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Original article

Resveratrol significantly improves cell survival in comparison to dexrazoxane and carvedilol in a h9c2 model of doxorubicin induced cardiotoxicity

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ABSTRACT

Cancer is one of the leading causes of deaths worldwide with 18.1 million deaths per year. Although there have been significant advances in anti-cancer therapies, they can often result in side effects with cardiovascular complications being the most severe. Dexrazoxane is the only currently approved treatment for prevention of anthracycline induced cardiotoxicity but there are concerns about its use due to the development of secondary malignancies and myelodysplastic syndrome. Additionally, it is only recommended in patients who are due to receive a total cumulative dose of 300 mg/m^2 of doxorubicin or 540 mg/m^2 of epirubicin. Thus, there exists an urgent need to develop new therapeutic strategies to counteract anthracycline induced cardiotoxicity. The h9c2 cardiomyoblast was investigated for its differentiation capacity and used to screen and compare promising prophylactics for doxorubicin induced cardiotoxicity. The half maximal inhibitory concentration of doxorubicin was determined in differentiated h9c2 cells after 24 h of exposure, to establish a model for drug screening. Cells were treated with dexrazoxane, resveratrol, and carvedilol either 3 h or 24 h prior to doxorubicin treatment. The ability of these cardioprotectants to prevent cardiotoxicity was analysed using the cck-8 cell viability assay and the dichlorofluorescin diacetate (DCFDA) reactive oxygen species (ROS) assay. There was no significant increase in survival in treatment groups after 3 h, however, at 24 h, resveratrol significantly improved survival compared to all other groups ($p < 0.05$). Additionally, dexrazoxane and resveratrol significantly decreased ROS formation at 3 h ($p < 0.05$) and all groups significantly decreased ROS production at 24 h ($p < 0.001$). This work is the first comparison of these cardioprotectants and suggests that resveratrol may be a more effective treatment in the prevention of anthracycline induced cardiotoxicity, compared to dexrazoxane and carvedilol. However, further work will be needed in order to decipher the exact mechanism and potential of this drug in the clinic.

Abbreviations: ATRA, All trans retinoic acid; CCK-8, Cell Counting Kit – 8; DCFDA, dichlorofluorescin diacetate; IC50, Half maximal inhibitory concentration; PBS, Phosphate buffered saline; ROS, Reactive oxygen species.

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1. Introduction

Cancer is a devastating disease resulting in 9.6 million new cases per year and 18.1 million deaths per year [1]. Cancer survival has increased by 27% since 1991 due to advances in cancer therapy equating to over 2.6 million lives saved in the United States alone [2]. Despite the success of anti-cancer therapy, chemotherapeutics such as anthracyclines can have drastic side effects including the development of cardiotoxicity [3]. Anthracyclines are one of the most commonly used class of chemotherapeutic agents which can result in cardiotoxicity that can range from mild hypertension to long term cardiotoxicity resulting in heart failure [3]. Doxorubicin, one of the most commonly used anthracyclines induces cardiotoxicity in a cumulative dose dependant manner resulting in increased rates of heart failure, which is reported to be 5%, 26%, and 48% with dosages of 400, 550, and 700 mg/M^2 respectively [4]. Heart failure induced by doxorubicin has a 3.5 fold higher mortality rate than idiopathic heart failure, thus creating an urgent need to develop new strategies to counteract cardiotoxicity [5].

There have been multiple theories as to the precise mechanism of doxorubicin induced cardiotoxicity. While this mechanism is still under investigation, it is generally accepted that free radical generation, the formation of an anthracycline-iron complex, and double stranded breaks in the DNA lead to cardiomyocyte dysfunction and even death resulting in doxorubicin induced cardiotoxicity [6]. It is thought that the heart is particularly sensitive to anthracyclines due to reduced concentrations of protective enzymes such as superoxide dismutase, making the heart susceptible to injury from oxidative stress by free radicals [7]. This can lead to myocardial vacuolisation, and progressive myofibril loss which is characteristic of doxorubicin induced cardiotoxicity [8].

Currently, dexrazoxane is the only drug approved by the Food and Drug Administration (FDA) for the prevention of anthracycline induced cardiotoxicity. Dexrazoxane functions by chelating iron involved in the formation of the anthracycline-iron complex, resulting in reduced free radical formation. Additionally, dexrazoxane inhibits the enzyme topoisomerase II without generating double stranded breaks [9]. Dexrazoxane has been shown to reduce the incidence of heart failure from anthracycline induced cardiotoxicity by an astounding 80% [7]. Despite the success of dexrazoxane as a cardioprotectant, it is only recommended in patients that are to receive a total cumulative dose of 300 mg/m2 of doxorubicin or 540 mg/m2 of epirubicin [10–12]. This is due to the concerns that the drug may result in secondary malignancies like acute myeloid leukaemia and myelodysplastic syndrome and may also have limited efficacy in children [13]. There thus exists an urgent need to develop new effective treatments for the prevention of anthracycline induced cardiotoxicity.

Two molecules that have potential in reducing anthracycline induced cardiotoxicity include an anti-oxidant, resveratrol and a beta-blocker, carvedilol. Resveratrol is a polyphenolic compound that can be found in grapes and red wine. It has potent antioxidant effects and has been shown to be anti-inflammatory, anti-fibrotic, and anti-apoptotic in cardiomyocytes and in vivo models of cardiotoxicity [14–23]. Additionally, resveratrol has been shown to be more effective as a prophylactic than as a therapeutic [24,25]. Animals exposed to doxorubicin were either treated prophylactically or therapeutically with resveratrol and it was found that animals treated prophylactically had reduced serum markers of myocardial damage, apoptosis, and fibrosis compared to controls and to animals treated therapeutically [25].

