Investigation of CA-4 metabolism and related β-lactam analogues in chemoresistant HT-29 colon cancer cells

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Introduction

Drug resistance is a common cause of the failure of chemotherapeutic agents to achieve cytotoxicity responses in human malignant disease.

Drug inactivation by metabolism within tumour cells is recognised as an important mechanism of drug resistance [1].

Glucuronidation is a major route for the metabolic inactivation of many drugs and also endogenous substances.

Combetastatin A4 (CA-4) undergoes direct glucuronidation in the presence of UGTs at the meta-hydroxy group of the B-ring and could cause an inherent resistance in HT-29 colon cancer cells [2].

Results and Discussion

Comparison of antiproliferative activity between m-hydroxy ring B and deletion of m-hydroxy ring B β-lactam compounds

The excellent activity of CA-4 in MCF-7 cells in nanomolar range and its resistance in HT-29 cells with IC50 = 4 μM is well known.

Compound 81 (Bearing m-hydroxyphenyl ring B) mimics the same manner of low cytotoxicity as CA-4 in HT-29 cells.

Compound 119 is an example of deletion of m-hydroxy in ring B that showed the significant improvement of cytotoxicity in HT-29 cells compared to its related m-hydroxyphenyl ring B β-lactam as well as CA-4.

Expression level of UGT protein is significantly higher in CA-4 resistant HT-29 cells as compared to CA-4 sensitive MCF-7 and HL-60 cells.

• The endogenous level of UGT in CA-4 resistant HT-29 cells was significantly higher when compared to UGT expression levels in CA-4 sensitive MCF-7 and HL-60 cells.

• The apparent abundant expression of UGT in HT-29 cells would confer resistance to CA-4 and derivatives of CA-4 that contain the required phenolic functional groups which facilitate glucuronate conjugation and subsequent inactivation.

Microsomal metabolic stability

CA-4 is rapidly metabolised by glucuronidation.

81 (m-hydroxy ring B) demonstrated 66% remaining at 45 min.

130 and 218 displayed considerable stability toward hepatic enzymes.

Evaluation of CA-4 stability in HT-29 cells using different UGT inhibitors

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Untreated</th>
<th>10 μM</th>
<th>20 μM</th>
<th>40 μM</th>
<th>60 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7</td>
<td>125%</td>
<td>115%</td>
<td>105%</td>
<td>95%</td>
<td>85%</td>
</tr>
<tr>
<td>HT-29</td>
<td>115%</td>
<td>105%</td>
<td>95%</td>
<td>85%</td>
<td>75%</td>
</tr>
<tr>
<td>HL-60</td>
<td>105%</td>
<td>95%</td>
<td>85%</td>
<td>75%</td>
<td>65%</td>
</tr>
</tbody>
</table>

• Many different glucuronidation inhibitors for CA-4 were used to evaluate UDP-glucuronosyltransferase activity toward CA-4.

• Three different known inhibitors of glucuronidation were used: boerenol, propofol and 4-nitrophenol.

• 4-Nitrophenol was found to be the most potent inhibitor of UGTs activity in HT-29 cells compared to other inhibitors which used boerenol and propofol.

• There is a significant improvement of CA-4 and β-lactam 81 cytotoxicity pretreated with 4-nitrophenol at 6, 12 and 24 h at higher concentration than 10 and 50 μM.

• Intrinsic clearance (CLint) (μL/min/mg protein) and half-life (t1/2) (min) for flufenamic acid, mfenamic acid as positive controls, together with CA-4 in HT-29 cells in the presence or absence of boerenol, propofol, and 4-Nitrophenol are shown in table 1.

• CA-4 pre-treatment with boerenol indicated weak inhibition

• There is a significant intracellular accumulation of CA-4 treated with propofol compared to CA-4 alone.

• 4-nitrophenol produced a significant inhibition of CA-4 metabolism by glucuronotransferase in HT-29 cells.

Conclusion

• CA-4 resistance mediated by glucuronidation could be inhibited weakly by a broad UGT inhibitor Borenol, and strongly inhibited by UGT1A9 competitive inhibitor propofol, or UGT1A6 substrate 4-nitrophenol.

• The strategic deletion of the ring B hydroxyl group can produce CA-4 analogues that are equally effective in cancer cells expressing UGTs as compared to those expressing little or undetectable levels of UGTs, offering a simple solution to overcoming resistance associated with glucuronidation of CA-4.

References:


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