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Connections between Aryl Hydrocarbon Receptor Activation and Induction of Carbonyl Reductase Expression

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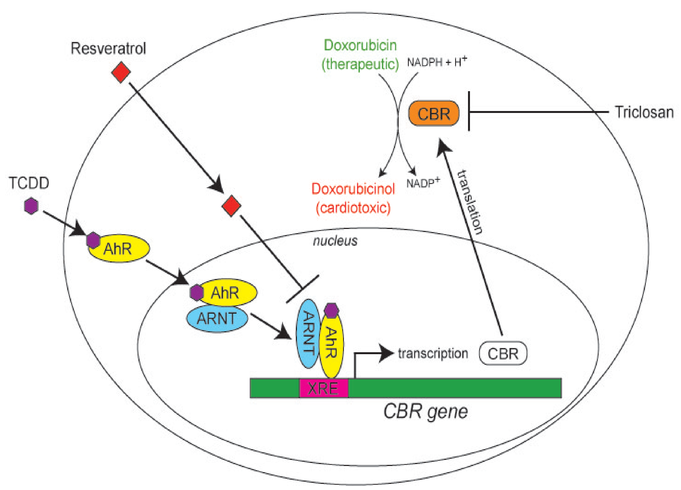
INTRODUCTION

The death rate due to cancer has not improved over the past five decades (3). Anthracyclines, such as doxorubicin, are some of the most effective anti-cancer chemotherapy agents used to treat several cancers. However, the use of anthracyclines is limited due to the chronic and potentially lethal cardiotoxicity associated with these drugs. The mechanism involves the two-electron reduction of the C-13 carbonyl group of the anthracyclines by carbonyl reductase (CBR). The resulting alcohol metabolites of anthracyclines (doxorubicinol and daunorubicinol) not only contribute to cardiotoxicity, but also possess diminished cytotoxicity against cancer cells (1). Therapeutic approaches to regulate CBR expression and/or activity may lead to increased efficacy of anthracyclines for cancer therapy and decrease the cardiotoxicity associated with the use of these drugs.

Recent evidence links aryl hydrocarbon receptor activation to the induction of CBR (2). The AhR is a soluble, ligand-activated transcription factor that modulates expression of genes involved in xenobiotic metabolism, apoptosis, and cell proliferation and differentiation (4). The goal of this study was to use an AhR agonist and antagonist to determine how AhR activation regulates CBR protein expression in a rat hepatoma cell line (5L cells). We also tested the hypothesis that doxorubicin induces CBR expression through a pathway that involves AhR activation. To this end, 5L cells were treated with the drugs/chemicals shown in Table 1, and cell extracts were analyzed by western blot to detect CBR, as well as Cyp1a1, which is an AhR-regulated gene whose expression is indicative of AhR activation.

PROPOSED MECHANISM

Figure 1. Proposed relationship between AhR activation and CBR induction.



AhR = Aryl Hydrocarbon Receptor
ARNT = Aryl Hydrocarbon Receptor Nuclear Translocator
CBR = Carbonyl Reductase
TCDD = 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
XRE = Xenobiotic Response Element

EXPERIMENTAL DESIGN

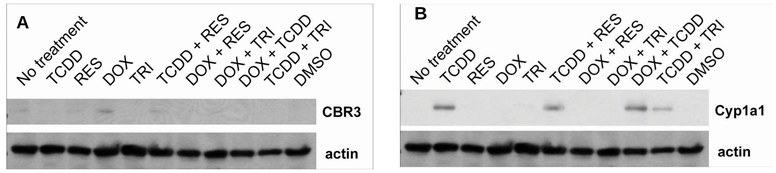
TABLE 1: Description of drugs/chemicals used in this study.

Drug or chemical	Abbreviation	Final concentration	Function
2,3,7,8-Tetrachorodibenzo-*p*-dioxin	TCDD	10 nm	AhR agonist
Resveratrol	RES	25 mm	AhR antagonist
Doxorubicin	DOX	5 mm	Induces CBR
Triclosan	TRI	25 mm	Inhibits CBR

Rat hepatoma 5L cells were grown to 70% confluency. Cells were washed with PBS and treated with the chemicals listed above, or combinations of these chemicals, for 24 hours. Cells were then washed and collected using RIPA lysis buffer that contained protease inhibitors. Cell extracts were quantified using a Lowry protein assay and resuspended in SDS loading buffer. Cell extracts were run on 10% SDS polyacrylamide gels at 50 ug protein per lane. Gels were transferred to PVDF membranes, blocked with Superblock (Fisher Scientific), and incubated with antibodies against CBR or Cyp1a1, followed by HRP-conjugated secondary antibodies. Protein bands were visualized with chemiluminescence.

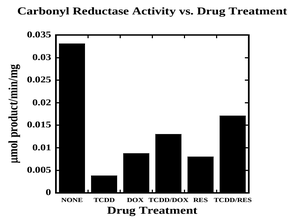
RESULTS

Figure 2. Consequences of chemical treatment on protein expression.



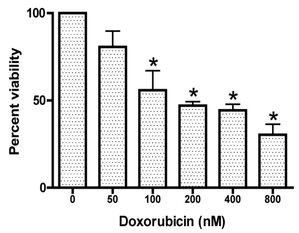
Western blot showing the induction of (A) CBR3 and (B) Cyp1a1 protein expression in rat hepatoma 5L cells treated for 24 hr with the indicated chemicals.

Figure 3. Consequences of chemical treatment on CBR enzyme activity.



Enzymatic study to measure carbonyl reductase activity in cell lysates.

Figure 4. Doxorubicin treatment diminishes viability of 5L cells.



Rat hepatoma 5L cells were grown to 80% confluency . Cells were treated with DOX for 24 hours . Alamar Blue was added to measured the percent viability of the cells.

SUMMARY AND CONCLUSION

• Our data confirm that DOX induces CBR protein expression, which agrees with previous studies. However, we don't know the exact mechanism by which this is taking place. Our proposed mechanism postulates that induction of CBR by DOX occurs through the AhR pathway. If DOX induces CBR through the AhR pathway then the inhibition of AhR will provide a means for controlling CBR protein expression during anthracycline treatment.

•TCDD is known for inducing Cyp1a1 protein expression via the AhR pathway, which is shown in our data (Fig. 3). However, we observed that when rat hepatoma 5L cells were cotreated with TCDD + TRI, a decrease in Cyp1a1 protein expression was observed. This could mean that TRI is an inhibitor of AhR signaling. TRI is a documented CBR inhibitor.

•Treatment of TCDD in combination with DOX or RES showed higher levels of CBR activity than in cells treated with DOX or RES alone. Even though RES is known to inhibit the AhR pathway, its inhibition may be masked by the potent induction of CBR activity by TCDD.

•Cell viability measurements using an Alamar Blue assay revealed that DOX has an IC50 value of 200nM (Fig.5), which indicates that 200nM is the amount of DOX required to inhibit half of the cellular metabolic activity.

FUTURE STUDIES

1. Future studies will consist of dose response and time course studies to determine the effects of DOX on CBR expression levels and AhR activation.
2. We will also perform DOX cytotoxicity studies for killing in presence/absence of AhR (using AhR-positive and AhR-defective cells) to see whether inhibiting the activity of AhR will impact DOX cytotoxicity.
3. We will also perform an Alamar Blue assay with a combination of DOX and RES to determine the IC50 value of DOX in the presence of RES. This will reveal whether RES can lower the IC50 value of DOX and enhance DOX cytotoxic activity through the inhibition of cellular metabolic activity.

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