

Supporting Information File 2

for

Drug targeting to decrease cardiotoxicity – determination of the cytotoxic effect of GnRH-based conjugates containing doxorubicin, daunorubicin and methotrexate on human cardiomyocytes and endothelial cells

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Supplementary tables – Comparative data of short- and long-term effects of GnRH-drug conjugates on cardiomyocytes and endothelial cells

Investigations of short- and long-term effects elicited by GnRH-drug conjugates provides information about diverse cell physiological responses induced by biologically active compounds. (i) The short-term effects are accomplished within several minutes or maximum 1–2 hours and they are mainly detected as acute responses (e.g., morphological changes like spreading or shrinking) of the cell. (ii) The long-term effects require more time to be detectable, the time required for development often depends on the cell cycle (24–72 hours). However, there is a long list of cell physiological responses induced in long-term relations, one of the most fundamental and significant response is cell proliferation. Therefore, in the present study we evaluate cytotoxic responses as chief characteristics of the long-term effects of GnRH-drug conjugates; nevertheless, provide comparative data of short- and long-term treatments as inducers of diverse cellular responses.

In the tables below, the results of impedimetric analysis on HUVEC and HCM cell lines are presented. The recorded data show spreading (short-term) and cytotoxic (long-term) responses. Statistically significant effects are highlighted in bold (one-way ANOVA test; * - $P < 0.05$; ** - $P < 0.01$; *** - $P < 0.001$).

Table S1: Short- and long-term cytotoxic effect of chemotherapeutic drugs (doxorubicin, daunorubicin, methotrexate) on (a) HUVEC and (b) HCM cell lines.

(a) HUVEC		10^{-12} mol/L	10^{-11} mol/L	10^{-10} mol/L	10^{-9} mol/L	10^{-8} mol/L	10^{-7} mol/L	10^{-6} mol/L
Short-term	Doxorubicin	0.008	0.021*	-0.004	0.003	-0.008	0.037***	0.063***
	Daunorubicin	-0.005	0.008	-0.008	-0.008	0.018	0.055**	0.047**
	Methotrexate	0.015*	0.013	0.012	0.019*	0.013	0.002	0.001
Long-term	Doxorubicin	0.017*	0.015*	0.007	0.008	0.002	0.001	-0.086***
	Daunorubicin	-0.001	0.002	0.001	-0.00113	-0.001	-0.007*	-0.019***
	Methotrexate	0.001	0.001	0.001	0.001	-0.001	-0.007	-0.112***
(b) HCM		10^{-12} mol/L	10^{-11} mol/L	10^{-10} mol/L	10^{-9} mol/L	10^{-8} mol/L	10^{-7} mol/L	10^{-6} mol/L
Short-term	Doxorubicin	0.001	0.002	0.002	-0.001	0.001	0.003	0.019***
	Daunorubicin	-0.001	-0.001	-0.001	-0.001	0.001	-0.001	0.031***
	Methotrexate	0.002	0.001	0.001	-0.001	0.001	0.001	0.005
Long-term	Doxorubicin	-0.001	-0.001	0.001	-0.001	-0.001	-0.006*	-0.016***
	Daunorubicin	-0.001	-0.001	-0.001	-0.001	-0.001	-0.007*	-0.019***
	Methotrexate	0.001	0.001	0.001	-0.001	0.001	0.001	-0.001

Table S2: Short- and long-term cytotoxic effect of GnRH conjugates containing doxorubicin on (a) HUVEC and (b) HCM cell lines.

(a) HUVEC		10^{-8} mol/L	10^{-7} mol/L	10^{-6} mol/L
Short-term	Doxorubicin	0.002	0.032	0.012
	An-152	-0.004	0.014	0.031
	GnRH-III(Dox-O-glut) (1)	-0.011	0.016	0.038
	GnRH-III(Dox-glut-GFLG) (2)	-0.004	0.016	0.058**
	GnRH-III(Dox=Aoa-GFLG) (3)	-0.002	-0.003	-0.006
Long-term	Doxorubicin	-0.007	-0.019***	-0.046***
	An-152	-0.005	-0.029***	-0.051***
	GnRH-III(Dox-O-glut) (1)	-0.003	-0.028***	-0.054***
	GnRH-III(Dox-glut-GFLG) (2)	-0.002	-0.017***	-0.042***
	GnRH-III(Dox=Aoa-GFLG) (3)	0.0004	-0.001	-0.009**
(b) HCM		10^{-8} mol/L	10^{-7} mol/L	10^{-6} mol/L
Short-term	Doxorubicin	0.001	-0.001	0.057***
	An-152	0.001	-0.001	0.008
	GnRH-III(Dox-O-glut) (1)	0.001	0.002	0.012**
	GnRH-III(Dox-glut-GFLG) (2)	0.009*	0.003	0.008*
	GnRH-III(Dox=Aoa-GFLG) (3)	0.004	0.001	-0.002
Long-term	Doxorubicin	-0.003*	-0.014***	-0.021***
	An-152	-0.001	-0.008***	-0.023***
	GnRH-III(Dox-O-glut) (1)	-0.002*	-0.008***	-0.025***
	GnRH-III(Dox-glut-GFLG) (2)	0.001	-0.004*	-0.019***
	GnRH-III(Dox=Aoa-GFLG) (3)	0.002	-0.001	-0.007***

Table S3: Short- and long-term cytotoxic effect of oxime bond-linked, bifunctional daunorubicin-GnRH-III conjugates on (a) HUVEC and (b) HCM cell lines.

