Do childhood cancer drugs kill heart cells and why?

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Background

Anthracyclines are potent anti-cancer drugs used in the treatment of childhood cancers and part of the treatment regime for more than 50 % of these children. Although these drugs are effective at killing cancer cells, they can also alter the function of heart cells and lead to heart failure. Almost 57 % of childhood cancer survivors treated with anthracyclines will develop cardiac problems; making today's cancer survivors, tomorrow's heart failure patients. The exact mechanism by which anthracyclines work to kill cancer cells is not fully understood, however they are known to elevate levels of molecules called reactive oxygen species (ROS). Excess ROS production can cause oxidative stress, a condition associated with various diseases, and may be the source of the toxicity in anthracyclines.

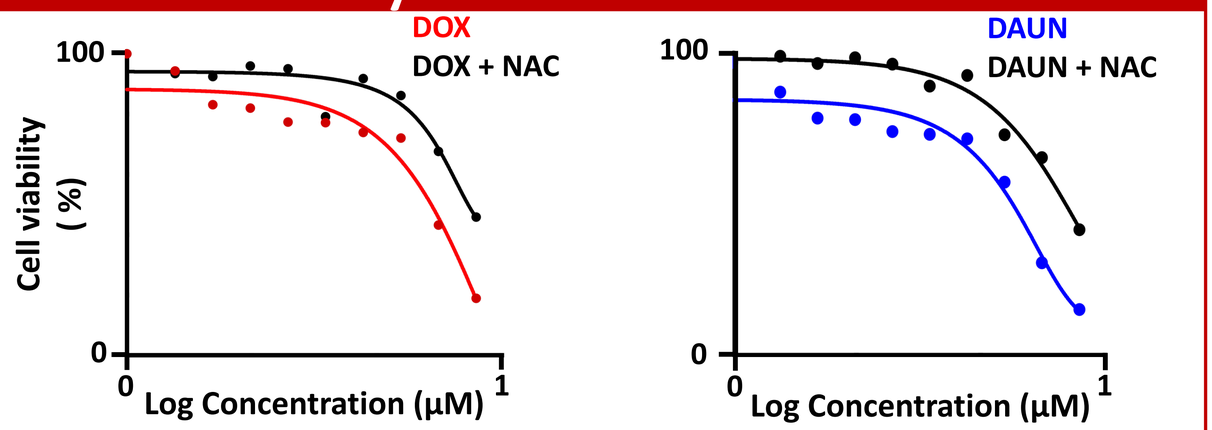
The objective of our study is to determine (1) if unwanted ROS elevations in heart cells lead to their death and (2) if this anthracycline-mediated heart cell death can be prevented with antioxidants; drugs that prevent the harmful effects of ROS.

Methods

U2-OS; a childhood cancer cell line and H9c2; an immortal rat cardiac muscle cell line were grown at 37 °C and at 5% CO_2.

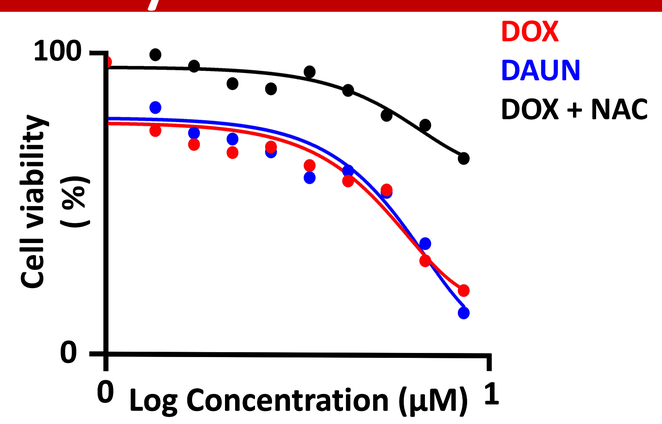
- Cell viability of both cell lines was assessed using a colorimetric MTT assay. Cells were treated with either anthracyclines or a combination of the antioxidant N-acetyl cysteine (NAC) and doxorubicin (DOX) or daunorubicin (DAUN) for 72 hours. Absorbance was measured at 570 nm and viability was calculated.
- To investigate the role of oxidative stress in anthracycline treatment 2',7' –dichlorofluorescin diacetate (DCF-DA), a fluorescent probe that emits light when oxidized by ROS was used. This light can then be detected using fluorescent spectroscopy with excitation/emission at 485/535 nm. The amount of light detected increases as levels of ROS increase corresponding to an increase in oxidative stress. Cells were stained with 1 µM DCF-DA and then treated with 5 µM DOX or DAUN for 45 minutes. For the combination treatments, 1mM NAC was added for 1 hour prior to the staining of the cells.