Carvedilol is a β and α1 adrenergic antagonist that acts as a nonselective blocker and is used to treat multiple cardiac conditions. It is thought to function by its β blocking effect and mitigating cardiac remodelling. In comparison to other β blockers, carvedilol has additional antioxidant and anti-inflammatory activity without compromising the activity of anthracyclines [26]. A number of studies have shown carvedilol to be an effective agent in the prevention of chemotherapy induced cardiotoxicity [27,28]. However, a recent large metanalysis concluded that carvedilol is not effective in preventing the decrease in initially asymptomatic left ventricular ejection fraction but it does have efficacy in decreasing the long term remodelling of the heart and the frequency of clinical cardiotoxicity [26].

Despite the use of these compounds and their success in preliminary studies the use of these cardioprotectants in the clinic still appears to be non-existent or ineffective. The aim of this study is to compare early prophylactic treatment of these cardioprotectants at the current recommended dexrazoxane timing, that is, 3 h before doxorubicin vs a longer-term prophylactic delivery at 24 h before doxorubicin in a h9c2 model of doxorubicin induced cardiotoxicity upon exposure to an IC50 dosage of doxorubicin. This will give an estimation of the optimal timing required for the delivery of these compounds and their effectiveness in the prevention of cardiomyocyte toxicity.

2. Material and methods

2.1. H9c2 culture

he h9c2 cell line was initially derived and immortalised from the ventricular section of an E13 BDIX rat heart thirteen days succeeding fertilisation. Upon exposure to all trans retinoic acid (ATRA) and low serum, the cells fuse to multinucleate and elongated myotubes and have been shown to be an effective model for studying cardiac diseases [29]. The h9c2 cell line was purchased from the American Tissue Type Collection (Manassas, VA, #CRL-1446) and maintained in Dulbecco's Modified Eagle's Medium containing 4 mM of L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate, 1500 mg/ml sodium bicarbonate and supplemented with 10% fetal bovine serum (FBS) and 1% penstrep (Sigma Aldrich). Cells were grown in T75 cm^2 tissue flasks at 37 °C in a humidified atmosphere of 5% CO_2. Cells were fed every 2–3 days and sub-cultured at 70–80% confluency to ensure differentiation potential. For differentiation experiments foetal bovine serum was reduced to 1% and ATRA was added daily for up to one week. ATRA concentrations of 10 nM, 50 nM, and 1 μM were used to examine phenotypic changes and 1 μM ATRA was used for further experimentation.

2.2. Immunofluorescence

Cells were grown on glass cover slips, fixed in 4% paraformaldehyde for 10 min, washed thrice in phosphate buffered saline (PBS), permeabilised by incubating cells for 5 min in 0.1% Triton-X and PBS solution, washed thrice in PBS, and blocked using 1% bovine serum albumin for one hour followed by using the appropriate staining method. For immunofluorescence staining, all materials were purchased from Abcam. Cells were stained with Hoechst 33342 for nuclear staining (1:1000), F-actin was stained using Phalloidin-iFluor 594 Reagent (ab176757) (1:1000), cardiac troponin-t was stained using ab8295 (1:100) and Alexa flour 488 goat anti-mouse secondary antibody followed by counterstaining with Hoechst 33342 and Phalloidin-iFluor 594 Reagent (ab150117) (1:1000). Following this, coverslip were carefully inverted onto a slide containing a drop of Fluoromount, labelled and fixed in position using ethyl acetate. Slides were stored at 4 °C until imaging. Imaging was performed on an Andor spinning disk confocal microscope using 10x air lens and a 40x oil lens.

2.3. Phenotypic analysis

For measurement of cell area, circularity, multinucleation, and percentage, troponin-t expression cells were grown in growth media or 1 μM of ATRA for 1, 4, or 7 days (N = 4) and stained using phalloidin and Hoechst, as previously described. Five random fields of view were taken per well per group. In order to determine the cell area and circularity the set measurements function on image J was chosen and measurements were set for area and shape descriptors. The border of every cell in each image was selected using the free hand selection tool and data exported to Microsoft excel for analysis. To determine the percentage of

multinucleation, the number of multinucleated cells was divided by the total number of cells and expressed as a percentage. A cell was considered multinucleated if there was more than one nucleus present in the cell. In order to determine the percentage of troponin-t expression, the number of cells that expressed troponin-t was divided by the total number of cells and expressed as a percentage.

2.4. Cell viability

For cell viability analysis, the cell counting kit 8 (CCK-8) (Abcam) was used. This kit works by intracellular dehydrogenases reducing the tetrazolum salt present in a kit which results in an orange product that can be measured using absorbance assays. In order to determine the half maximal inhibitory concentration, cells were seeded at 5000 cells per well in a 96 well plate and exposed to either no doxorubicin, 1 μM, 5 μM, 10 μM, 20 μM, 40 μM, 80 μM, or 100 μM (N = 5 per group) for 24 h and the experiment was repeated three times. After 24 h, doxorubicin containing media was replaced and 10 μl of CCK-8 reagent was added to 90 μl of fresh media. Cells were incubated with the reagent for three hours and absorbance was read at 450 nm using a Hidex-sense plate reader. In order to determine viability, each absorbance measurement was expressed as a percentage of the negative control. In order to determine the half maximal inhibitory concentration, the percentage survival was graphed against the log of the doxorubicin concentration and the point of 50% survival was determined to be the half maximal inhibitory concentration. In order to determine cell survival upon prophylactic treatment with cardioprotectants, cells were seeded at 5000 cells per well in a 96 well plate. Cells were pre-treated with either 70 μM dexrazoxane, 25 μM resveratrol, or 25 μM carvedilol for 3 h or 24 h. Media containing cardioprotectants was removed prior to exposure to 7.045 μM doxorubicin (IC50) for 24 h. Cell viability was determined by removing doxorubicin containing media and adding 10 μl of cck-8 assay to 90 μl of fresh media, incubating for 3 h, and reading the absorbance at 450 nm (N = 6). Cell viability was expressed as a percent of the negative control.

