In vitro assessment of anthracycline-induced cardiotoxicity and mitigation by angiotensin blockade

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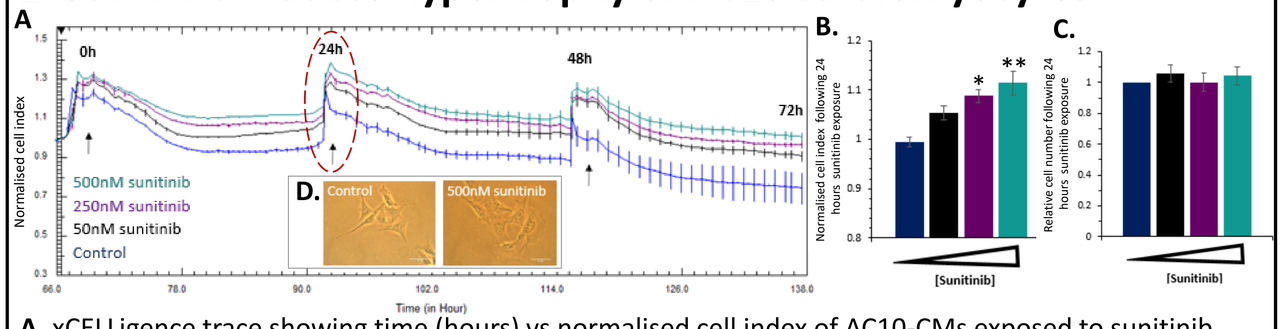
Cardiotoxicity is a major complication of many anticancer therapies such as sunitinib and anthracyclines, which frequently impacts the quality of life and overall survival of patients. Consequently, accurate prediction of structural and functional cardiac liabilities pre-clinically, and identification of therapeutic strategies to mitigate the toxicities are of crucial importance. Recent clinical studies have demonstrated that medicines acting upon the angiotensin signalling pathway may reduce anthracycline-induced cardiotoxicity and improve clinical outcomes. However, despite showing promise, the molecular mechanisms and pathways responsible for angiotensin-mediated mitigation of anthracycline toxicity are currently unclear.

STUDY AIMS

- Investigate the structural and functional cardiotoxicities induced by sunitinib and anthracyclines in two *in vitro* models
- Changes in cell survival, morphology and drug response were evaluated against the AC10 human adult ventricular cardiomyocyte cell line (AC10-CMs) using real-time impedance-based cell analyses (xCELLigence systems)
- Human iPSC-derived cardiomyocytes were also evaluated for changes in contractility, morphology and drug response using the CARDIO xCELLigence system
- The influence of angiotensin receptor blockade on doxorubicin (anthracycline) induced cardiotoxicity was evaluated in the cardiomyocytes in addition to its effect on expression of the angiotensin receptor (ATR1)

