Human Metabolism of a Novel Chemotherapeutic: Illuminating the Mechanisms of P450-Catalyzed Deboronation

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Multiple Myeloma (MM)
plasma cell malignancy

- 5 cases in 100,000 people
- YR 2005 : 16,000 new cases
- 50,000 with MM in USA

Multiple Myeloma
(amplified proteasome activity)
(1) uncontrolled division
(2) traffic to bone
(3) cell adhesion
(4) ↑ M protein

www.multiplemyeloma.com (MMRF)
MM Cells Depend on 26S Proteasome

cell cycle control

antigen processing

stress response

Threonine protease

courtesy of Dr. M. Groll
Ludwig-Maximilians-University of Munich
Bortezomib : Transition State Analog of 26S

- approved in 2003 for treatment of multiple myeloma*
- currently approved for 2nd line treatment
- undergoing multiple clinical trials

<table>
<thead>
<tr>
<th>enzyme</th>
<th>$K_i$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20S proteasome</td>
<td>0.62</td>
</tr>
<tr>
<td>Leuk. elastase</td>
<td>2,300</td>
</tr>
<tr>
<td>cathepsin G</td>
<td>630</td>
</tr>
<tr>
<td>chymotrypsin</td>
<td>320</td>
</tr>
<tr>
<td>thrombin</td>
<td>13,000</td>
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</tbody>
</table>

*Bortezomib : Transition State Analog of 26S


*relapsed, refractory
Human Disposition of Bortezomib

- ~ 83% protein bound
- high $V_d$; long elim. $t_{1/2}$
- not subjected to drug efflux (e.g., Pgp)
- does not inhibit efflux in Caco-2 cells
- No obsvd inhibition of P450 (M1/M2-2C19)
- P450 phenotype (3A4>2C19>1A2>2D6>2C9)
- renal elimination of metabolites
Human Metabolism of Bortezomib

Drug Metab. Dispos. 2005, 33, 771-77.
Routes of Borane/Boronate Oxidation
(H.C. Brown)

A

\[
\begin{align*}
R\text{BOH} & \xrightarrow{\text{H}_2\text{O}_2, \text{OH}} R\text{BOH}^+ & \text{peroxidation} \\
& \xrightarrow{\text{B(OH)}_3} \text{ROH} & \text{retention}
\end{align*}
\]

B

\[
\begin{align*}
R\text{BOH} & \xrightarrow{\text{XO} \cdot} \text{ROX} & \text{autooxidation} \\
& \xrightarrow{\text{reduction, } \text{H}^+} \text{ROH} & \text{no stereocontrol}
\end{align*}
\]

\[\text{XO} \cdot = \text{O}_2 \text{ or peroxyl radical}\]

No published accounts of boronic acid metabolism (i.e., P450)
Human Liver Metabolism of Bortezomib

What is the mechanism of this novel P450 reaction?

Near equal ratio of M1:M2

No observed interconversion
Use of $^{18}\text{O}_2$ resulted in the formation of $^{18}\text{O}$-labeled M1 and M2.
Stability of Bortezomib in Formulation

Fortified solutions of bortezomib with ascorbate & EDTA.

Ascorbate & EDTA

reactive oxygen species mediate deboronation.

Schematic stability behavior.” V. Stella


Fenton-type rxn:

Fe(II)SO₄, O₂, buffer (pH 7.4)

-O₂/H₂O₂ produced
Oxidase Activity of P450: ROS Formation ("uncoupling")

P450 2E1 Metabolism of Bortezomib “the leaky CYP”

2E1 reaction

2E1 reaction + SOD/catalase

HLMs convert to M1 & M2

M1

M2

M3
Evidence of Radical Intermediate?

[Chemical diagram showing the reaction of GSH with NADPH and a drug, leading to the formation of carbinolamides and GSH conjugates, with mass spectrometric analysis (LC/MS/MS)].

- Metabolites obtained from rat bile
- 1H/13C NMR-confirmed
- Regiochemistry of carbinolamides
Zen and the art of P450 Catalysis…

“The real purpose of the scientific method is to make sure Nature hasn’t misled you into thinking you know something you don’t actually know. If you get careless or go romanticising scientific information, giving it a flourish here or there, Nature will soon make a fool out of you.”

Robert Pirsig

Zen and the Art of Motorcycle Maintenance (Copyright© 1974, Pirsig)
3A4 Metabolism of Bortezomib

3A4 reaction

3A4 reaction + SOD/catalase

2° metabolites

partial contribution by ROS?
Enzyme-bound oxidants contributing?

Peroxoxo-iron

Oxo-iron

FeTPP:PhIO

6-fold increase in M1:M2 over control (PhIO)

Oxometalloporphyrin biomimetic

see references within Chem. Res. Toxicol. 2001, 14, 611.
Summary of this Novel P450 Reaction

[We can teach you metabolism; we can’t teach you chemistry.]
G.T. Miwa

Thank you Gerald.

Sincerely,

A Grateful Chemist
Acknowledgements

Millennium DMPK

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