ONLINE SUPPLEMENT: Mechanism of doxorubicin cardiotoxicity evaluated by integrating multiple molecular effects into a biophysical model

To provide a detailed description of the reference models and the simulated effects of acute doxorubicin (DOX), acute doxorubicinol (DOXL) and chronic DOX on cardiac myocyte function we have additional phonotype synopsises predicted by simulations of cellular function for human (Tables 1-6) and rabbit (Tables 7-12) cardiac myocytes. We report the APD (action potential duration), resting membrane potential (RMP), systolic Ca^{2+} (peak Ca^{2+}), Ca^{2+} RT (Ca^{2+} relaxation time), diastolic Ca^{2+} (minimum Ca^{2+}concentration) and the mean intracellular sodium concentration ($[Na^+]_i$) for basic cycle length (BCL) of 1300, 1000, 800, 650 and 600ms. The stimulation rates correspond to heart rates of 45, 60, 75, 90 and 100 beats per minute (BPM).

Table 1: References cellular read outs for human cardiac myocyte model for differing heart rates.

BCL (ms)	APD (ms)	RMP (mV)	Ca^{2+} Systolic (μM)	Ca^{2+} RT (ms)	Diastolic Ca^{2+} (μM)	Mean $[Na^+]_i$ (mM)
1300 (45 BPM)	308	-85.67	0.47	387	0.09	8.66
1000 (60 BPM)	305	-85.42	0.80	356	0.10	9.79
800 (75 BPM)	301	-85.22	1.10	343	0.12	10.79
650 (90 BPM)	293	-85.03	1.32	332	0.13	11.59
600 (100 BPM)	289	-84.95	1.37	326	0.13	11.82

Table 2: Acute Dox Cellular Phenotypes for human model for differing heart rates.

BCL (ms)	APD (ms)	RMP (mV)	Ca^{2+} Systolic (μM)	Ca^{2+} RT (ms)	Diastolic Ca^{2+} (μM)	Mean $[Na^+]_i$ (mM)
1300 (45 BPM)	343	-84.89	0.78	573	0.18	13.26
1000 (60 BPM)	344	-84.48	1.18	564	0.21	15.56
800 (75 BPM)	345	-84.01	1.57	536	0.26	17.61
650 (90 BPM)	343	-83.30	1.93	480	0.36	19.11
600 (100 BPM)	338	-82.92	2.05	455	0.41	19.44

Table 3: Chronic Dox Cellular Phenotypes for human model for differing heart rates.

BCL (ms)	APD (ms)	RMP (mV)	Ca^{2+} Systolic (μM)	Ca^{2+} RT (ms)	Diastolic Ca^{2+} (μM)	Mean $[Na^+]_i$ (mM)
1300 (45 BPM)	294	-84.50	0.25	385	0.18	13.56
1000 (60 BPM)	285	-83.92	0.50	365	0.23	15.75
800 (75 BPM)	275	-83.42	0.81	337	0.28	17.90
650 (90 BPM)	261	-82.86	1.12	303	0.33	19.85
600 (100 BPM)	256	-82.59	1.22	288	0.36	20.51

Table 4: Acute DOXL Cellular Phenotypes for human model for differing heart rates

BCL (ms)	APD (ms)	RMP (mV)	Ca^{2+} Systolic (μM)	Ca^{2+} RT (ms)	Diastolic Ca^{2+} (μM)	Mean $[Na^+]_i$ (mM)
1300 (45 BPM)	213	-83.67	0.37	456	0.17	18.70
1000 (60 BPM)	189	-83.36	0.49	421	0.20	20.55
800 (75 BPM)	166	-82.99	0.62	389	0.24	22.69
650 (90 BPM)	158	-82.42	0.88	347	0.32	26.05
600 (100 BPM)	180	-81.99	1.18	325	0.40	28.64

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Table 5: Acute 100μM DOX Cellular Phenotypes for human model for differing heart rates.

