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Nonclinical Drug-Drug Interaction Profile of BCX4208, an Oral, Once-Daily, Novel Nonmetabolized Enzyme Inhibitor for Chronic Management of Gout

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BACKGROUND

- Common comorbidities associated with gout, including obesity, hypertension, diabetes, hyperlipidemia, and chronic kidney disease, may confer a greater risk of drug-drug interaction (DDI) through both polypharmacy and disease-associated alterations in drug absorption, distribution, metabolism, or excretion[1]
- Currently available antiinflammatory and urate-lowering therapies (such as colchicine, allopurinol, and probenecid) used for management of gout are associated with significant DDIs[1-3]
- BCX4208 is a novel, oral, once-daily purine nucleoside phosphorylase inhibitor in clinical development for the chronic management of gout. In a phase 1 pharmacokinetic (PK) study of BCX4208, a dose-dependent reduction in serum uric acid concentration was observed[4]

OBJECTIVES

- In this study, we have characterized the following:
  - The potential for BCX4208 to act as an inducer or inhibitor of cytochrome P450 enzymes
  - The potential for BCX4208 to act as a substrate or inhibitor of drug transporters
  - The in vitro metabolism of BCX4208

METHODS

CYP inhibition and induction

CYP inhibition

- BCX4208 (1, 10, 25, and 50 μM) was incubated in pooled human liver microsomes with marker substrates for CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4/5, and the catalytic activity of these isoforms was determined
- Probe substrate K_m concentrations were: 1.7 μM ethoxyresorufin (CYP1A2), 3.6 μM coumarin (CYP2A6), 1.5 μM amodiaquine (CYP2C8), 166 μM tolbutamide (CYP2C9), 40 μM S-mephenytoin (CYP2C19), 30 μM (+)-bufuralol (CYP2D6), 40 μM chlorzoxazone (CYP2E1), and 5 μM midazolam (CYP3A4/5)
- Selective inhibitors of each CYP enzyme were used as positive controls
- Rate of formation of probe substrate metabolite was determined using LC-MS/MS

CYP induction

- BCX4208 was incubated with human primary hepatocytes from 3 donors (Source: CellzDirect™, Pittsboro, NC) for 48 hours at 0.1, 1, and 10 μM
- Induction of CYP1A2, CYP2B6, CYP2C9, CYP3A4/5, MDR1 (P-glycoprotein [P-gp]), and MRP2 activity was assessed by both RT-PCR and LC-MS/MS methods. Enzymatic activity of CYP1A2, CYP2C9, and CYP3A4/5, with phenacetin deacetylase, diclofenac hydroxylase, and midazolam hydroxylase activity, were measured by LC-MS/MS methods

Transporter studies

OAT1 transporter studies

- To determine whether BCX4208 is an inhibitor for the OAT1 transporter, *Xenopus laevis* oocytes were injected with OAT1 cRNA or water, and p-aminohippuric acid (PAH) uptake was assessed in the absence or presence (1 or 10 μM) of BCX4208
- To determine whether BCX4208 is a substrate of OAT1 transport, 5 μM [14C]-BCX4208 was incubated with OAT1- and water-injected oocytes in the absence and presence of varying concentrations of probenecid

OCT2 transporter studies

- To examine whether BCX4208 is a substrate for OCT2, CHO cells and 2 stably transfected CHO-OCT2 cell lines (clone 6 and clone 7) were incubated with [14C]-TEA or [14C]-BCX4208 for 48 hours at 37°C in a humidified atmosphere of 5% CO_2
- To assess whether BCX4208 is an inhibitor of OCT2, 10 μM of [14C]-TEA as probe substrate was tested in cells following 1-minute incubation with BCX4208 (50 and 200 μM) and cimetidine (100 and 500 μM)

MDR1 (P-gp)

- Bidirectional (apical to basolateral and basolateral to apical; BA/AB ratio) transport of BCX4208 (10 μM) was assessed in Caco-2 cell monolayers grown to confluence on collagen-coated transwell plates
- Digoxin was used as a positive control