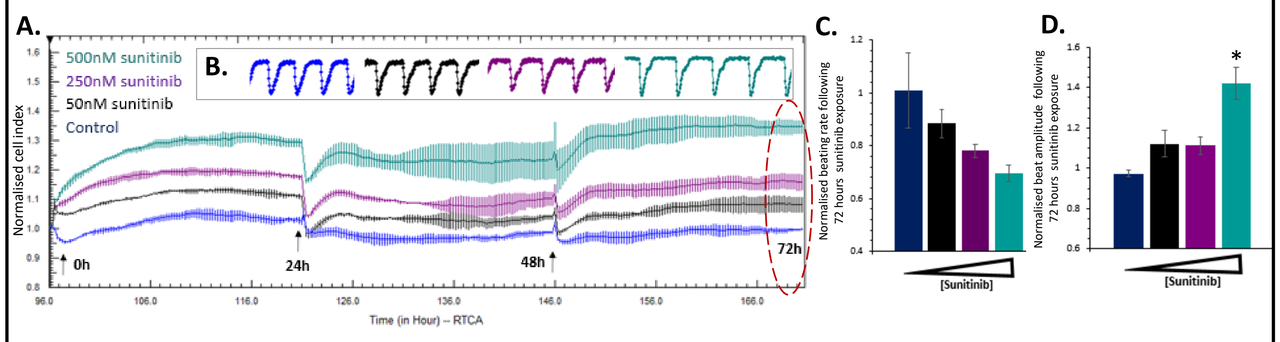
RESULTS

1. Sunitinib induces hypertrophy of AC10 cardiomyocytes



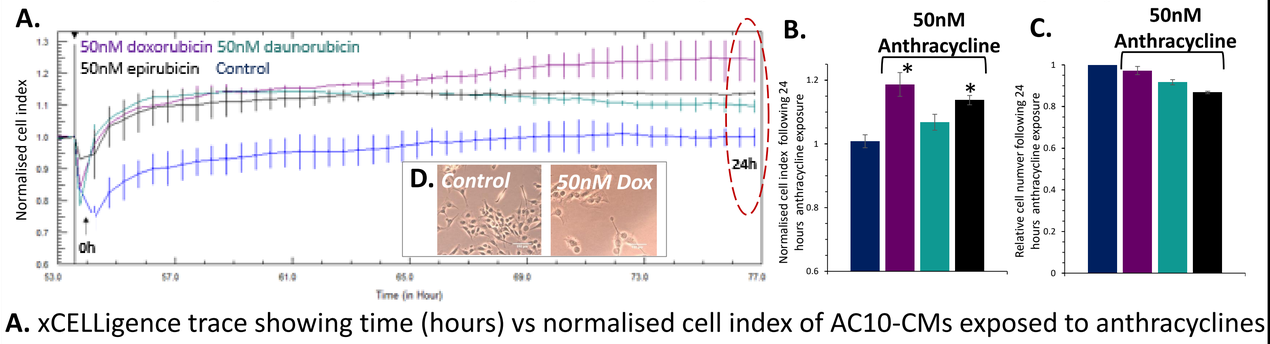
A. xCELLigence trace showing time (hours) vs normalised cell index of AC10-CMs exposed to sunitinib with points of sunitinib addition (arrows) and exposure time indicated, data representative of n=3, data points are average ± SD. **B.** Normalised cell index following 24 hours exposure to sunitinib (n=3 ± SE). **C.** Relative cell number following 24 hours exposure to sunitinib measured by MTT assay (n=3 ± SE). **D.** Images of untreated and sunitinib treated AC10-CMs showing a morphology change.

2. Sunitinib induces hypertrophy of hiPSC-derived cardiomyocytes and induces contractility changes



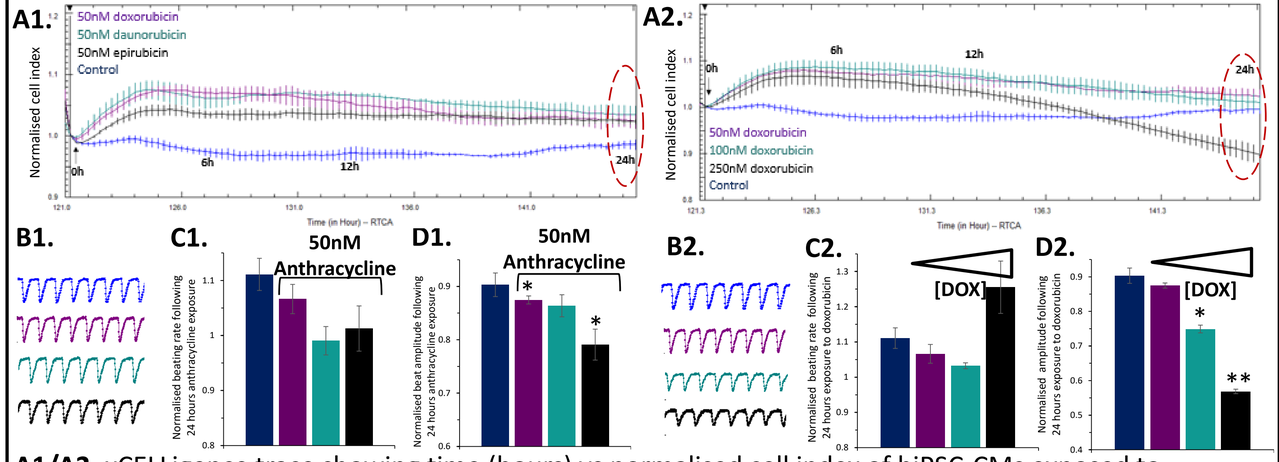
A. xCELLigence trace showing time (hours) vs normalised cell index of hiPSC-CMs exposed to sunitinib with points of addition (arrows) and exposure time indicated, data points are average ± SD.
B. Contractility of control cells and cells exposed to sunitinib for 72 hours. **C.** Normalised beating rate following 72 hours exposure to sunitinib (3 wells ± SE). **D.** Normalised beat amplitude following 72 hours exposure to sunitinib (3 wells ± SE).