2.5. Reactive oxygen species analysis

In order to determine the production of reactive oxygen species (ROS) 2′,7′ –dichlorofluorescin diacetate (DCFDA) assay was used (Abcam). DCFDA is a fluorogenic dye that measures intracellular ROS production including hydroxy and peroxyl species. Cells were seeded at a density of 20,000 cells per well in 24 well black clear bottom imaging plates and exposed to prophylactic treatment and doxorubicin as previously described (N = 4). Following this, cells were stained with DCFDA for 45 min and imaged using an Andor spinning disk confocal microscope. In order to determine the ROS production, fluorescence intensity analysis was performed on image J. Five random fields of views were taken per sample and individual cells were selected using the freehand drawing tool with mean intensity value selected under the set measurements tab. Cell fluorescence was determined by subtracting cellular fluorescence from mean background fluorescence.

2.6. Statistics

For all experiments conducted, graphs were presented as a mean and error bars as standard deviations. For bar charts, the means of each well were presented as individual points on bar charts. All graphs and statistical analysis were performed using GraphPad Prism software. A Sharpio-Wilks test was performed on each data set in order to test normality with all data passing normality testing. In order to compare groups at the same time point, a one-way ANOVA with post-hoc Tukeys testing was used to compare the mean of each sample against every other mean. In order to compare different time points, a two-way ANOVA was performed with multiple comparisons. The significance was determined at p-value < 0.05 (*), p-value < 0.01 (**), and p-value < 0.001(***).

3. Results

3.1. Differentiation of h9c2 cells

Qualitative morphological assessment of the differentiation capacity of the h9c2 cell line revealed that multinucleation and myotube formation increased with increasing dosages of ATRA after 7 days. Limited multinucleation and myotube formation was observed in the growth media group and increasing levels of multinucleation and myotube formation were observed with increasing ATRA dose. 1 μM was selected as the optimal ATRA dose for continuing studies (Fig. 1). Quantitation of the differentiation capacity of the h9c2 cells over time upon exposure to 1 μM ATRA revealed that the cells become increasingly elongated, multinucleated, and express increased amounts of the cardiac marker troponin-t over time. A significant change in the cell area was observed compared to the growth media as early as one day after exposure to ATRA and low serum media [$P < 0.05$ (0.026)]. The cell area significantly increased between day 1 and day 4 ($P < 0.01$). There was no significant differences in the area between day 4 and 7, however, there was a significant difference in the cell area between days 1 and 4 and days 1 and 7 ($P < 0.001$). Upon assessment of the circularity, it was shown that the cells were significantly less circular at day 7 in comparison to growth media and day 1 cells ($P < 0.001$) and day 4 ($P < 0.01$). Multinucleation was significantly increased at day 7 in comparison to all other timepoints ($P < 0.001$) and was significant in comparison to growth media by day 4 ($P < 0.01$). There was a significant increase in the expression of the cardiomyocyte marker troponin-t at day 7 compared to all other groups ($P < 0.001$) (Fig. 2).

3.2. Induction of chemotherapy induced cardiotoxicity

To induce a model of chemotherapy induced cardiotoxicity, cells were exposed to increasing dosages of the chemotherapeutic doxorubicin. The data was expressed as a log transformation of the doxorubicin concentration in μM (Fig. 3A). From this graph, the half maximal inhibitory concentration (IC50) was determined to be 7.045 μM. This dosage was used in further experiments in order to assess cardiotoxicity reversal by various agents.

3.3. Cell viability

Cell viability, as assessed by the CCK-8 assay showed that in comparison to non-treated controls, the cell viability was decreased to 50% in doxorubicin only treatment group ($P < 0.001$). Assessment of the cell viability in the 3 h pre-incubation group showed that although doxorubicin resulted in the development of cardiotoxicity as shown by significant changes between the non-treated group and all other groups, prophylactic treatment failed to improve viability (Fig. 4A). After 24 h pre-incubation with the cardioprotective drugs, it was revealed that cardiotoxicity had successfully been reduced and that a 24 h pre-incubation with resveratrol resulted in significantly improved viability in comparison to all groups (Fig. 4B), [Resveratrol vs doxorubicin ($P < 0.01$), Resveratrol vs Doxorubicin-Dexrazoxane (Dox Dex) ($P < 0.01$), Resveratrol vs Doxorubicin-Carvedilol (Dox Carv) ($P < 0.05$ (0.027)]. Comparison of the viability across samples at 3 h and 24 h pre-incubation revealed that pre-treatment with resveratrol at 24 h significantly increased the viability of the cells in comparison to dexrazoxane pre-treatment at 3 h [$P < 0.05$ (0.031)] and 24 h [($P < 0.05$ (0.026)] and significantly improved viability in comparison to 3 h prophylactic treatment with carvedilol but not 24 h carvedilol [$P < 0.05$ (0.048)], as shown in Fig. 4C.

