Computational model of the anthracycline-binding site in carbonyl and aldo-keto reductases: a structural basis for designing inhibitors

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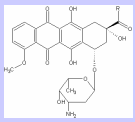
INTRODUCTION

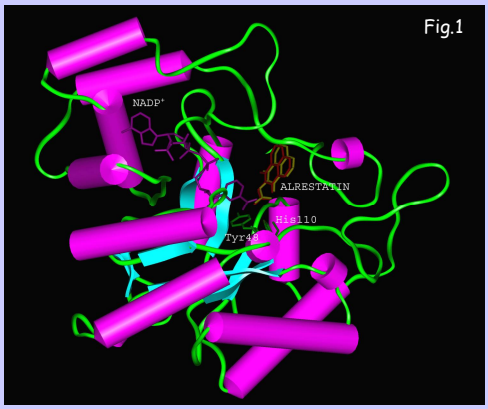
Anthracycline antibiotics such as doxorubicin (DOX) and daunorubicin (DAU) are widely used antineoplastic agents in the treatment of various types of cancer. However their use is limited by the major side effect of cardiac toxicity which has not been effectively prevented by pharmacologic intervention with cardioprotective drugs. The mechanism by which DOX and DAU or their metabolites cause chronic cardiomyopathy is not fully understood. Cytosolic reductases have been implicated in the development of anthracycline-induced cardiotoxicity. Carbonyl reductase 1 (CBR1) and aldo-keto reductases AKR1 are known to catalyze the reduction of DOX and DAU to the corresponding C13-hydroxyanthracycline. It has been demostrated (Olson et al. *Cancer Research*, 2003, 63, 6602-6606) that a decrease in CBR1 would limit doxorubicin-induced toxicity. Thus, diminution of CBR1 activity using pharmacologic inhibitors may be a useful means of ameliorating the side effects of doxorubicin in patients undergoing chemotherapy. Until now, no evidence has been provided for the spatial location of the DOX and DAU binding site in the carbonyl and aldo-keto reductases . The aim of this work was therefore to investigate the interaction mechanism of the anthracyclines with the cytosolic reductases. Predictive models have been constructed by means of a molecular docking study, that offer utility in guiding the rational design of inhibitors of reductase activity.

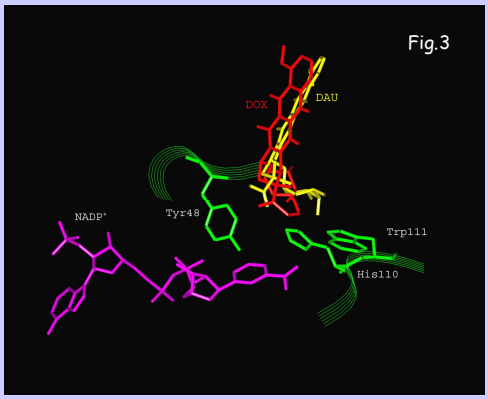
METHODS

Docking of DOX and DAU into carbonyl and aldo-keto reductases was carried out using the software GRID. The GRID program (Goodford, P.J. *J. Med. Chem.* 1985, 28, 849-857) determines the interaction energy of a chemical fragment or "probe" at points on a grid which encloses a target macromolecule. A molecular docking module, GLUE, implemented in GRID, identifies potential ligand binding sites by fitting the maps generated by GRID for the probes which mimic the structure of the ligand. The program was tested on the crystal structures of human aldose reductase bound to alrestatin (pdb code 1AZ1) and of human carbonyl reductase CBR1-hydroxy-PP complex (pdb code 1WMA). The same computational approach was then used to investigate potential binding sites of DOX and DAU in both aldose and carbonyl reductases. The ligands were built up in InsightII (Accelrys Inc.) and energy-minimized *in vacuum* using the CHARMM force field (Brooks et al. *J. Comput. Chem.* 1983, 4, 187-217). The following single atom probes which mimic the structure of DOX and DAU were used in the GRID calculations : H, OH2, DRY, C3, N2:, O, O1, OH, OC2.



