Cardiac microtissue models for the improved prediction of drug induced cardiac hypertrophy

Stephanie Ryder, Samantha Bevan, Taylor Forster, Benjamin Park, Clive Dilworth & Paul Walker

cyprotex AN EVOTEC COMPANY

INTRODUCTION

Drug-induced structural cardiotoxicity

- Cardiotoxicity is a major cause of pre-clinical and clinical drug attrition suggesting current *in vitro* models lack the complexity required for accurate toxicity prediction.
- Drugs can exert functional toxicities (e.g. arrhythmias) and/or morphological (structural) damage.
- Cardiac left ventricular hypertrophy resulting from an increase in cardiomyocyte mass is a major risk factor for heart failure and current pre-clinical detection is poor.
- The myocardial cell population comprises 70% non-myocytes such as endothelial and fibroblasts. The role of these in drug-induced cardiac hypertrophy is yet to be established.
- B-type natriuretic peptide (pro-BNP) has been highlighted as a surrogate indicator of a hypertrophic response *in vitro* (Carlson, 2013).
- Pointon *et al.*, 2013 highlighted calcium homeostasis, mitochondrial function and ATP content as major targets for structural cardiotoxicity.
- *In vitro* three-dimensional (3D) cell cultures more accurately reflect the complex *in vivo* microenvironment than traditional two-dimensional (2D) cell monolayer cultures.

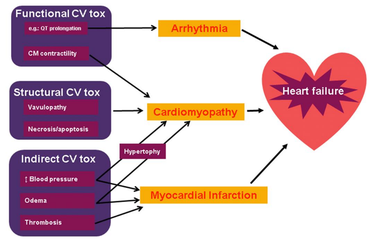

Figure 1. Drug induced cardiotoxicity overview

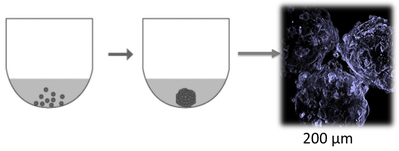

Figure 2. Microtissue formation in ultra-low attachment (ULA) plates

AIMS

- Develop cardiac microtissues of various cell compositions with a spontaneous beat, uniform size, shape and longevity amenable to chronic compound exposure.
- Develop a single plate based 3D high content structural cardiotoxicity assay capable of detecting early and late hypertrophy responses through multi- time point imaging combined with multiparametric fluorescent imaging and cytotoxicity readouts for enhanced predictivity and improved *in vitro* to *in vivo* extrapolation (IVIVE).

RESULTS

High content screening (HCS) assay design

- Cardiac microtissues were formed using scaffold free 96-well ultra low attachment round bottom plates (Corning®) using induced pluripotent stem cell derived cardiomyocytes (iPSC-CMs).
- Microtissues were exposed to a panel of 10 known pathophysiological hypertrophy inducing cardiotoxins and 4 other structural cardiotoxins (plus 2 negatives) for 336hrs. Chronic exposure over 14 days incorporated 3 repeat doses at days 3, 7 and 10.
- Brightfield images of microtissues were captured at each repeat dose to allow time course monitoring of hypertrophy (microtissue area).
- Following compound exposure fluorescent probes TMRE (mitochondrial function), Fluo-4 AM (calcium homeostasis) and Hoechst (Nuclei/ DNA structure) were incorporated into each cell model for 30 minutes. Calcium homeostasis and mitochondrial membrane potential have previously been defined as major distal targets of structural cardiotoxicity (Pointon *et al*., 2013).
- Fluorescent images were acquired using the confocal mode of an ArrayScan™ XTI HCS reader (ThermoScientific) following which cellular ATP was measured using CellTiter-Glo® (Promega).