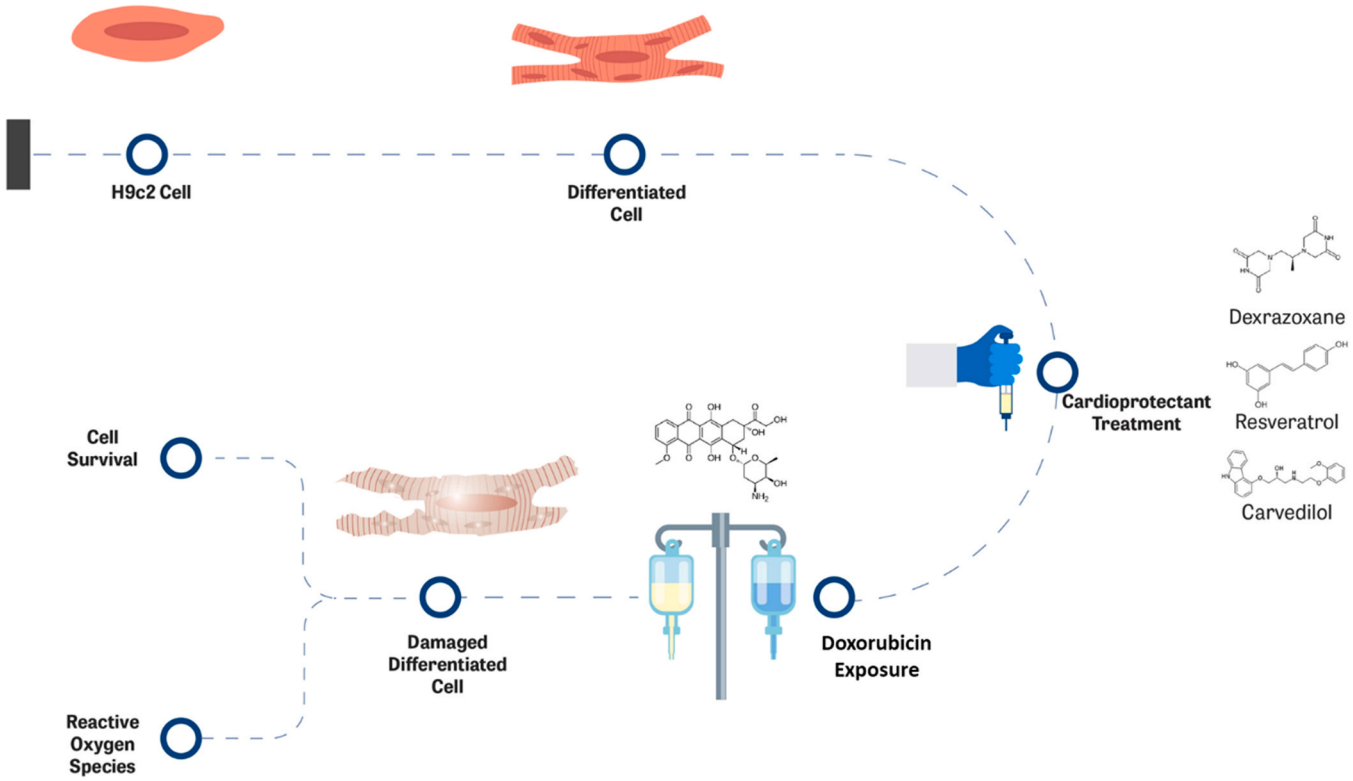

Fig. 1. Overview of prophylactic treatment of differentiated h9c2 cells with cardioprotectants ahead of doxorubicin treatment.

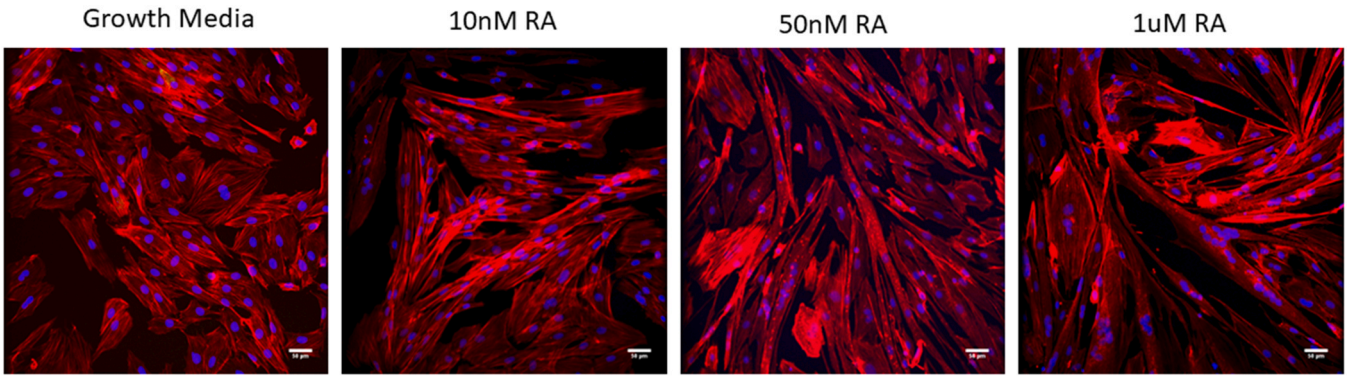

Fig. 2. Maximum intensity projections of the h9c2 cell line after 7 days differentiation upon exposure to growth media, 10 nM, 50 nM, and 1 μM ATRA. Growth media group shows small, circular, and mononucleate cells. Increasing dosages of ATRA correlates with increased fusion of myotubes and increased multinucleation. Scale bar represents 50 μm.

