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How is childhood cancer treatment related to cardiomyopathies?

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According to the World Health Organization (WHO) 149 per million children will be diagnosed with cancer worldwide annually, while 90,000 will die of cancer and its associated complications every year[3]. Despite the continual advances in cancer research and treatment resulting in an impressive increase in childhood cancer survival, approximately 75% of these surviving children will suffer from the late effects of chemotherapies such as cardiac complications and develop chronic life-threatening illnesses.

Cancer research continues to thrive as an enduring and captivating field of study with the pursuit of new developments and innovation offering hope for a brighter future. Research on the long-term cardiac implications following cancer treatment marks a new beginning in the quest to mitigate these side effects and extend the lifespans of survivors.

Anthracyclines are a class of anti-tumour compounds, commonly used as part of the treatment regime for childhood cancers, like osteosarcoma and rhabdomyosarcoma[4]. Daunorubicin (DAUN) and doxorubicin (DOX) are the most widely utilised compounds within the family of anthracyclines. Unfortunately, exposure to these drugs has been linked to development of heart failure, affecting majority of childhood cancer patients[7].This cardiac dysfunction typically appears following remission and can occur up to 20 years after treatment[7].

The mechanisms that underpin this toxicity are not fully understood. Nonetheless, it is suspected that anthracycline mediated cardiotoxicity is a result of excess production of reactive oxygen species (ROS) and thus elevated levels of oxidative stress (OS). These elevations damage cellular DNA, leading to dysregulation of cellular functions, and ultimately cell death[1]. Previous research has demonstrated that oxidative stress “significantly contributes” to cancer cell cytotoxicity[2]. Surprisingly, not many studies have been conducted on the specific role of reactive oxygen species production following treatment with anthracyclines in cardiac cells. Therefore, the debate about the actual mechanism of action of these anticancer agents continue.

The aim of our study was to address (1) if anthracyclines; DOX and DAUN, kill cancer based on oxidative stress alterations and (2) if anthracycline-mediated heart cell death can be prevented with the use of commercially available antioxidants, such as N-acetylcysteine (NAC).

Viability of both cancer (data not shown) and cardiac cells was initially assessed using a colour-based assay. As expected, both anthracyclines reduced cancer cell viability, specifically by 50 % at 0.312 μM for doxorubicin and 0.156 μM for daunorubicin. In this case, a concentration dependent mode of action is observed for doxorubicin.

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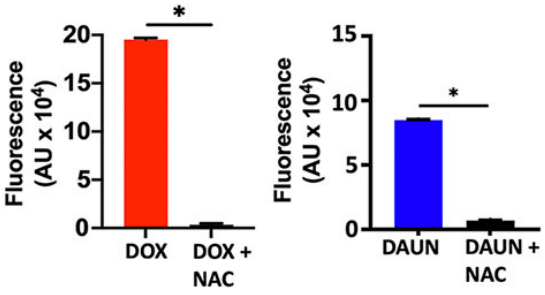

Figure 1: Oxidative stress of different treatments in cardiac cells. Different anthracycline treatments and their corresponding levels of fluorescence, as assessed via the DCF-DA assay.

To answer how exactly anthracyclines induce cell death in both cancerous and cardiac cells, we aimed to indirectly measure oxidative stress levels induced by DOX and DAUN using the DCF-DA assay. The specific technique relies on the oxidation of the dye by the ROS present, causing it to emit fluorescence proportional to oxidative stress levels[5,6].

Doxorubicin treatment in osteosarcoma cells, showed a significant dose-dependent increase in oxidative stress between different concentrations ($p<0.05$, $n = 3$), but daunorubicin did not, compared to control cells (data not shown). The same alterations in fluorescence in the presence of anthracyclines were detected in cardiac cells (Figure 1). Doxorubicin and daunorubicin increased oxidative stress significantly compared to the combination treatments with N-acetylcysteine ($p<0.05$, $n = 3$).

Interestingly, cardiac cell viability was also reduced in a similar pattern to that of the cancer cells. Doxorubicin and daunorubicin decreased the viability of the cardiomyocytes, with a 50 % decline recorded at 0.156 µM for both compounds. The addition of N-acetylcysteine in combination with anthracycline treatment decreased its cardiotoxic effects, as cell viability was not declined to the previous extent with just anthracyclines (Figure 2).

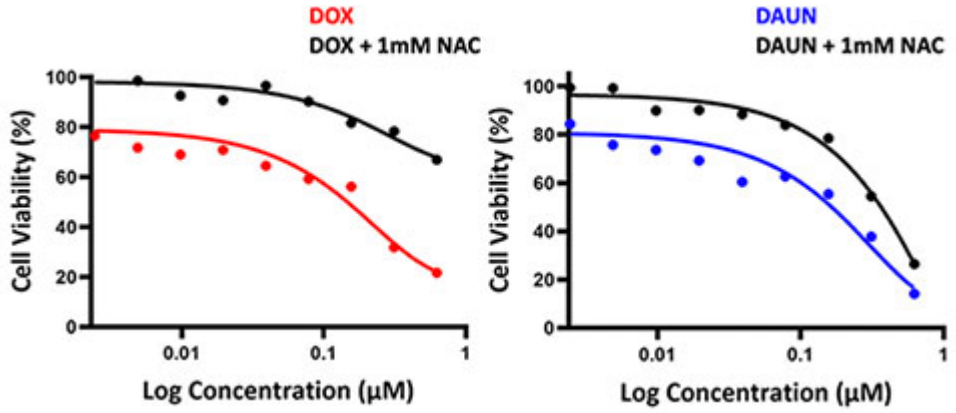

Figure 2: The cardiotoxic effect of anthracyclines was mitigated through co-treatment with N-acetylcysteine. Cardiac cells were exposed to various treatments of anthracyclines alone or combined with 1mM NAC and viability was assessed employing MTT assays.

Increased cardiac cell viability and extremely low ROS levels in its presence suggests compelling evidence that elevated oxidative stress results in cancer and cardiac cell death.

Our findings propose that oxidative stress has a pivotal role in the way anthracyclines kill cancer cells and damage cardiac cells, leading to cardiotoxic side effects. This reveals that further targeting of reactive oxygen species production may decrease the likelihood for long term cardiac complications in childhood cancer survivors, providing a new beginning for mitigating these side effects and increasing survivor lifespans. This study also highlights that the use of the commercially available antioxidant N-acetylcysteine may offer a cheap, safe, and readily available strategy to mitigated cardiac complications. Consequently, further research on cardioprotective strategies related to oxidative stress is strongly advised to better understand the global impacts of this treatment strategy and to evaluate if more specific reactive oxygen

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species pathway inhibitors are more effective than N-acetylcysteine at alleviating chemotherapy induced cardiac dysfunction.

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