Triclosan and Resveratrol Binding to Human Carbonyl Reductase 1

Human Carbonyl Reductase 1 (HCBR1) is a monomeric, NADPH-dependent enzyme with a very broad substrate specificity with carbonyl compounds. Two such substrates are the anticancer drugs daunorubicin and doxorubicin. HCBR1 reduces both drugs to the less effective and cardiotoxic alcohol metabolites daunorubicinol and doxorubicinol. Earlier work by Forrest et al. (Forrest et al. (2000) Cancer Res. 60, 5158-5164) showed that overexpression of HCBR1 in transgenic mice advances the development of doxorubicin-induced cardiotoxicity more so than in non-expressing mice. These findings suggest that inhibition of HCBR1 may lower the risk of cardiotoxicity associated with treatment with daunorubicin and doxorubicin. Triclosan, a widely used antimicrobial compound, has been found to be a potent inhibitor of HCBR1 in vitro and in vivo. Resveratrol, a polyphenol found in many seed plants is another compound with the recently discovered property of inhibiting HCBR1 both in vitro and in vivo, although not as effectively as triclosan. (Charlier and Ewing 2009, patent number WO2009048760(A2)) This research attempts to better understand how triclosan and resveratrol bind to and inhibit HCBR1 by determining which form of HCBR1 binds to these inhibitors and the binding constants of these bonds. Based on inhibition studies, triclosan and resveratrol are predicted to bind to the enzyme-NADP⁺-product complex. Intrinsic protein fluorescence quenching studies of three forms of HCBR1 (the HCBR1-NADPH and HCBR1-NADP⁺ holoenzymes and the apoenzyme) with triclosan failed to directly confirm binding to the enzyme-NADP⁺ complex. However these same studies performed with resveratrol succeed in showing that resveratrol binds to the HCBR1-NADP⁺ holoenzyme. This shows that resveratrol is mimicking an alcohol product and perhaps other product analogs could effectively inhibit HCBR1.

Anthracyline cardiotoxicity may be caused by human carbonyl reductase 1.

Human Carbonyl Reductase 1 (HCBR1) reduces the carbonyls of the valuable anticancer drugs doxorubicin and daunorubicin to the cardiotoxic and less medicinally effective alcohols doxorubicinol and daunorubicinol respectively.

- Inhibition of HCBR1 is a promising and effective solution to this problem.
- Triclosan and trans-resveratrol have been found to inhibit HCBR1 both in vitro and in vivo.

Inhibition of HCBR1 may significantly reduce anthraclyine cardiotoxicity.

The goals of this research are to:

1. Determine the form(s) of HCBR1 with the greatest affinity for triclosan and resveratrol.
2. Determine the binding constants associated with this form of HCBR1 and inhibitor.
3. Suggest possible paths for future research.

Resveratrol binds to the HCBR1-NADP⁺ complex.

A fluorescence spectrophotometer or fluorimeter was used to perform fluorescence quenching titrations which measure the decline in fluorescence intensity of the various forms of HCBR1 at each addition of titrant.

- The triclosan titration data failed to show any real quenching so it has been omitted here.
- The resveratrol HCBR1-NADP⁺ graph (3) is most likely the only one that shows real quenching due to inhibitor binding. The quenching shown in graphs 1 & 2 is most likely entirely due to suspended resveratrol particles since resveratrol is poorly soluble in water.

Resveratrol may be a useful uncompetitive inhibitor of HCBR1.

- Based on the binding constants and quenching curves resveratrol primarily binds to the HCBR1-NADP⁺ holoenzyme.
- This is useful because it confirms that resveratrol does not compete with daunorubicin or doxorubicin and therefore resveratrol’s effectiveness as an inhibitor is independent of the amount of drug present.
- Quenching of the apoenzyme and HCBR1-NADPH holoenzyme is most likely entirely due to the methanol dissolved resveratrol slightly coming out of solution when added to the water based phosphate buffer in the cuvette.
- Because it has been shown that resveratrol binds to the HCBR1-NADP⁺ holoenzyme future studies may now investigate the binding mechanism or resveratrol’s specificity as an inhibitor.

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<tr>
<th>Resveratrol Binding Constants Kd (µM)</th>
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<tr>
<td>1) HCBR1</td>
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<td>2) HCBR1-NADPH</td>
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<td>3) HCBR1-NADP⁺</td>
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