3.4. Reactive oxygen species production

ROS was significantly increased when cells were treated with doxorubicin compared to a non-treated control ($P < 0.0001$) (Fig. 5B and C). After 3 h pre-incubation with cardioprotectants, there was a significant decrease in ROS production in the dexrazoxane [$P < 0.05$ (0.047)] and resveratrol [$P < 0.05$ (0.012)] groups but not the carvedilol group (Fig. 5B). After 24 h prophylactic treatment prior to doxorubicin exposure, ROS production was significantly decreased across all the groups (Fig. 5C) ($p < 0.001$). A comparison across 3 h and 24 h prophylactic treatment groups revealed that there was no significant differences in ROS production between the cardioprotectants. Furthermore, there was a significant change in ROS production when comparing the differences between carvedilol use at 3 h and 24 h prophylactic treatment [$P < 0.05(0.04)$] (Fig. 6).

4. Discussion

Doxorubicin treatment often results in cardiac damage for which current treatment options are limited [3–5]. Although dexrazoxane has been shown to be an effective prophylactic treatment its use has been restricted due to safety concerns creating a need for alternative options [7,10–12]. This paper presents for the first time a comparison of the use of dexrazoxane and two promising cardioprotectants to prevent chemotherapy induced cardiotoxicity. A h9c2 model was used in order to assess these cardioprotective effects. A comparison of these cardioprotectants show that resveratrol may be more effective than dexrazoxane and the beta blocker carvedilol in cell toxicity prevention as shown by increased cell survival and reduced ROS. Furthermore, the delivery time and mode of delivery of cardioprotectant is important [30] as this study has found that delivering cardioprotectants 24 h prior to