Biotransformation

Liver microsomal incubations, liver S9 fractions, and hepatocytes for metabolite profiling

- Liver microsomes, liver S9 fractions, and cryopreserved hepatocytes from male CD1 mouse, male Wistar rat/Sprague-Dawley rat, male Beagle dog, male Cynomolgus monkey, and pooled (male and female) human sources were incubated with [14C]-BCX4208 (10 μM)
- Midazolam (20 μM) was used as a positive control
- Samples were analyzed by an HPLC coupled to a Radiomatic Flo-one-Beta model 525A flow scintillation analyzer
- Metabolic identification was performed using a Micromass Q-TOF II mass spectrometer

RESULTS

- BCX4208 is not an inhibitor of CYP450 enzymes (Tables 1 and 2)

Table 1. Summary of IC_{50} (μM) Values for BCX4208 Toward Human Liver Microsomal P450 Activity

	IC_{50} Values (μM)							
CYP isoform	CYP1A2	CYP2A6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4/5
BCX4208	>50	>50	>50	>50	>50	>50	>50	>50

Table 2. Summary of Time-Dependent Inactivation of CYP3A4/5 by BCX4208[a]

CYP3A4/5	Concentration (μM)	% Loss of enzyme activity at 24 min of preincubation	K_{obs} (min^{-1})
BCX4208	10	No inactivation	0
Ethinylestradiol[b]	10	24.1	0.0116
Verapamil[b]	10	46.3	0.0269

[a] Values represent the mean of n=4. [b] Positive control.

- Lack of induction of CYPs, MDR1, and MRP2 in human hepatocytes by BCX4208 (Table 3)

Table 3. Summary of Enzyme Induction Activity Expressed as Fold Change in Human Hepatocytes[a]

Treatment	CYP1A2 mRNA	CYP1A2 enzyme activity	CYP2B6 mRNA	CYP2C9 mRNA	CYP2C9 enzyme activity	CYP3A4 mRNA	CYP3A4/5 enzyme activity	MDR1 mRNA	MRP2 mRNA
Rifampicin 0.1 μM	0.70-2.38	1.26-1.49	1.81-2.45	1.21-4.70	1.12-1.24	1.11-8.64	1.66-4.11	0.93-1.56	1.14-2.13
Rifampicin 1 μM	0.53-1.83	1.60-1.87	3.64-5.26	1.21-5.21	1.24-1.88	5.14-24.14	2.29-8.95	1.19-1.93	1.25-2.50
Rifampicin 10 μM	0.91-2.69	2.21-2.27	5.88-9.70	1.36-5.74	1.08-1.62	11.0-30.22	2.16-10.47	1.49-2.39	1.72-2.12
Omeprazole 0.1 μM	1.43-5.12	1.18-1.51	1.13-1.79	1.04-5.23	0.89-1.09	0.72-1.27	0.81-1.13	0.95-1.24	1.03-1.32
Omeprazole 1 μM	5.81-21.93	1.61-5.04	1.45-1.65	1.11-4.24	0.87-1.20	0.79-1.52	0.82-1.18	0.91-1.19	1.10-1.29
Omeprazole 10 μM	58.7-269.3	10.7-37.1	6.12-6.64	1.53-4.48	1.07-1.29	2.09-4.89	0.45-1.27	1.09-1.25	1.43-1.64
BCX4208 0.1 μM	0.90-1.94	1.02-1.04	0.71-1.44	0.98-1.60	0.95-1.15	0.73-0.98	0.85-1.09	0.63-1.12	0.89-1.08
BCX4208 1 μM	1.02-1.26	1.10-1.27	0.72-1.20	0.91-1.27	0.87-1.14	0.68-0.83	0.83-1.03	0.49-0.98	0.85-1.06
BCX4208 10 μM	1.01-2.39	0.91-0.94	0.86-2.02	0.90-1.63	0.96-1.27	0.81-1.12	0.87-1.22	0.45-1.15	0.89-1.29

[a] Ratio of test article activity to vehicle activity was calculated, and the range of ratios observed in triplicate experiments was reported.

