described for metformin PK, in which iohexol with abemaciclib was the test and iohexol with placebo was the reference. The eGFR was calculated using SCr or cystatin-C using the CKD-EPI creatinine or cystatin-C equations.

Statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC).

Analysis of exploratory biomarkers

End points for biomarker and safety analyses included serum concentrations of cystatin-C, NGAL, and creatinine, creatinine CL, and urinary concentrations of NGAL, KIM-1, and creatinine. Renal biomarker concentrations at the time of maximal creatinine concentration were calculated for each dosing period. Ratios of urine renal biomarkers to urine creatinine were calculated.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

Figure S1. Clinical study subject disposition.

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CONFLICT OF INTEREST

J.C.C., P.K.T., Y.A.P., J.B., A.Y.C., S.D.H., and P.K. are current or former employees of Eli Lilly.

AUTHOR CONTRIBUTIONS

J.C.C., P.K.T., Y.A.P., J.B., A.Y.C., J.R., S.D.H., P.K., and J.V.B. wrote the manuscript. J.C.C., P.K.T., Y.A.P., J.B., A.Y.C., J.R., S.D.H., P.K., and J.V.B. designed the research. J.C.C., Y.A.P., J.B., and A.Y.C. performed the research. J.C.C., P.K.T., Y.A.P., J.B., A.Y.C., J.R., S.D.H., P.K., and J.V.B. analyzed the data.

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