3. Anthracyclines induce hypertrophy of AC10 cardiomyocytes



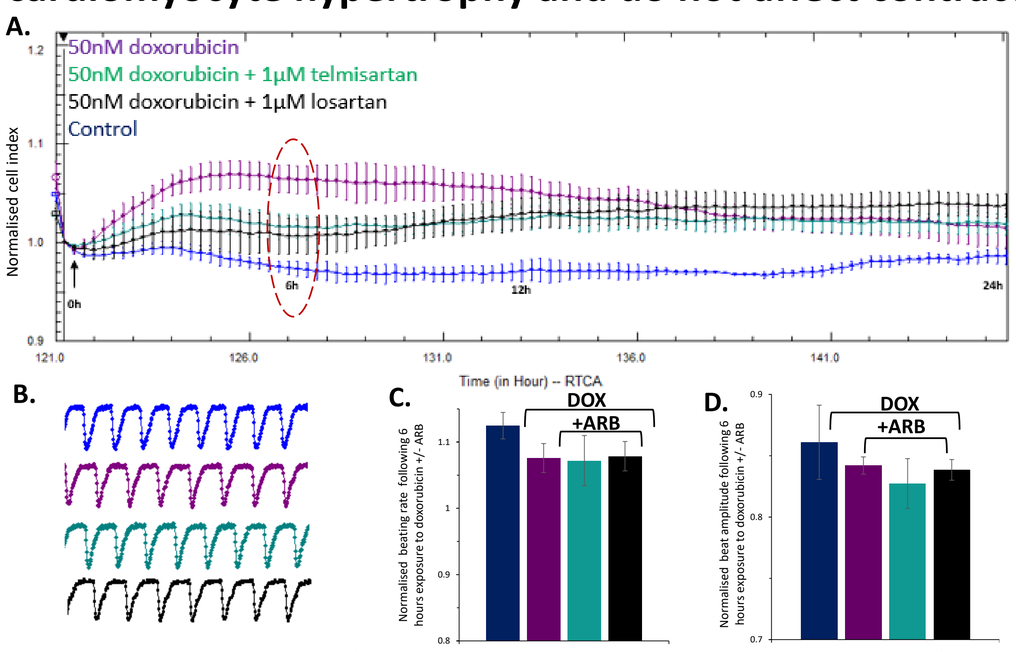
A. xCELLigence trace showing time (hours) vs normalised cell index of AC10-CMs exposed to anthracyclines with point of addition (arrow) and exposure time indicated, data representative of n=3, data points are average ± SD. **B.** Normalised cell index following 24 hours exposure to anthracyclines (n=3 ± SE).
C. Relative cell number following 24 hours exposure to anthracyclines measured by MTT assay (n=3 ± SE).
D. Images of untreated and doxorubicin treated AC10-CMs showing a morphology change.

4. Anthracyclines induce hypertrophy of hiPSC-derived cardiomyocytes and induce contractility changes



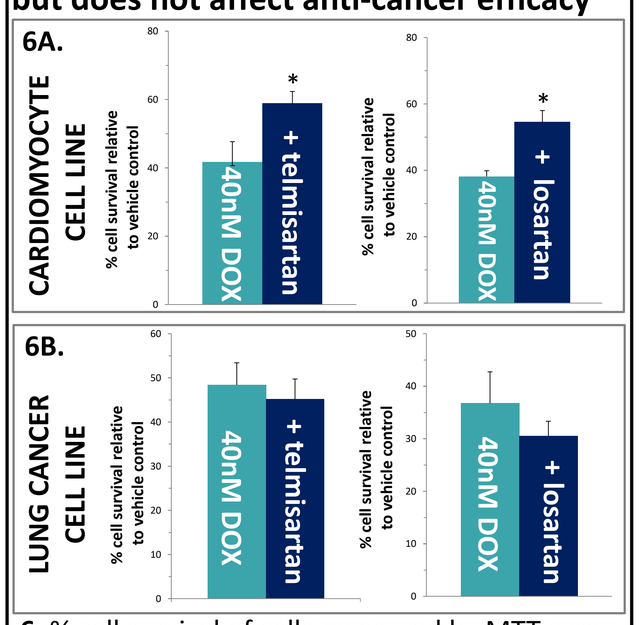
A1/A2. xCELLigence trace showing time (hours) vs normalised cell index of hiPSC-CMs exposed to anthracyclines with points of addition (arrows) and exposure time indicated, data points are average ± SD
B1/B2. Representative traces showing contractility of control cells and cells exposed to anthracyclines for 24 hours. **C1/C2.** Normalised beating rate following 24 hours exposure to anthracyclines (3 wells ± SE).
D1/D2. Normalised beat amplitude following 24 hours exposure to anthracyclines (3 wells ± SE).