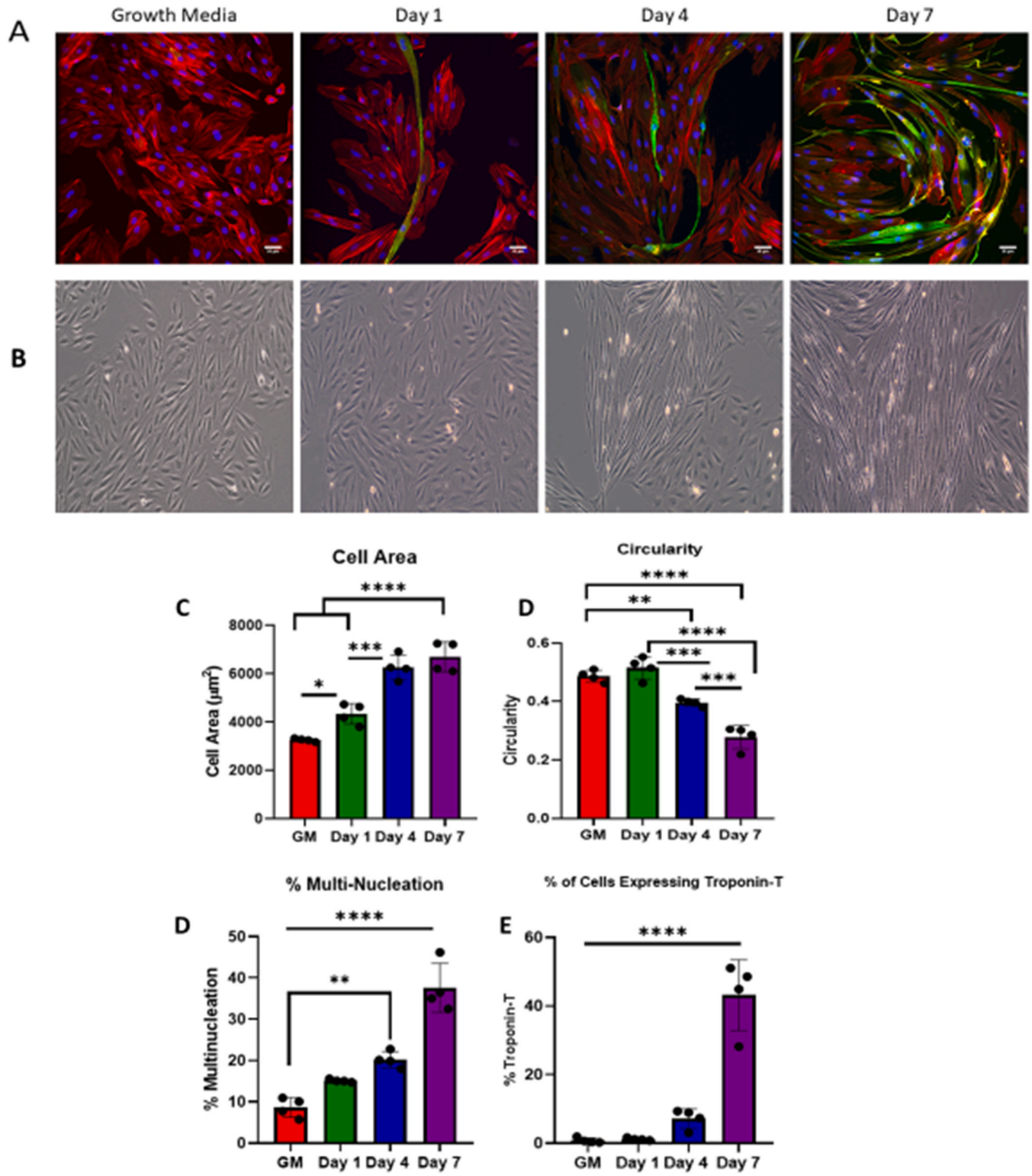

Fig. 3. Representative images of the differentiation capacity of the h9c2 cell line showing increased elongation and multinucleation over time (A and B) [Nuclei (blue), F-actin (red), troponin-t (green)]. Cell area significantly increases over time with means of 3254 μm^2, 4336 μm^2, 6261 μm^2, and 6694 μm^2 in GM, day 1, day 4, and day 7 groups respectively (growth media vs day 1 [$P < 0.05$ (0.026)], day 1 vs day 4 ($P < 0.001$), day 7 vs GM and day 1 ($P < 0.0001$)) (C). Cells become significantly elongated over time as measured by circularity with values of 0.515, 0.396, and 0.278 at day 1, day 4, and day 7 respectively (Day 1 vs day 4 ($P < 0.001$), day 1 vs day 7 ($P < 0.0001$), day 4 vs day 7 ($P < 0.001$) (D). Cells become significantly more multinucleated over time with values of 15.083%, 20.157%, and 37.542% at day 1, day 4, and day 7 (E). Cells significantly increase troponin-t expression over time with values of 0.822%, 1.180%, 7.162%, and 43.125% at day 1, day 4, and day 7 respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

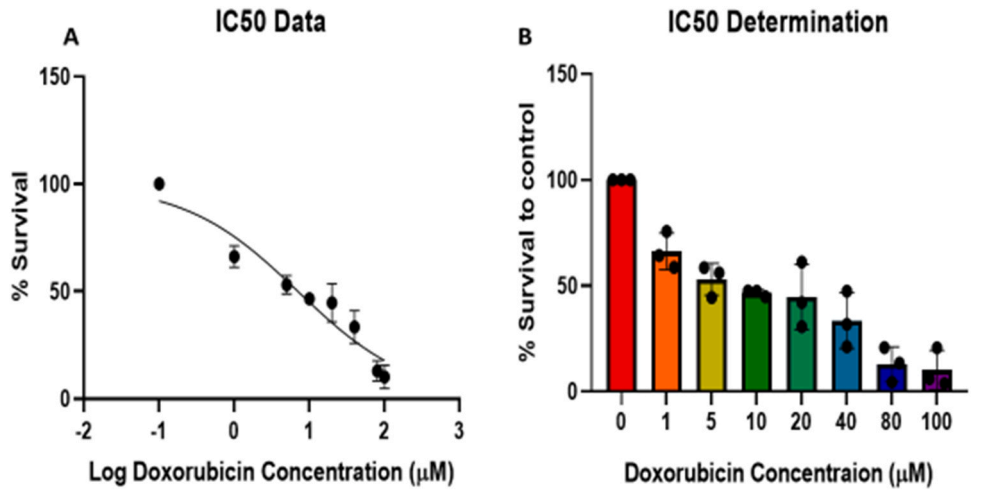

Fig. 4. Determination of the half maximal inhibitory concentration. Logarithmic survival curve representing percentage survival of cells in response to increasing doxorubicin concentrations for IC50 determination (A). Bar chart representing the percentage of live cells in response to increasing doxorubicin concentration (B).

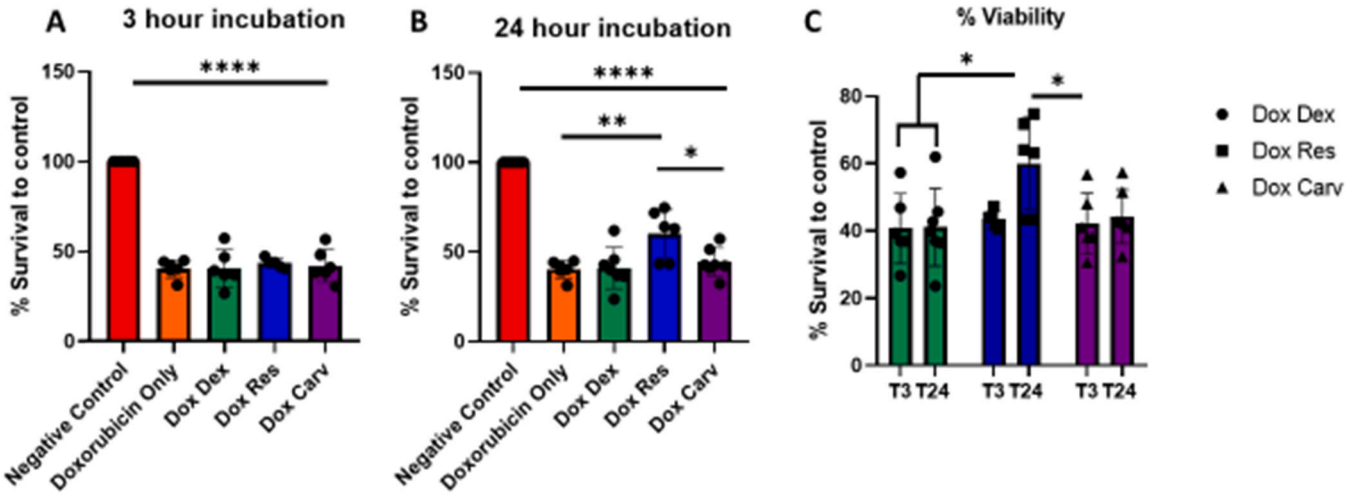

Fig. 5. Cell viability as determined by cell counting kit 8. Bar chart representing cell viability in response to 24 h treatment with doxorubicin and either 3 h prophylactic treatment (A) or 24 h prophylactic treatment (B). A comparison of cell viability after 3 h and 24 h prophylactic treatment with cardioprotectants prior to exposure to doxorubicin (C).

doxorubicin treatment may be more effective than delivering 3 h in advance, which is the current administering time of dexrazoxane.

