Do anthracycline's elevate intracellular oxidative stress?

Anthony Purcell, Amy Foster and Dr David Greensmith

School of Science, Engineering and Environment, University of Salford, Manchester, M5 4WT

Introduction

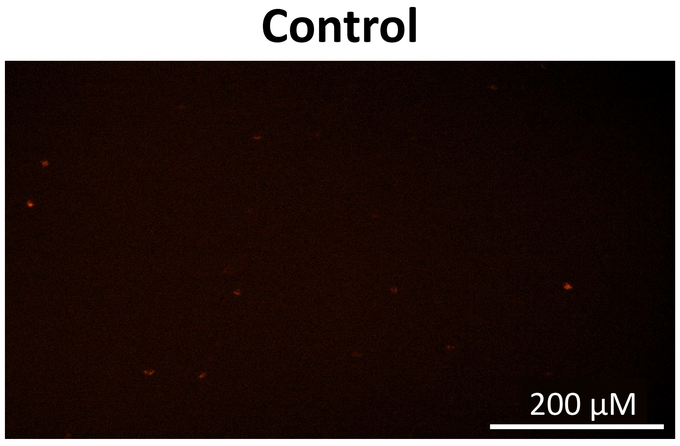
Anthracyclines are a type of anti-cancer drug. While they are highly effective at treating cancer, they can lead to heart failure in some patients. Anthracyclines are known to increase the number molecules in cells called reactive oxygen species (ROS). These are highly reactive molecules derived from oxygen. When the amount of ROS exceeds the body's ability to remove them this leads to a condition called oxidative stress (OS). Oxidative stress damages cells and in turn organs. To prevent heart failure as a result of OS we must understand to what extent anthracyclines increase cellular oxidative stress.

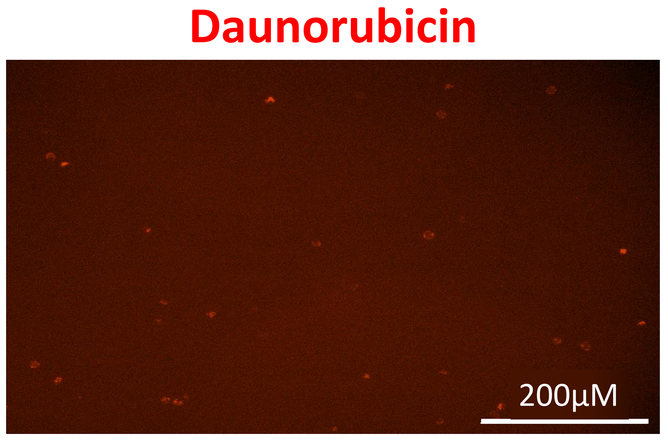
Methods

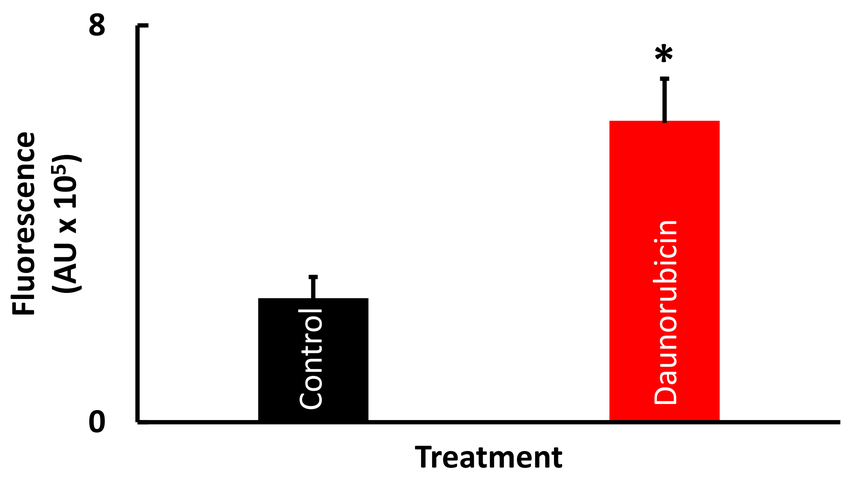
A lymphoblastic leukaemia cancer cell line called MOLT-4 was kept in culture at 37°C in 5% CO_2. The cancer cells were treated with the anthracycline daunorubicin and ROS was detected using a high content microscope that allows live cell imaging. The cells were loaded with CellRox; a dye that emits light in the presence ROS and images were captured to quantify this light. The next set of experiments investigated the use of pre-treating cells with anti-oxidants prior to daunorubicin treatment. Cell death was determined using a separate assay. Statistical analysis was carried out using SPSS and results were found to be significant if less than <0.05.

Does daunorubicin increase oxidative stress?