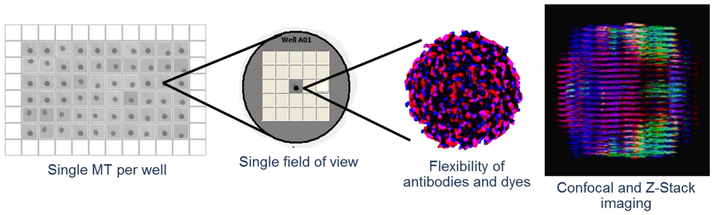

Figure 3. Principles of three dimensional (3D) confocal high content screening (HCS)

Spontaneously beating cardiac microtissues allow the pathophysiological hypertrophy prediction of structural cardiotoxins in a single plate based assay

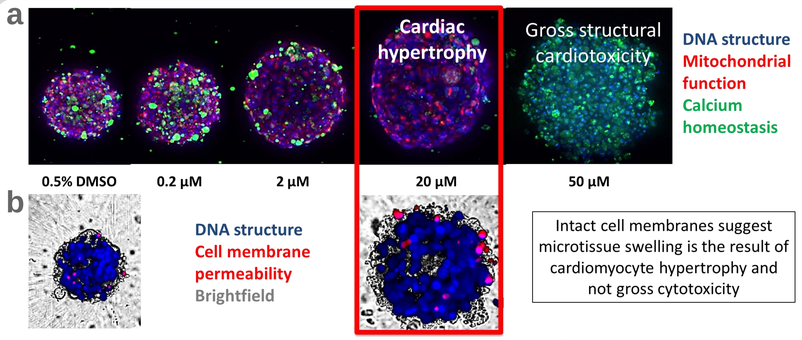

Figure 4. (a) Representative high content screening images (HCS) of the known structural cardiotoxin, dasatinib, inducing pathophysiological hypertrophy in cardiac microtissues (MT's) following 14 day compound exposure. Calcium homeostasis shown in green, mitochondrial function shown in red and DNA structure shown in blue. (b) Brightfield imaging of cardiac microtissues with overlayed staining of DNA structure and cell membrane permeability.

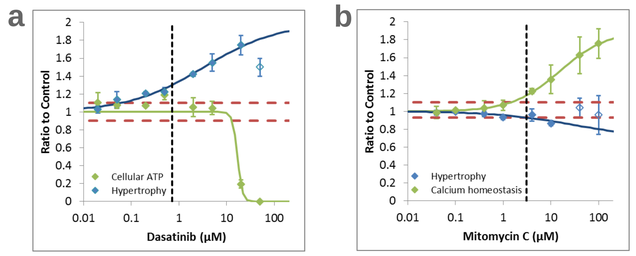

Figure 5. Graphical representation of (a) hypertrophy and cellular ATP response to dasatinib and (b) hypertrophy and calcium homeostasis response to mitomycin C in cardiac mono-MTs following 336hr exposure

Cardiac tri-MTs fail to predict pathophysiological hypertrophy of structural cardiotoxins using brightfield time course monitoring of MT area

Table 1. Summary of structural cardiotoxicity responses in monoculture and tri-culture MTs

Drug	Human exposure (C_{max}; µM)	Cardiotoxicity category	Most sensitive structural cardiac mono-MTs MEC (µM)	Most sensitive hypertrophy cardiac mono-MTs MEC (µM)	Most sensitive mechanism	Most sensitive structural cardiac tri-MTs MEC (µM)	Most sensitive hypertrophy cardiac tri-MTs MEC (µM)	Most sensitive mechanism
sunitinib	0.25	P	0.38	0.16	hypertrophy	0.73	NR	Calcium
dasatinib	0.72	P	0.15	0.02	hypertrophy	0.58	0.86	ATP
imatinib	3.54	P	0.04	0.05	ATP	10.40	NR	MMP
doxorubicin	15.34	P	0.01	1.46	ATP	0.05	NR	MitoMass
norepinephrine	0.17	P	0.10	0.06	hypertrophy	1.63	NR	Calcium
amphotericin B	9.00	P	7.85	0.25	hypertrophy	0.27	NR	ATP
lapatinib	4.18	P	0.19	37.40	ATP	2.15	NR	Calcium
clozapine	2.40	P	32.40	6.67	hypertrophy	37.40	4.11	hypertrophy
isoproterenol	0.01	P	0.10	26.30	ATP	7.80	NR	Calcium
cyclophosphamide	153.20	P	381.00	NR	ATP	239.00	NR	Size
amiodarone	5.30	P	7.76	3.51	hypertrophy	1.33	2.28	ATP
mitomycin C	3.12	P	0.21	NR	ATP	0.43	NR	ATP
idarubicin	0.12	P	0.004	1.45	ATP	0.006	NR	MitoMass
fluorouracil	4.61	P	10.30	NR	ATP	16.20	NR	MitoMass
acyclovir	6.66	N	NR	NR	-	NR	NR	-
buspirone	0.03	N	NR	NR	-	NR	NR	-
Correct prediction of structural cardiotoxicity using a 10x Cmax cut off			**94%**	**81%**		**88%**	**44%**	