BCL (ms)	APD (ms)	RMP (mV)	Ca^{2+} Systolic (μM)	Ca^{2+} RT (ms)	Diastolic Ca^{2+} (μM)	Mean $[Na^+]_i$ (mM)
1300 (45 BPM)	338	-85.28	0.52	449	0.12	10.25
1000 (60 BPM)	338	-84.93	0.91	427	0.15	11.73
800 (75 BPM)	338	-84.65	1.28	419	0.17	13.01
650 (90 BPM)	333	-84.33	1.56	400	0.19	13.93
600 (100 BPM)	329	-84.15	1.64	389	0.21	14.14

Table 6: Acute 10μM DOXL Cellular Phenotypes for human model for differing heart rates.

BCL (ms)	APD (ms)	RMP (mV)	Ca^{2+} Systolic (μM)	Ca^{2+} RT (ms)	Diastolic Ca^{2+} (μM)	Mean $[Na^+]_i$ (mM)
1300 (45 BPM)	219	-83.66	0.43	429	0.17	18.83
1000 (60 BPM)	193	-83.30	0.60	401	0.21	21.07
800 (75 BPM)	173	-82.92	0.77	374	0.25	23.47
650 (90 BPM)	Alternans					
600 (100 BPM)	Alternans					

Table 7: References cellular read outs for rabbit cardiac myocyte model for differing heart rates.

BCL (ms)	APD (ms)	RMP (mV)	Ca^{2+} Systolic (μM)	Ca^{2+} RT (ms)	Diastolic Ca^{2+} (μM)	Mean $[Na^+]_i$ (mM)
1300 (45 BPM)	219	-85.69	0.37	358	0.09	8.71
1000 (60 BPM)	209	-85.74	0.40	344	0.09	9.20
800 (75 BPM)	200	-85.80	0.43	325	0.10	9.68
650 (90 BPM)	191	-85.85	0.46	303	0.11	10.21
600 (100 BPM)	187	-85.87	0.48	291	0.11	10.43

Table 8: Acute Dox Cellular Phenotypes for rabbit model for differing heart rates.

BCL (ms)	APD (ms)	RMP (mV)	Ca^{2+} Systolic (μM)	Ca^{2+} RT (ms)	Diastolic Ca^{2+} (μM)	Mean $[Na^+]_i$ (mM)
1300 (45 BPM)	243	-85.58	0.43	457	0.10	10.12
1000 (60 BPM)	223	-85.57	0.49	404	0.12	10.80
800 (75 BPM)	Alternans					
650 (90 BPM)	Alternans					
600 (100 BPM)	Alternans					

Table 9: Chronic Dox Cellular Phenotypes for rabbit model for differing heart rates.

BCL (ms)	APD (ms)	RMP (mV)	Ca^{2+} Systolic (μM)	Ca^{2+} RT (ms)	Diastolic Ca^{2+} (μM)	Mean $[Na^+]_i$ (mM)
1300 (45 BPM)	206	-85.58	0.27	429	0.10	10.17
1000 (60 BPM)	187	-85.62	0.35	374	0.11	10.76
800 (75 BPM)	174	-85.65	0.45	331	0.12	11.42
650 (90 BPM)	163	-85.66	0.59	290	0.13	12.24
600 (100 BPM)	163	-85.67	0.67	273	0.14	12.64

Table 10: Acute DOXL Cellular Phenotypes for rabbit model for differing heart rates.

BCL (ms)	APD (ms)	RMP (mV)	Ca^{2+} Systolic (μM)	Ca^{2+} RT (ms)	Diastolic Ca^{2+} (μM)	Mean $[Na^{+}]_i$ (mM)
1300 (45 BPM)	176	-85.50	0.22	652	0.14	12.50
1000 (60 BPM)	159	-85.48	0.28	541	0.17	13.71
800 (75 BPM)	146	-85.43	0.39	449	0.23	15.28
650 (90 BPM)	133	-85.34	0.62	364	0.42	17.97
600 (100 BPM)	133	-85.30	0.84	341	0.60	19.58

Table 11: Acute 100μM DOX Cellular Phenotypes for rabbit model for differing heart rates.

BCL (ms)	APD (ms)	RMP (mV)	Ca^{2+} Systolic (μM)	Ca^{2+} RT (ms)	Diastolic Ca^{2+} (μM)	Mean $[Na^{+}]_i$ (mM)
1300 (45 BPM)	268	-85.67	0.52	371	0.09	9.11
1000 (60 BPM)	248	-85.72	0.59	344	0.09	9.61
800 (75 BPM)	233	-85.76	0.66	320	0.10	10.12
650 (90 BPM)	219	-85.80	0.74	292	0.11	10.70
600 (100 BPM)	219	-85.82	0.78	281	0.12	10.96

Table 12: Acute 10μM DOXL Cellular Phenotypes for human model for differing heart rates.

