Poster

In vitro and in vivo characterization of the novel cathepsin C inhibitor BI 1291583 for use in bronchiectasis

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In vitro and in vivo characterization of the novel cathepsin C inhibitor BI 1291583 for use in bronchiectasis

Stefan Kreideweiss,[1] Gerhard Schänzle,[1] Gisela Schnapp,[1] Viktor Vintonyak,[1] Marc A. Grundl[1]

[1]Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany

INTRODUCTION

- Airway inflammation in chronic inflammatory lung diseases such as bronchiectasis is partly mediated by an imbalance between neutrophil-derived serine proteases (NSPs) and antiproteases.[1,2]
- The protease cathepsin C (CatC; also known as dipeptidyl peptidase 1) is the major enzyme activating NSPs (neutrophil elastase [NE], cathepsin G and proteinase 3 [PR3]) during myelopoiesis in the bone marrow.[3]
- Inhibition of CatC is an attractive target to improve the protease-antiprotease balance in the lungs of patients with bronchiectasis by decreasing the activation of NSPs in neutrophils;[2] this is expected to reduce distal airway destruction with possible secondary anti-inflammatory and anti-mucus hypersecretory effects.[4,5]

AIM

- To present preclinical in vitro/in vivo characterization of BI 1291583, a novel CatC inhibitor currently undergoing clinical testing.

METHODS

- **Binding kinetics** of BI 1291583 (at increasing doses of 0.08, 0.4, 2, 10 and 50 nM) to human CatC were assessed by surface plasmon resonance using a Biacore T200 system.
- **In vitro inhibition of human cathepsin enzymatic activity** in the presence of BI 1291583 was measured by conversion of fluorescent substrates specific for cathepsins C, B, F, H, K, L and S. The concentration of BI 1291583 that inhibited 50% of cathepsin activity (IC_{50}) was calculated using GraphPad Prism software with a non-linear regression curve fit.
- **Activity against a panel of unrelated proteases** from different classes was assessed by enzyme assays with BI 1291583 (10 µM), using validated fluorometric or photometric techniques as appropriate.
- **Inhibitory activity on the level of active NE** was investigated with BI 1291583 (0.064, 0.32, 1.6, 8, 40, 200 and 1000 nM) in the human myeloid neutrophil progenitor cell line U937. Cells were incubated for 48 hours and cell viability was measured using the CellTiter-Glo® Luminescent Cell Viability Assay. NE activity was measured by conversion of a fluorescent substrate.
- **In vivo inhibition of active NE and PR3 production** was tested in mice treated orally with BI 1291583 (0.00005, 0.0001, 0.001, 0.01, 0.03, 0.1, 0.5 or 5 mg/kg) or vehicle once daily for 11 consecutive days, followed by a lipopolysaccharide (LPS) challenge via inhalation on Day 12. Neutrophils were collected 4 hours post-challenge from bronchoalveolar lavage fluid (BALF), and the activities of NE and PR3 were measured. The dose of BI 1291583 that inhibited 50% of NE activity (ED_{50}) was calculated using GraphPad Prism software with a non-linear regression curve fit. The dose inhibiting 99% of NE activity (ED_{99}) was calculated from the regression curve using the equation $ED_{99}=99^{(1/hill\ slope)} \times ED_{50}$. A one-way ANOVA with Dunnett's multiple comparisons test analysis was performed to determine statistical significance.
- **Mean exposure in target tissue bone marrow and plasma** was measured, by liquid chromatography–tandem mass spectrometry, at efficacious doses of BI 1291583 (0.1 mg/kg, 0.5 mg/kg and 5 mg/kg) 6 hours after administration on Day 12.

CONCLUSIONS

- BI 1291583 is a potent, fully reversible and highly selective inhibitor of CatC, which has the potential to ameliorate neutrophilic inflammation and tissue destruction mediated by uncontrolled NSP activity in the airways.
- Results of this preclinical study support further clinical investigation of BI 1291583 in patients with bronchiectasis; of note, BI 1291583 has completed Phase I studies[6] and entered a Phase II trial.[7]

RESULTS

BINDING KINETICS

BI 1291583 binds human CatC in a covalent, fully reversible manner

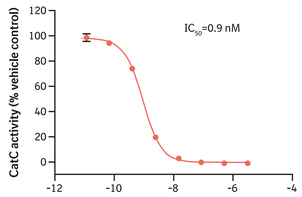
- BI 1291583 bound recombinant human CatC with mean k_{on} and k_{off} values at pH 4.5 of 6.36 x 10^5/M/s and 2.29 x 10^{-3}/s, respectively.
- The K_D value was 0.43 nM.
- Mean $t_{1/2}$ was 5.19 minutes.

IN VITRO INHIBITION OF HUMAN CATHEPSIN ENZYMATIC ACTIVITY

BI 1291583 selectively inhibits human CatC enzymatic activity in vitro

- The IC_{50} of BI 1291583 was 0.9 nM (Figure 1).

