P1524

ECCMID 2011

BC-3781: Evaluation of the CYP3A Interaction Potential

U. Schmidt, W.W. Wicha, F. Obermayr, R. Novak, W. Prince

Nabriva Therapeutics AG, Vienna, Austria

Nabriva Therapeutics AG
Leberstrasse 20
1110 Vienna, Austria
www.nabriva.com

Abstract

Background: *In vitro* and *in vivo* assessment of the interaction potential of BC-3781, an investigational pleuromutilin, with CYP450 enzymes.

Methods: Interaction of BC-3781 with CYP450 enzymes was investigated by reaction phenotyping using human recombinant CYP450 enzymes. Inhibition and induction was investigated *in vitro* using microsomes and human hepatocytes. The *in vivo* interaction of BC-3781 on the pharmacokinetics (PK) of midazolam and the interaction of ketoconazole on the PK of BC-3781 were investigated in two cross over phase I studies in healthy subjects.

Results: Reaction phenotyping using CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4 demonstrated exclusive metabolism of BC-3781 by CYP3A4 in this setting. Microsomal inhibition experiments identified CYP3A as primary target of BC-3781. While CYP3A mediated testosterone hydroxylation was very weakly affected by BC-3781 (IC_{50} > 300 µM) midazolam hydroxylation was inhibited with an IC_{50} value of 5.5 µM. Induction experiments with human hepatocytes from three single donors did not reveal any induction of CYP1A2 or 3A4 by BC-3781.

A clinical study investigating the effect of BC-3781 on midazolam PK identified BC-3781 as a very weak inhibitor of CYP3A. The AUC of midazolam increased by 1.17 (CI_{90} 0.82-1.67) while C_{max} was increased by a factor of 1.04 (CI_{90} 0.82-1.30) in the presence of BC-3781. Inhibition of CYP3A with ketoconazole identified BC-3781 as a weak CYP3A substrate in vivo with geometric mean AUC ratio estimates of 1.29 (CI_{90} 1.20-1.40). A C_{max} increase of 1.06 (CI_{90} 0.98-1.14) was below the weak CYP3A interaction threshold of 1.25 and within the no-effect boundaries.

Conclusions: While BC-3781 did not induce CYP1A2 and 3A4 it could be demonstrated that BC-3781 serves as a CYP3A4 substrate and inhibitor in vitro. Two clinical drug interaction studies with BC-3781 showed that the PK of BC-3781 is only marginally affected in the presence of ketoconazole and only a weak inhibition of CYP3A could be suggested from the study where midazolam was co-administered. The data obtained in both studies suggest that BC-3781 can be classified as having only a weak interaction with either CYP3A substrates or inhibitors in a clinical setting. Taken together, these results suggest that no major CYP450 mediated drug-drug interactions are expected with BC-3781.

Introduction and Purpose

BC-3781 is an investigational semi-synthetic pleuromutilin derivative. Pleuromutilin antimicrobial agents inhibit protein synthesis through interaction with the 50S ribosomal subunit and cross resistance with other antimicrobial classes is uncommon. BC-3781 has demonstrated potent antimicrobial activity against Gram-positive cocci relevant for skin and skin structure infections and Gram-negative pathogens associated with community-acquired bacterial pneumonia.

BC-3781 is being developed for the treatment of serious skin infections and bacterial pneumonia caused by *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma*, *Legionella* and other bacteria, including drug resistant strains such as MRSA and Vancomycin resistant *Enteroccocus faecium*. BC-3781 is under development for treatment of acute bacterial skin and skin structure infections (ABSSSI) and hospital treated community-acquired pneumonia (HCAP) with intravenous and oral dosing formulations.

Many drugs interact with CYP450 enzymes as substrates, inhibitors or inducers. Thus, we evaluated if BC-3781 can affect or might be affected by other CYP450 interacting drugs in order to prevent adverse drug reactions caused by drug-drug interactions by standard *in vitro* assays and in two clinical drug-drug interaction studies.

Methods

CYP reaction phenotyping: The metabolic stability of BC-3781 was tested *in vitro* against a battery of commercially available human CYP isoenzymes. BC-3781 was incubated in duplicates at a final concentration of 0.5 µM (0.25 µg/ml) for 60 min at 37 °C. The final CYP enzyme concentrations were between 10-50 pmol/ml, respectively. In parallel, the robustness of the assay was confirmed by testing respective standard CYP substrates (positive controls).

CYP inhibition: The potential to of BC-3781 to inhibit CYP enzymes was analyzed by determining IC_{50} values using a mixed gender pool of human liver microsomes. Inhibition experiments were carried out at a final protein concentration of 0.2 mg/ml for 15 min at 37 °C using recommended standard substrates.