Investigation of the ability of ATRA to differentiate the h9c2 cells into myotubes revealed increased myotube formation with increased ATRA concentrations. A previous study investigating 10 nM ATRA failed to detect troponin-t expression by fluorescent microscopy, which could be due to the low dosage used [31]. This is consistent with the data presented in this paper, as low amount of myotubes were observed at this dose. Genotypic analysis has previously shown the upregulation of cardiac genes upon exposure to 1 μM ATRA while genes associated with cell cycle, cell division, and DNA repair were significantly down-regulated, further validating this model with the 1 μM dosage [32]. Phenotypic analysis has validated the h9c2 line as a model of cardiotoxicity by showing that this cell successfully transitions into a cardiomyocyte-like-cell as shown by increased area, decreased circularity, increased multinucleation, and increased troponin-t expression. These quantitative parameters validate the increased elongation, multinucleation, and cardiac marker expression over time which is associated with myotube formation and is consistent with those shown in another study [29]. Exposure to doxorubicin decreased viability with increasing dosages, therefore, validating this model for cardiotoxicity development and the testing of prophylactic cardioprotectants.

Several studies have tested prophylactic treatment using dexrazoxane, resveratrol, and carvedilol in h9c2 models and in rodent models of cardiotoxicity [15,17,22,25,33–38]. Although each treatment has been successful, they have never been compared. This paper presents comparison of cardioprotectants as prophylactic treatments at 3 h and 24 h in response to high dosages of doxorubicin. Surprisingly, following 3 h of prophylactic dexrazoxane, resveratrol, and carvedilol, there was no significantly increased survival in comparison to the doxorubicin only group. However, prophylactic treatment with resveratrol 24 h prior to doxorubicin exposure significantly increased survival in comparison to doxorubicin only and 24 h prophylactic treatment with dexrazoxane or carvedilol. Doxorubicin is known to produce ROS, leading to DNA damage and cell death. Doxorubicin significantly increased the levels of ROS compared to untreated controls. Groups were treated with dexrazoxane, resveratrol, or carvedilol for 3 h or 24 h prior to doxorubicin treatment and it was found that in the 3 h group, ROS were significantly reduced in the dexrazoxane and resveratrol groups but not in the carvedilol group. After 24 h pre-incubation, ROS was significantly reduced in all groups compared to doxorubicin treated controls and reduced ROS levels to those in the untreated negative control. This indicates that