≤ 1x C_{max}
≤ 3x C_{max}
≤ 10x C_{max}
≥ 10x C_{max}

P — Structural cardiotoxin with hypertrophic features
P — Structural cardiotoxin, no known hypertrophy
N — Non-structural cardiotoxin

proBNP highlights hypertrophic responses in all MTs, however MT area increase is restricted in MTs comprising cardiac fibroblasts

Table 2. Summary of hypertrophic responses in cardiac mono-, co- and tri-MTs

	Hypertrophy responses (Size increase MEC; µM)			
	Pathophysiological hypertrophins			Cytotoxin
Microtissue model cell composition	dasatinib	clozapine	sunitinib	mitomycin C
Cardiomyocytes (mono-MT)	0.02	6.67	0.16	NR
Cardiomyocytes + endothelial cells (co-MT)	0.168	5.44	NR	NR
Cardiomyocytes + fibroblasts (co-MT)	NR	NR	NR	NR
Cardiomyocytes + endothelials + fibroblasts (tri-MT)	NR	NR	NR	NR
10x human exposure (C_{max}; µM)	**7.2**	**24**	**2.5**	**31.2**

≤ 10x C_{max}
≥ 10x C_{max}

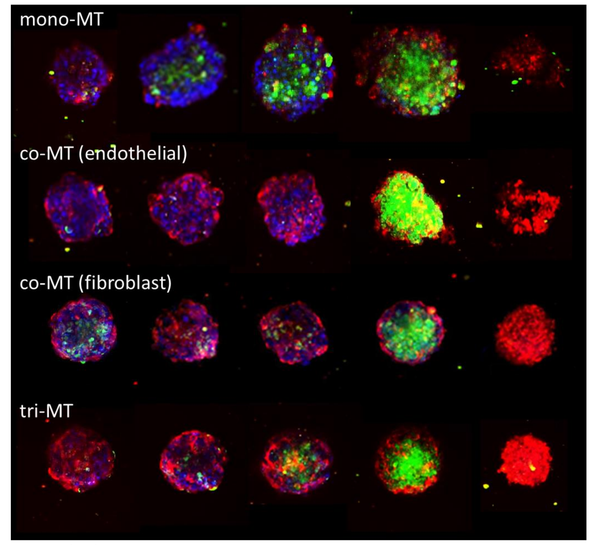

Figure 6. Representative high content screening images (HCS) of the known structural cardiotoxin, sunitinib, inducing pro-BNP expression in cardiac mono-MT's, co-MT's (endothelial), co-MT's (fibroblast) and tri-MT's following 14 day compound exposure. Hoechst shown in blue, pro-BNP expression shown in green and collagen 1a shown in red.

SUMMARY/CONCLUSIONS

- Cardiac microtissues display a spontaneous beat with uniform size, shape and longevity.
- High content brightfield imaging of cardiac mono-MTs (cardiomyocytes alone) enables the detection of cardiac hypertrophy by measuring microtissue area (81% accuracy with a 10x Cmax cut off). The non-invasive principles of brightfield imaging allow repeat imaging of a single plate over a time course of chronic compound exposure to permit hypertrophy detection prior to gross structural cardiotoxicity detected on day 14 with calcium, mitochondrial and DNA probes in combination with a cellular ATP content measurement. Cardiac tri-MTs failed to accurately predict hypertrophy.
- All compound toxicities were correctly predicted in the 3D cardiac mono-MTs with a 10x Cmax cut off using the combined assay approach with chronic compound exposure. This includes isoproterenol (MEC 0.1 µM) and cyclophosphamide (MEC 381 µM) which were previously undetected in 2D hESC-CM's by Pointon et al (2013).
- Microtissues comprising variations in cellular composition (mono-, co & tri-cultured models) displayed an increase in the hypertrophy associated marker pro-BNP with sunitinib treatment, however only MTs without cardiac fibroblasts exhibit an increase in MT area.
- This study shows how using a single organotypic human derived 3D model per well and automated, multiplexed confocal HCS can enhance the *in vitro* to *in vivo* understanding and extrapolation of structural cardiotoxicity.

REFERENCES

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