Do anthracyclines kill cancer cells ?



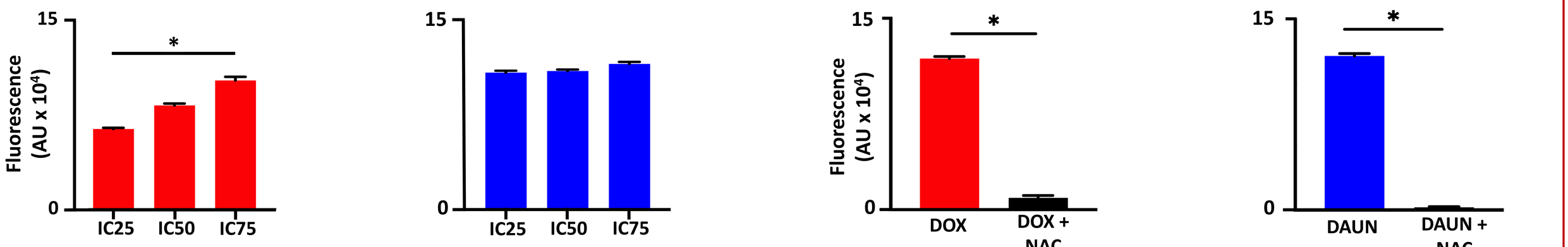
Cell viability of U2-OS is reduced by 50 % when treated with 0.312 µM DOX and 0.156 µM DAUN (n = 9). The viability is increased when cells are treated with a combination of anthracycline and N-acetyl cysteine. At 0.15 µM DOX and 1 mM NAC viability is increased by 19.54 % compared to DOX alone ($p<0.05$, n = 9). At 0.15 µM DAUN and 1 mM NAC cell viability is increased by 27.8 % compared to DAUN alone ($p<0.05$, n = 9).

Do anthracyclines kill heart cells ?



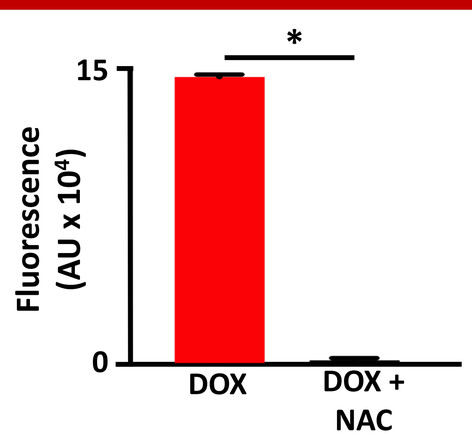
Cell viability of H9c2 is reduced by 50 % when treated with 0.156 µM DOX and 0.156 µM DAUN (n = 9). When pre-treated with 1 mM NAC and then 0.156 µM DOX viability is increased by 45 % compared to the DOX treatment alone ($p<0.05$, n = 9).

How do anthracyclines kill cancer cells?



There was a significant increase in fluorescence when U2-OS cells were treated with 0.625 µM DOX compared to 0.07 µM DOX ($p<0.05$, n = 17), while no concentration dependent change was detected with DAUN. Fluorescence detected was significantly reduced for cells that were pre-treated with the antioxidant when exposed to the anthracyclines ($p<0.05$, n = 3).

How do anthracyclines kill heart cells?



The combination treatment of DOX and NAC showed a significant decrease in fluorEscence ($p<0.05$, n = 2) and thus ROS levels compared to the anthracycline treatment alone.

Conclusion

Oxidative stress was increased in both cancer and heart cells when treated with DOX and DAUN. For the cancer cell line, the increase in oxidative stress was dose dependent for DOX but not DAUN. Treatment with NAC prior to anthracycline exposure significantly reduced oxidative stress and increased cell viability for both cell lines. These results suggest that oxidative stress plays a vital role in the mechanism by which anthracyclines kill cancer cells and may play a pivotal role in the cardiotoxic effects of these drugs. Future experiments will explore the different pathways involved in anthracycline-mediated toxicity and how to eliminate it.

References

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