5. ATR1 blockers reduce doxorubicin-induced hiPSC-derived cardiomyocyte hypertrophy and do not affect contractility



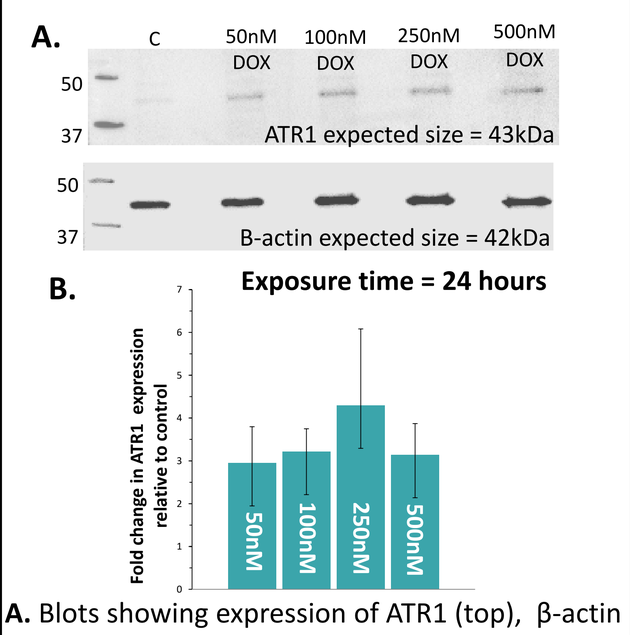
A. xCELLigence trace showing time (hours) vs normalised cell index of hiPSC-CMs exposed to doxorubicin +/- ARBs with points of addition (arrow) and exposure time indicated, data points are average ± SD. **B.** Representative traces showing contractility of control cells and cells exposed to doxorubicin +/- ARB for 6 hours. **C.** Normalised beating rate following 6 hours exposure to doxorubicin +/- ARB (3 wells ± SE). **D.** Normalised beat amplitude following 6 hours exposure to doxorubicin +/- ARB (3 wells ± SE).

6. ATR1 blockade reduces toxicity of doxorubicin in AC10 cardiomyocytes, but does not affect anti-cancer efficacy



6. % cell survival of cells measured by MTT assay treated with 40nM doxorubicin +/- losartan & telmisartan (1µM) in **A.** AC10 cardiomyocytes and **B.** H460 lung cancer cells (n=3±SE)

7. Doxorubicin increases expression of ATR1 in AC10 cardiomyocytes



A. Blots showing expression of ATR1 (top), β-actin (bottom) in DOX treated AC10 cardiomyocytes.
B. Densitometric analysis expressed as fold change in ATR1 expression relative to control (n=3±SE)

CONCLUSION

- Sunitinib and the anthracyclines doxorubicin, daunorubicin and epirubicin induce cardiomyocyte hypertrophy in AC10 and hiPSC-derived cardiomyocytes
- Changes in contractility of hiPSC-derived cardiomyocytes also occur with clinically relevant doses of sunitinib and doxorubicin
- Blockade of the ATR1 by telmisartan and losartan mitigates the hypertrophic and cardiotoxic effects of doxorubicin, but does not affect anti-cancer efficacy
- In addition, increased expression of the ATR1 following doxorubicin treatment strongly implies a relationship between doxorubicin-mediated toxicity and angiotensin II activity
- These data support blockade of angiotensin signalling as a therapeutic strategy for managing anthracycline-induced cardiotoxicity.

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