Figure 1. Inhibition of recombinant human CatC



- BI 1291583 has a >6,000-fold selectivity for CatC versus related cathepsins (Table 1).

Table 1. Comparison of BI 1291583 enzyme selectivity for recombinant human cathepsins

Enzyme	IC_{50} (nM)
Cathepsin C	**0.9**
Cathepsin B	> 100,000
Cathepsin F	> 100,000
Cathepsin H	> 100,000
Cathepsin K	6,695
Cathepsin L	7,225
Cathepsin S	25,200

ACTIVITY AGAINST UNRELATED PROTEASES

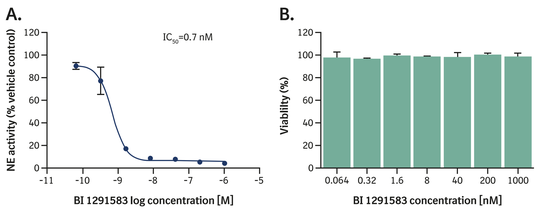
- No relevant inhibition or stimulation of unrelated proteases from different classes was detected at 10 µM (of 33 tested).
- In addition, no direct inhibition of NE or CatG was displayed.

INHIBITORY ACTIVITY ON THE LEVEL OF ACTIVE NE IN U937 CELLS

BI 1291583 inhibits the production of active NE in a neutrophil progenitor cell line, with no effects on cell viability

- Inhibition of active NE production was concentration dependent, with a mean IC_{50} of 0.7 nM (Figure 2a).
- Cell viability was >93% at all concentrations of BI 1291583 (>94% at highest concentration of 1000 nM) (Figure 2b).

Figure 2. (a) Inhibition of the production of active NE in U937 cells; (b) effect of BI 1291583 on cell viability

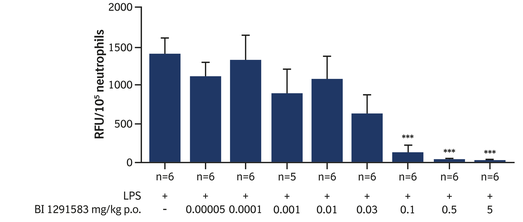


IN VIVO INHIBITION OF ACTIVE NE AND PR3 PRODUCTION IN MICE

BI 1291583 inhibits the production of active NE and PR3 in vivo

- In vivo production of active NE in mice BALF neutrophils was completely attenuated by BI 1291583 in a dose-dependent manner (ED_{50}=0.03 mg/kg and ED_{99}=0.3 mg/kg) (Figures 3 and 4).
- Levels of active PR3 were similarly reduced (Figure 5).

Figure 3. NE activity in mouse BALF neutrophil lysate after treatment with BI 1291583 and subsequent LPS challenge, 11-day dosing



Data are shown as mean ± standard error of mean; ***P<0.001 vs LPS control; NE activity shown as RFU normalized to the number of neutrophils.

Figure 4. Dose-response for inhibition of active NE production in mouse BALF neutrophil lysate after treatment with BI 1291583 and subsequent LPS challenge, 11-day dosing

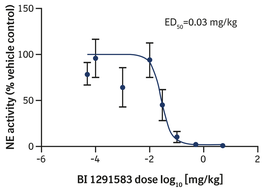
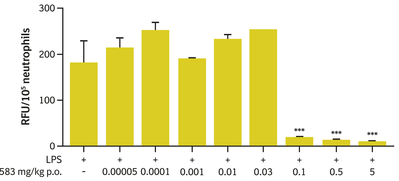


Figure 5. PR3 activity in mouse BALF neutrophil lysate after treatment with BI 1291583 and subsequent LPS challenge, 11-day dosing

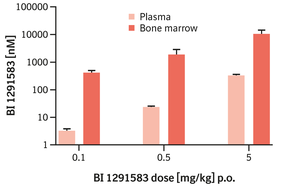


MEAN EXPOSURE IN BONE MARROW AND PLASMA

BI 1291583 distributes preferentially to bone marrow

- Due to its physicochemical properties, BI 1291583 shows over 100x higher exposure in the bone marrow compared with plasma at efficacious doses (0.1, 0.5 and 5 mg/kg), which may reduce the risk of unwanted effects of inhibiting CatC in the periphery (Figure 6).

Figure 6. Bone marrow and plasma distribution of BI 1291583 at 6 hours post-administration



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ABBREVIATIONS:
BALF, bronchoalveolar lavage fluid; CatC, cathepsin C; ED_{50}, effective dose that inhibits 50% of production of active NE; ED_{99}, effective dose that inhibits 99% of production of active NE; IC_{50}, concentration of BI 1291583 resulting in 50% inhibition of activation of NE; K_D, equilibrium dissociation constant; k_{off}, dissociation rate constant; k_{on}, association rate constant; LPS, lipopolysaccharide; NE, neutrophil elastase; NSP, neutrophil-derived serine protease; P.O., orally; PR3, proteinase 3; RFU, relative fluorescence units; $t_{1/2}$, half-life.

DISCLOSURES:
All authors are employees of Boehringer Ingelheim.

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