- BCX4208 is not an inhibitor of OAT1 kidney transporter (Table 4)

Table 4. Summary of Inhibition of PAH Transport by OAT1 Kidney Transporter[a]

	OAT1-injected oocytes		Water-injected oocytes	
	n	Uptake (pmol/oocyte/h)	n	Uptake (pmol/oocyte/h)
No inhibitor	7	1.836 ± 0.610	9	0.178 ± 0.072
10 μM probenecid	9	1.044 ± 0.373	8	0.248 ± 0.085
1 μM BCX4208	9	1.984 ± 0.995	8	0.150 ± 0.089
10 μM BCX4208	10	2.298 ± 0.786	8	0.153 ± 0.118

[a] Uptake values are mean ± standard deviation.

- BCX4208 is not a substrate of OAT1 kidney transporter (Table 5)

Table 5. Summary of [14C]-BCX4208 Uptake by OAT1 Kidney Transporter[a]

		OAT1-injected oocytes		Water-injected oocytes	
Substrate	Probenecid (μM)	n	Uptake (pmol/oocyte/h)	n	Uptake (pmol/oocyte/h)
PAH	0	10	1.557 ± 0.563	11	0.171 ± 0.056
PAH	10	9	0.867 ± 0.348	10	0.175 ± 0.092
[14C]-BCX4208	0	9	0.089 ± 0.050	9	0.185 ± 0.045
[14C]-BCX4208	1	6	0.088 ± 0.044	6	0.139 ± 0.052
[14C]-BCX4208	10	7	0.066 ± 0.034	9	0.122 ± 0.042

[a] Uptake values are mean ± standard deviation.

- BCX4208 is not a substrate of OCT2 kidney transporter (Figures 1A and 1B)

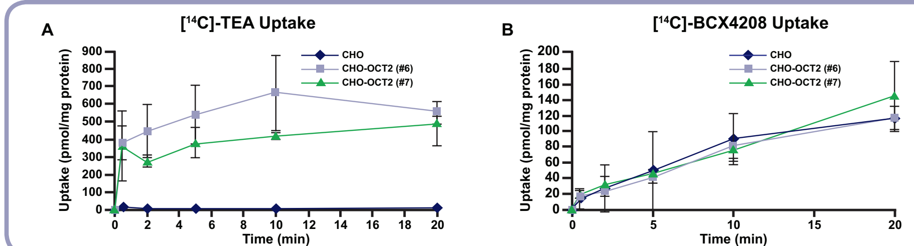

Figure 1. Compound uptake by CHO and CHO-OCT2 cells. **(A)** A time-course study of [14C]-TEA (positive control OCT2 substrate) in CHO, CHO-OCT2 (#6), and CHO-OCT2 (#7) cells. 3.125 μM of [14C]-TEA was used in this study, and the values represent mean ± standard deviation (n=3). **(B)** A time-course study of [14C]-BCX4208 in CHO, CHO-OCT2 (#6), and CHO-OCT2 (#7) cells. 5 μM of [14C]-BCX4208 was used in this study, and the values represent mean ± standard deviation (n=3).

- BCX4208 is a weak inhibitor of OCT2 kidney transporter at very high concentrations (Figure 2)

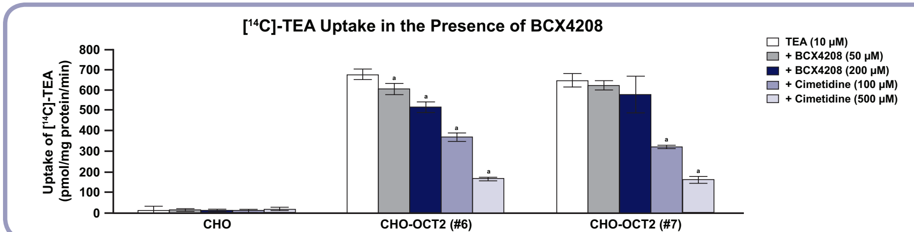

Figure 2. [14C]-TEA concentration was 10 μM. BCX4208 was used at 50 and 200 μM. Cimetidine was used at 100 and 500 μM. The values represent mean ± standard deviation (n=3). * Statistically significantly different from no inhibitor control. $P<0.001$ vs TEA alone.