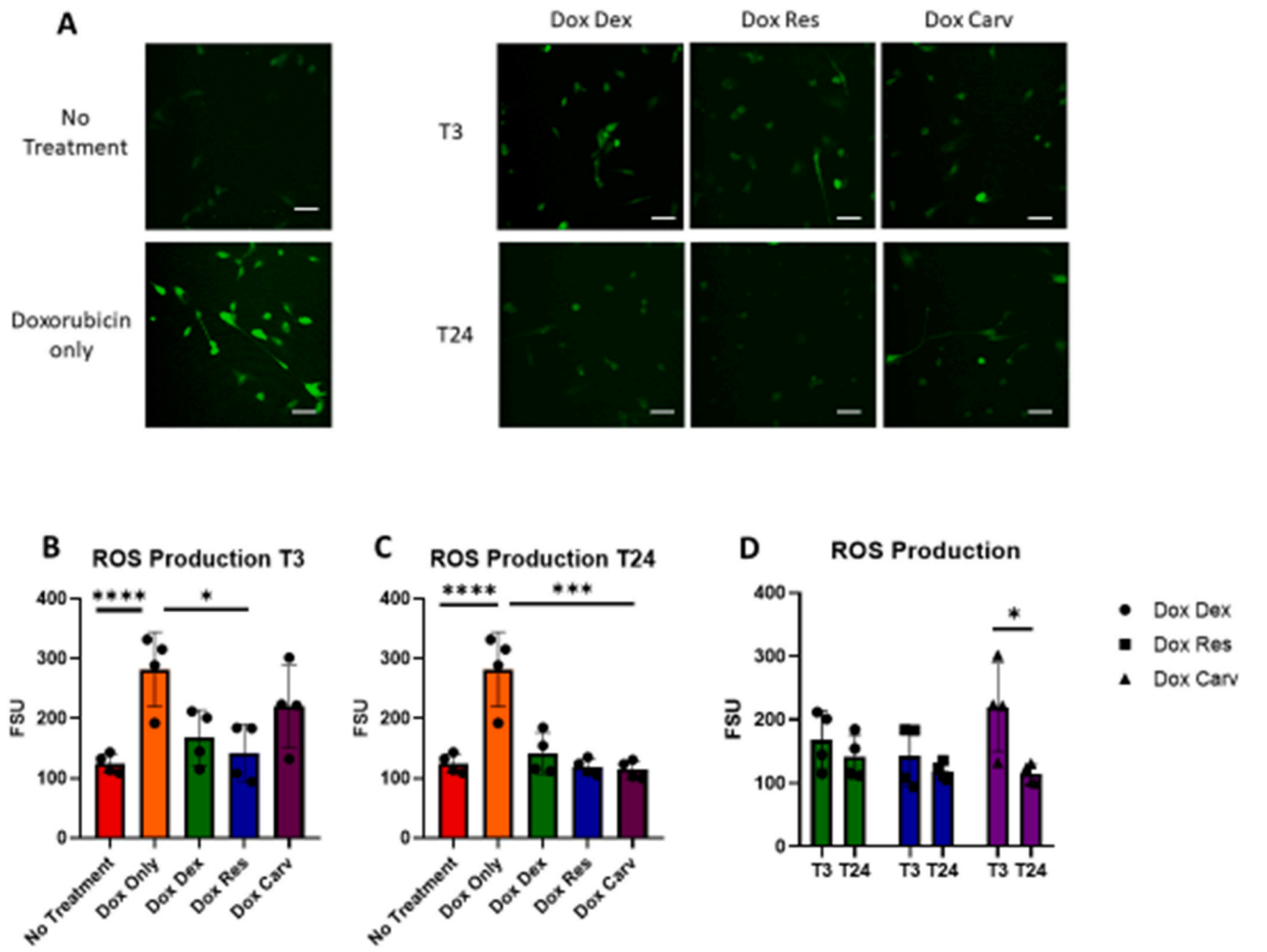

Fig. 6. Comparison of ROS production after prophylactic treatment at 3 h and 24 h. Representative images of ROS production in response to doxorubicin and prophylactic treatment of cardioprotectants (A). ROS production in response to doxorubicin after three hours prophylactic treatment (B). ROS production in response to doxorubicin after 24 h prophylactic treatment (C). Comparison of ROS production between 3 h prophylactic treatment at 24 h prophylactic treatment (D).

prolonged prophylactic treatment using these sets of cardioprotectants be more beneficial than shorter term treatment. We hypothesise that this could be due to an increased uptake of cardioprotectants into the cells at the 24 h timepoint. Each cardioprotectant in this study has an antioxidant capacity which would result in the prevention of intracellular ROS production and theoretically prevent downstream cell death. Although, each of these treatments had reduced ROS after 24 h, there was still more cell death in the dexrazoxane and carvedilol groups. It is likely that resveratrol also upregulates protective enzymes such as SIRT1 which has been previously shown to be beneficial in similar models [15,16].

Dexrazoxane is the only clinically approved drug for the prevention of doxorubicin induced cardiotoxicity. Dexrazoxane has been shown to be an effective agent by improving left ventricular ejection fraction (LVEF) in patients receiving doxorubicin [39]. This paper assessed the effects of dexrazoxane on cell survival in doxorubicin induced cardiotoxicity in differentiated h9c2 cells. Our results suggest although dexrazoxane may have beneficial effects by reducing ROS production albeit significant cell death still occurred [40,41]. This could be due to the high dose of doxorubicin used in this study.

Resveratrol has been largely investigated in preclinical models of chemotherapy induced cardiotoxicities. The prophylactic use of resveratrol has been shown to be more beneficial than its use as a therapeutic agent [25]. Resveratrol was given to male Wister rats at the same time as doxorubicin treatment for two weeks and doxorubicin was then stopped after two weeks, and resveratrol was supplemented for another four weeks. This group was compared to a group that received resveratrol only after doxorubicin treatment was stopped to compare its prophylactic vs therapeutic use. It was found that both groups were effective in preventing chemotherapy induced cardiotoxicities, demonstrated by a significant reduction in serum levels of the myocardial damage markers CK-MB and LDH [25]. However, prophylactic use of resveratrol also showed decreased apoptosis and fibrosis in comparison to doxorubicin only control and therapeutic resveratrol groups, showing that it is more effective as a prophylactic agent [25]. Resveratrol is thought to function through free radical scavenging through anti-oxidant properties [23,25,36]. However, multiple mechanisms have been reported including inhibition of nicotinamide adenine dinucleotide phosphate (NADPH), prevention of lipid peroxidation, decreasing NFAT levels, and increasing SIRT1 levels [23,25,33,35,36,42]. The results presented in this work have shown that resveratrol may be more effective than current treatments resulting in increased cell survival compared to dexrazoxane and carvedilol. This prompts further investigation into comparing the effectiveness of resveratrol in comparison to dexrazoxane and carvedilol in more clinically relevant models of chemotherapy induced cardiotoxicities.

Limited studies have shown the effects of carvedilol in h9c2 cells in the development of chemotherapy induced cardiotoxicity. To our knowledge, this is the first time that carvedilol has been used in

differentiated h9c2 cells and used in comparison to other groups. Carvedilol showed poor protective effects compared to other groups included in our study. Carvedilol has failed to show any significant improvement in LVEF in a large network analysis comparing the use of various cardioprotectants in chemotherapy induced cardiotoxicity [43]. Additionally, the largest published double blind control trial of 192 HER-2 negative breast cancer patients receiving anthracyclines failed to show significant effects on LVEF after six months follow up [27]. However, this study did show reduced levels of troponin I which indicates reduced myocardial damage [27]. A large meta-analysis looking at the effects of carvedilol on chemotherapy induced cardiotoxicity found that there was significantly reduced rates of left ventricular systolic dysfunction and prevention of LVEF decline. However, the authors warned that the results should be interpreted with caution due nominal significance and due to the fact that most of the studies included were single blinded, single centre, and often lacked effect size and power calculations [28].

5. Conclusion

This paper presents a comparison of cardioprotectants in a differentiated h9c2 model of doxorubicin induced cardiotoxicity. The paper shows that 3 h prophylactic treatment was ineffective in preventing cell death and that 24 h prophylactic treatment is most beneficial. Additionally, resveratrol was found to be a more promising treatment for chemotherapy induced cardiotoxicity based on a significant increase in cell survival compared to both dexrazoxane and carvedilol treatment groups and also reduced ROS production. Further work should be done to understand the precise mechanisms of why resveratrol is more effective in improving cell survival compared to the other two cardioprotectants, in order to provide more evidence of the possible benefits of resveratrol for use in the clinic.

Financial interests

The authors declare they have no financial interests.

Conflict of interest statement

The authors declare no conflicts of interest and have no relation to industry.

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