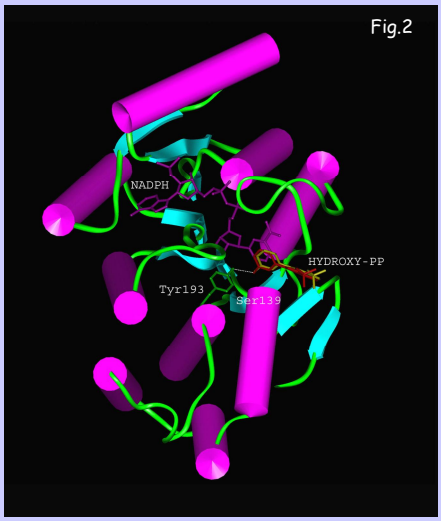
R = CH3 Daunorubicin
R = CH_2OH Doxorubicin

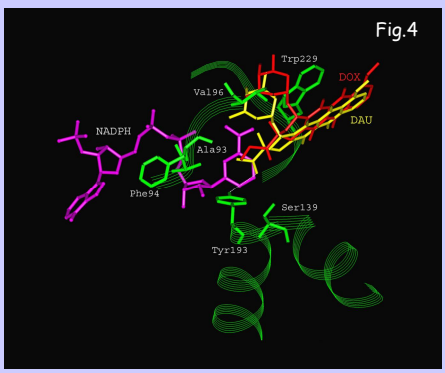




RESULTS

The docking program GLUE was successful in generating multiple poses that include binding modes similar to the crystallographically determined bound structure. **Figure 1** shows that the alrestatin molecule is correctly positioned within the binding site of aldose reductase. The predicted orientation of alrestatin is shown in yellow, superimposed onto the crystallographic position (red). Also hydroxy-PP is correctly docked into carbonyl reductase structure. The overlay of the docking pose (yellow) and the crystal structure binding mode (red) of hydroxy-PP is shown in **Figure 2**. The same computational protocol was then applied to characterize anthracycline binding sites in cytosolic reductases. The optimal binding position for DOX and DAU in human aldose reductase is reported in **Figure 3** which shows that C13 carbonyl group of both anthracyclines is close to the C4 of the coenzyme nicotinamide ring and to the binding site formed by Tyr 48 and His 110. Estimated binding energies by GLUE of DOX and DAU with aldose reductase were –15.1 kcal/mol and –16.8 kcal/mol respectively. The preferred docking orientation of DOX and DAU in carbonyl reductase 1 are reported in **Figure 4**. Molecular docking reveals anthracycline C13 carbonyl group close to the C4 of the coenzyme nicotinamide ring and to the the active site of CBR1 which involves Ser 139 and Tyr 193. It is worth noticing that DAU is hydrogen bonded to Tyr 193. Estimated binding energies of DOX and DAU with CBR1 were –15.4 kcal/mol and –17.9 kcal/mol respectively.





CONCLUSIONS

To design reductase inhibitors that would limit DOX and DAU- induced cardiotoxicity, initial investigations have focused on the molecular mechanism by which the cytosolic reductases bind the anthracyclines. So far, no structural model for the anthracycline-reductase interaction has been proposed. In the current study we generated theoretical complexes of DOX and DAU bound to human aldose and carbonyl reductases. Molecular modelling provides a rationale for the affinity differences pointed out by docking studies:

- A stacking interaction between the anthracycline molecule and the indole ring of Trp229 of human carbonyl reductase may account for the predicted major affinity of DOX and DAU toward CBR1 when compared to aldose reductase.
- The presence of a polar OH group in C14 position of DOX molecule which locates at the base of the hydrophobic cavity representing the enzyme's active site may decrease DOX binding in both reductases when compared to DAU.