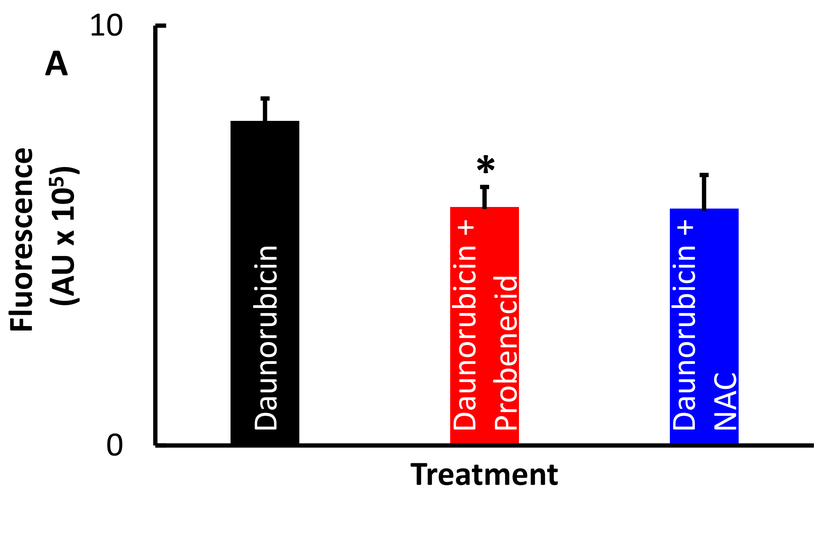
As CellRox dye used in the Cytation is designed to detect reactive oxygen species and therefore oxidative stress. When compared to the control cells, cells treated with 2nM daunorubicin showed a significant increase in CellRox dye fluorescence (n=44, P<0.05). This therefore showed ROS to be increased in cells treated with daunorubicin. As more cells are killed the higher the concentration of daunorubicin used, it would stand to reason that increased oxidative stress plays a role in cell death.

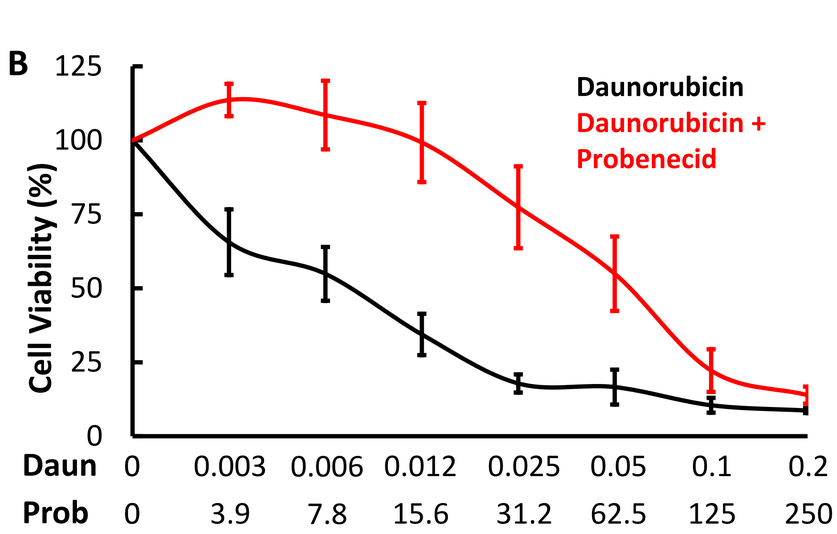


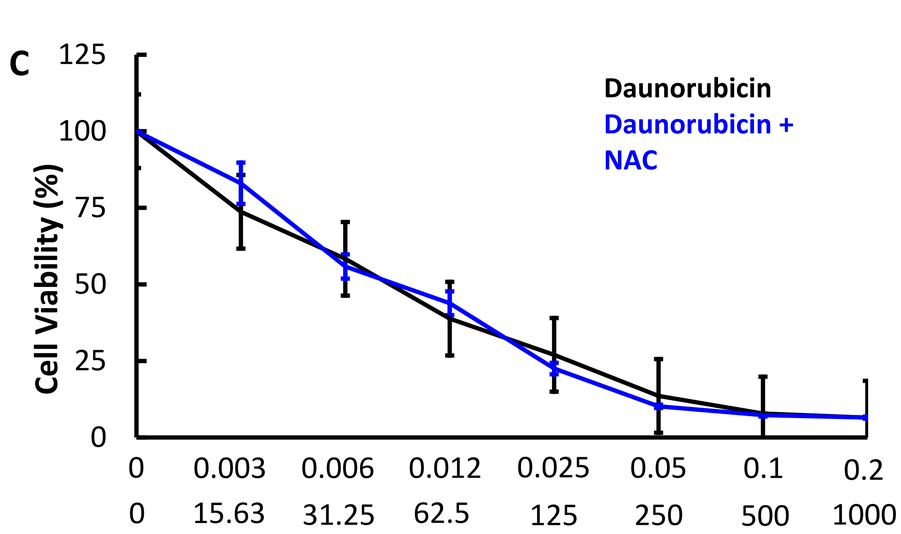




Do anti-oxidants reduce oxidative stress caused by daunorubicin?







N-Acetyl cysteine (NAC) and probenecid were the two anti-oxidants used. (A) Cells pre-treated with probenecid showed a significant decrease in oxidative stress in both control and 2 nM daunorubicin treated conditions (n=45, P<0.05). Cells pre-treated with NAC also showed a decrease in oxidative stress. However this was not found to be significant. (B) Cells treated with probenecid showed a decrease in cell death. (C) However, those pre-treated with NAC did not.

Conclusion

Our data demonstrates that one mechanism in which anthracyclines kill cancer cells is by increasing oxidative stress. It is likely that this is also the case in heart cells, which may contribute to anthracycline-induced heart failure. Though we need to confirm this, reducing oxidative stress in the heart remains a logical way to prevent heart failure. However, our data also shows we must specifically target oxidative stress in the heart so as not to reduce the effectiveness of anthracyclines as an anti-cancer drug.