BCL (ms)	APD (ms)	RMP (mV)	Ca^{2+} Systolic (μM)	Ca^{2+} RT (ms)	Diastolic Ca^{2+} (μM)	Mean $[Na^{+}]_i$ (mM)
1300 (45 BPM)	180	-85.49	0.22	605	0.11	13.04
1000 (60 BPM)	159	-85.47	0.29	505	0.14	14.41
800 (75 BPM)	141	-85.43	0.40	426	0.18	16.18
650 (90 BPM)	123	-85.29	0.63	350	0.28	19.14
600 (100 BPM)	123	-85.20	0.84	315	0.43	21.40

The effect of acute DOX on I_{Kr} and I_{Ks}

Due to experimental uncertainty in the effect of acute DOX on I_{Ks} and I_{Kr} we have performed a number of additional simulations in the main body of the manuscript and here to test what effect different permutations of I_{Ks} and I_{Kr} inhibition would have on model predictions of acute DOX cellular phenotypes. Due to the use of different data sets to constrain human cardiac cell models, there is variability in model predictions that reflect the uncertainty in the underlying experimental data. To provide a reference for the effect of I_{Ks} and I_{Kr} inhibition alone on the human and rabbit cell model we have performed simulations with 10%, 25% and 50% inhibition of each channel on the APD and calcium transient as reported in Table 13 and 14.

Table 13: Effect of either I_{Kr} or I_{ks} inhibition in human cell model on APD and calcium transient

Condition	ADP (ms)	Ca^{2+} Systolic (μM)	Ca^{2+} RT (ms)
Control	305	0.80	356
I_{Kr} scaled by -10%	309	0.82	357
I_{Kr} scaled by -25%	315	0.84	359
I_{Kr} scaled by -50%	327	0.87	361
I_{Ks} scaled by -10%	312	0.84	359
I_{Ks} scaled by -25%	326	0.89	362
I_{Ks} scaled by -50%	355	1.02	372

Table 14: Effect of either I_{Kr} or I_{ks} inhibition in rabbit cell model on APD and calcium transient

Condition	ADP (ms)	Ca^{2+} Systolic (μM)	Ca^{2+} RT (ms)
Control	209	0.40	344
I_{Kr} = -10%	214	0.41	343
I_{Kr} = -25%	223	0.43	341
I_{Kr} = -50%	240	0.47	339
I_{Ks} = -10%	210	0.40	344
I_{Ks} = -25%	211	0.40	343
I_{Ks} = -50%	214	0.41	343

In both consensus models of acute DOX exposure (derived using method 1 or 2) that we used to predict an increase in SR leak, we did not include any effect of DOX on I_{Ks}. To ensure that the predicted increased SR leak result was not dependent on how the effect of acute DOX exposure on K^+ channels was modelled we estimate the SR leak and corresponding APD and Ca^{2+} transient phenotypes for the case where acute DOX exposure inhibits I_{Ks} and not I_{Kr} and the case where acute DOX exposure inhibits I_{ks} and I_{kr}. All simulations results are based on the acute DOX 100μM model. The results are presented in Table 15.

Table 15: Predicted SR leak in the rabbit and human acute DOX 100μM exposure model for different postulated models of acute DOX on K^+ channels. Consensus values wer a 50% increase in APD, 50% increase in systolic Ca^{2+} and a 30% increase in Ca^{2+} RT. Where I_K = X% corresponds to an X% change in the conductivity of the corresponding K^+ channel.

Species	Estimated SR leak	Effect on K^+ channels	ADP	Ca^{2+} Systolic	Ca^{2+} RT
Human	3x Leak	I_{Ks} = -95%	+69%	+28%	+58%
Human	3x Leak	I_{Ks} = -95%, I_{Kr} =-50%	+117%	+63%	+77%
Rabbit	2x Leak	I_{Ks} = -95%	+11%	+39%	+0%
Rabbit	3x Leak	I_{Ks} = -95%, I_{Kr} =-50%	+34%	+33%	+8%