CYP induction: The potential of BC-3781 to induce CYP1A2 and 3A4 was investigated using single human cryopreserved hepatocytes from three independent donors. Determinations were performed in triplicates with 0.75 x 10^6 viable cells each. The viability of the used hepatocytes was not significantly impaired over the treatment period (48 h) as determined by neutral red assays (data not shown). Positive control inducers rifampin and omeprazole, respectively, confirmed that the hepatocytes were inducible. Results of one representative donor batch are depicted (Figure 1).

Midazolam interaction study – study design: Midazolam (2 mg) was administered as a single oral dose alone or together with a single dose of 150 mg BC-3781 i.v. to 8 healthy female and 8 healthy male subjects aged 18-55 years. The study was designed as a single site randomized cross over study.

Ketoconazole interaction study – study design: The study was designed as a single site randomized cross over study on 12 male subjects. All subjects received a single dose of 150 mg BC-3781 administered as a single i.v. infusion on day 1 or day 2 and on day 7, day 4 to 7 all subjects received 200 mg ketoconazole *bid*.

Results

In vitro analysis of the interaction potential with CYP enzymes

CYP reaction phenotyping

- Among the isoenzymes tested only CYP3A4 was capable of metabolizing BC-3781

Table 1. Metabolism of BC-3781 by single CYP isoenzymes

BC-3781 [0.5 µM / 0.25 µg/ml]		
Isoenzyme	BC-3781 remaining after 60 min [%]	Positive control remaining after 60 min [%]
CYP1A2	106	5
CYP2B6	116	5
CYP2C8	102	13
CYP2C9	117	0
CYP2C19	107	8
CYP2D6	114	1
CYP3A4	0.4	1

CYP inhibition

- No significant inhibition of CYP1A, 2B6, 2C9, 2D6 and 2E1 was detected in a pre-inhibition screen at 10 µM (5.08 µg/ml) of BC-3781 (data not shown)
- Weak inhibition of CYP2C8 with an IC_{50} value of 66 µM (33.5 µg/ml) (Table 2)
- IC_{50} determinations identified CYP3A as primary target of BC-3781 displaying an IC_{50} value of 5.5 µM (2.79 µg/ml) for midazolam hydroxylation while testosterone hydroxylation was affected to a much lesser extend (IC_{50} value ≥ 300 µM (≥ 152 µg/ml))

Table 2. Inhibition of CYP isoenzymes by BC-3781

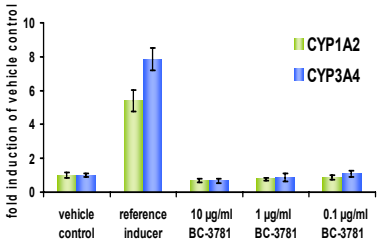
Enzyme	CYP2B6	CYP2C8	CYP2C19	CYP2D6	CYP2E1	CYP3A[a]	CYP3A[b]
IC_{50} [µM]	> 300	66	> 300	> 300	> 300	5.5	> 300

[a] substrate: midazalom, [b] substrate: testosterone

CYP induction

- BC-3781 did not induce CYP1A2 and 3A4 *in vitro*

Figure 1. Induction of CYP1A2 and CYP3A4 by BC-3781



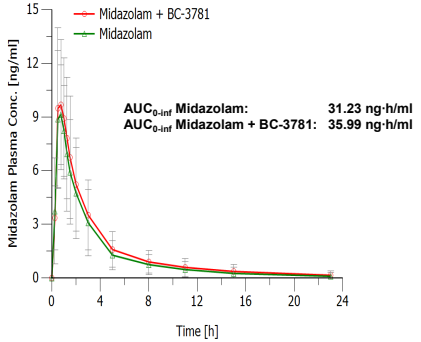
A clinical study to evaluate if BC-3781 is an inhibitor of CYP3A using midazolam

- Exposure of midazolam alone was not significantly altered when administered together with BC-3781
- The geometric mean ratio estimates of $AUC_{0\text{-}inf}$ were with 1.17 below threshold of 1.25 describing a weak CYP3A inhibitor as defined in the FDA and the EMA guidelines [1,2]
- The $AUC_{0\text{-}inf}$ CI_{90} exceeded the default no-effect boundaries of 0.8-1.25; therefore, a small effect of BC-3781 on midazolam PK could not be excluded
- The study drugs were safe and well tolerated

Table 3. Statistical analysis describing the drug-drug interaction potential of BC-3781 on midazolam