- BCX4208 is not a substrate of MDR1 (P-gp; Table 6)

Table 6. Summary of BCX4208 Transport by MDR1 in Caco-2 Monolayers

Test article	Direction	Papp (10^{-6} cm/s)[a]	Efflux ratio	Significant efflux
BCX4208 10 μM	A-to-B	0.16		
	B-to-A	0.29	1.8	No
Digoxin 10 μM[b]	A-to-B	1.62		
	B-to-A	11.7	7.2	Yes

A, apical; B, basolateral. [a] Average of duplicate determinations. [b] Digoxin is provided as a positive control.

RESULTS SUMMARY

- BCX4208 is not an inducer or inhibitor of CYP isoforms
  - No significant change in substrate metabolism was observed in any tested CYP isoform (Table 1). In addition, BCX4208 was not shown to be a time-dependent inhibitor of CYP3A4/5 (Table 2)
  - BCX4208 did not induce protein synthesis or enzyme activity for all tested CYP isoforms in primary hepatocyte cultures (Table 3)
- BCX4208 is neither a substrate nor an inhibitor of OAT1 kidney transporter
  - BCX4208 did not significantly inhibit OAT1-mediated uptake of PAH, a good substrate for OAT1 (Table 4)
  - No measurable uptake of 5 μM [14C]-BCX4208 by OAT1-injected oocytes compared with negative control oocytes, indicating that BCX4208 is not an OAT1 substrate (Table 5)
- BCX4208 is not a substrate, but it is a weak inhibitor of OCT2 kidney transporter at very high concentration
  - Unlike [14C]-TEA, [14C]-BCX4208 levels were almost identical in OCT2 transfected cells and the control CHO cells (Figures 1A and 1B)
  - Uptake of [14C]-TEA (10 μM) was strongly inhibited by cimetidine at 100 μM (45%-50%) and 500 μM (75%-76%), but was only weakly inhibited by BCX4208 at 50 μM (4%-11%) and 200 μM (11%-24%) in CHO-OCT2 (#6) and (#7) cells (Figure 2)
- BCX4208 is a poor substrate of MDR1 (P-gp) with a BA/AB ratio of 1.8 (Table 6)
- BCX4208 is not metabolized in liver microsomes, S9 fractions, or hepatocytes (data not shown)

DISCUSSION AND CONCLUSIONS

- Potential for hepatic or renal DDIs is low given that BCX4208 does not induce or inhibit CYP isoforms, has low potential as a P-gp substrate or inducer, and is not a substrate or inhibitor of renal organic anion and cation transporters. BCX4208 undergoes renal elimination and is not metabolized by liver cells
- Furthermore, at the maximum dose (40 mg) used in phase 2B studies, the plasma C_{max} of BCX4208 was 42 ng/mL (0.159 μM), a concentration that is at least 30- to 100-fold lower than IC_{50} values determined in CYP or renal transporter inhibition studies
- These results provide good assurance that DDIs are not expected in the clinical setting with BCX4208 in a chronic gout patient population. Indeed, in a phase 2 trial of BCX4208 administered in combination with allopurinol, a first-dose PK assessment revealed no DDI with allopurinol or its active metabolite oxypurinol
- There is a low risk of DDIs between BCX4208 and coadministered medications

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References: **1.** Grosser T, Smyth E, FitzGerald GA. In: *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. Brunton LL, ed. 12th ed. New York, NY: McGraw-Hill Medical; 2011:994-1000. **2.** Li M, Anderson GD, Wang J. *Expert Opin Drug Metab Toxicol*. 2006;2(4):505-532. **3.** Vessel ES, Passananti GT, Greene FE. *N Engl J Med*. 1970;283(27):1484-1488. **4.** Fitz-Patrick D, Drummond W, Pappas J, Hollister AS. *Arthritis Rheum*. 2010;62(suppl 10):150.

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