Midazolam PK parameters	Midazolam with/without BC-3781	
	Geometric mean ratio	CI_{90} lower, upper
$AUC_{0\text{-}inf}$	1.17	0.82, 1.67
C_{max}	1.03	0.82, 1.30
$t_{1/2}$	1.20	0.82, 1.75

Figure 2. Plasma-concentration curve of midazolam administered with and without BC-3781



A clinical study to evaluate if BC-3781 is a substrate of CYP3A using ketoconazole

- The C_{max} of BC-3781 was not significantly affected by the concomitant administration of ketoconazole whereas a mild effect on exposure was observed
- The geometric mean ratio estimates of C_{max} between co-medication and baseline was with 1.06 below the weak CYP3A interaction threshold of 1.25 and thus were not considered as clinically relevant
- The geometric mean ratio estimates of $AUC_{0\text{-}inf}$ were with a 1.29-fold only slightly above the threshold of 1.25, suggesting only a small contribution of CYP3A to the total clearance of BC-3781
- The study drugs were safe and well tolerated

Figure 3. Plasma-concentration curve of BC-3781 administered with and without ketoconazole

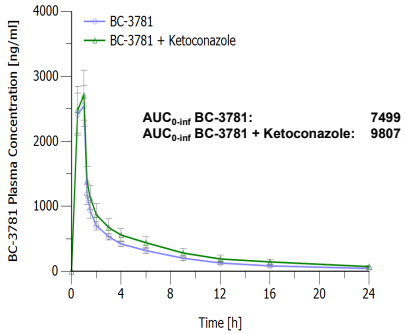


Table 4. Statistical analysis describing the drug-drug interaction potential of ketoconazole

Ketoconazole PK parameters	BC-3781 with/without ketoconazole	
	Geometric mean ratio	CI_{90} lower, upper
$AUC_{0\text{-}inf}$	1.29	1.20 ,1.40
C_{max}	1.06	0.98, 1.14
$t_{1/2}$	1.14	1.07 ,1.22

Discussion

The CYP450 interaction potential of BC-3781 was investigated *in vitro* and *in vivo*. *In vitro* experiments identified an interaction of BC-3781 with CYP3A. Furthermore, BC-3781 did not induce CYP1A2 or 3A4 in primary human hepatocytes *in vitro*. Consequently, it is not expected that these as well as co-inducible CYP isoenzymes and co-inducible p-gp will be upregulated in a clinical setting.

A potential interaction of CYP3A with BC-3781 was investigated in more detail in human. Midazolam was used as a substrate investigating the potency of BC-3781 to inhibit midazolam metabolism by CYP3A while ketoconazole was applied to investigate the magnitude of CYP3A mediated BC-3781 metabolism. The geometric mean ratio estimates of midazolam AUCs of 1.17 showed no significant CYP3A inhibition potential of BC-3781 *in vivo*. Due to high intersubject variability the CI_{90} of the $AUC_{0\text{-}inf}$ exceeded the no effect bounderies and a small effect of BC-3781 on midazolam pharmacokinetics cannot be ruled out. When co-administered with ketoconazole no increase in BC-3781 C_{max} was observed. The geometric mean ratio of C_{max} and its CI_{90} values where within the no effect bounderies. The geometric mean ratio estimates of BC-3781 $AUC_{0\text{-}inf}$ of 1.29 was only slightly above the threshold of 1.25. The observed interaction of ketoconazole with BC-3781 is classified as weak and implies only minor a contribution of CYP3A to the total clearance of BC-3781.

In summary, we demonstrated that BC-3781 interacted with CYP3A *in vitro*. However, the more detailed analysis in a clinical setting revealed only a marginal interaction of BC-3781 and CYP3A.

Conclusions

- BC-3781 was identified as a CYP3A interacting compound *in vitro*
- No induction of CYP1A2 or 3A4 by BC-3781 is expected in humans
- BC-3781 showed no effect on midazolam pharmacokinetics
- The pharmacokinetics of BC-3781 were only mildly affected by co-administration of ketoconazole
- No dose adjustment is required when BC-3781 is dosed with CYP3A substrates or inhibitors

References

1. Guidance for Industry: Drug Interaction Studies - Study Design, Data Analysis, and Implications for Dosing and Labeling 2006 (DRAFT). FDA.
2. Guideline on the Investigation of Drug Interactions, Draft version April 2010. EMA.

Acknowledgements:
In vitro studies were performed in part at Cerep, USA and clinical studies were conducted at FOCUS Clinical Drug Development GmbH, Germany