(a) HUVEC		10^{-8} mol/L	10^{-7} mol/L	10^{-6} mol/L
Short-term	Daunorubicin	-0.008	-0.002	0.003
	GnRH-III(Dau=Aoa) (4)	0.001	-0.003	-0.003
	GnRH-III(Dau=Aoa-K(Dau=Aoa)) (12)	0.009	0.010	-0.003
	[⁴ Lys(Ac)]-GnRH-III(Dau=Aoa) (5)	-0.001	0.004	0.002
	[⁴ Lys(Dau=Aoa)]-GnRH-III(Dau=Aoa) (14)	0.002	-0.005	0.006
Long-term	Daunorubicin	0.001	0.001	-0.010***
	GnRH-III(Dau=Aoa) (4)	-0.001	-0.001	-0.001
	GnRH-III(Dau=Aoa-K(Dau=Aoa)) (12)	-0.001	-0.001	-0.001
	[⁴ Lys(Ac)]-GnRH-III(Dau=Aoa) (5)	-0.001	-0.001	-0.001
	[⁴ Lys(Dau=Aoa)]-GnRH-III(Dau=Aoa) (14)	0.001	-0.001	-0.001
(b) HCM		10^{-8} mol/L	10^{-7} mol/L	10^{-6} mol/L
Short term	Daunorubicin	-0.001	-0.001	0.028***
	GnRH-III(Dau=Aoa) (4)	-0.002	-0.001	0.001
	GnRH-III(Dau=Aoa-K(Dau=Aoa)) (12)	-0.001	-0.001	-0.001
	[⁴ Lys(Ac)]-GnRH-III(Dau=Aoa) (5)	-0.002	-0.001	-0.005
	[⁴ Lys(Dau=Aoa)]-GnRH-III(Dau=Aoa) (14)	0.001	-0.002	-0.001
Long term	Daunorubicin	-0.001	-0.006***	-0.021***
	GnRH-III(Dau=Aoa) (4)	-0.001	-0.001	-0.001
	GnRH-III(Dau=Aoa-K(Dau=Aoa)) (12)	-0.001	-0.001	-0.002
	[⁴ Lys(Ac)]-GnRH-III(Dau=Aoa) (5)	0.001	-0.001	-0.003*
	[⁴ Lys(Dau=Aoa)]-GnRH-III(Dau=Aoa) (14)	-0.0011	-0.001	-0.002

Table S4: Short- and long-term cytotoxic effect of GnRH-III conjugates containing daunorubicin on (a) HUVEC and (b) HCM cell lines.

(a) HUVEC		10^{-8} mol/L	10^{-7} mol/L	10^{-6} mol/L
Short-term	Daunorubicin	0.005	0.016*	0.009
	GnRH-III(Dau=Aoa-GFLG) (7)	-0.002	-0.012	0.003
	GnRH-III(Dau=Aoa-YRRL) (8)	-0.010	-0.004	0.003
	{GnRH-III(Dau=Aoa-YRRL-C)} ₂ (11)	0.022	0.002	0.014
	{GnRH-III(Dau=Aoa-C)} ₂ (9)	0.001	0.008	0.014
	[⁴ N-MeSer]-GnRH-III(Dau=Aoa) (6)	0.011	0.002	0.006
	{[⁴ N-MeSer]-GnRH-III(Dau=Aoa-C)} ₂ (10)	0.008	0.011	0.017*
Long-term	Daunorubicin	0.006	0.004	-0.058***
	GnRH-III(Dau=Aoa-GFLG) (7)	-0.001	0.001	-0.013***
	GnRH-III(Dau=Aoa-YRRL) (8)	-0.007	-0.002	-0.018***
	{GnRH-III(Dau=Aoa-YRRL-C)} ₂ (11)	0.001	-0.005*	-0.017***
	{GnRH-III(Dau=Aoa-C)} ₂ (9)	-0.002	-0.001	0.001
	[⁴ N-MeSer]-GnRH-III(Dau=Aoa) (6)	0.001	-0.001	0.001
	{[⁴ N-MeSer]-GnRH-III(Dau=Aoa-C)} ₂ (10)	0.001	0.002	0.004

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(b) HCM		10 ⁻⁸ mol/L	10 ⁻⁷ mol/L	10 ⁻⁶ mol/L
Short term	Daunorubicin	-0.001	-0.009***	0.030**
	GnRH-III(Dau=Aoa-GFLG) (7)	-0.001	-0.001	0.003
	GnRH-III(Dau=Aoa-YRRL) (8)	-0.003	-0.001	-0.002
	{GnRH-III(Dau=Aoa-YRRL-C)} ₂ (11)	-0.001	-0.001	-0.004
	{GnRH-III(Dau=Aoa-C)} ₂ (9)	-0.001	0.002	0.003*
	[⁴ N-MeSer]-GnRH-III(Dau=Aoa) (6)	-0.005	-0.001	0.001
	{[⁴ N-MeSer]-GnRH-III(Dau=Aoa-C)} ₂ (10)	-0.002	-0.001	0.001
Long term	Daunorubicin	-0.001	-0.009***	-0.024***
	GnRH-III(Dau=Aoa-GFLG) (7)	0.001	-0.002	-0.002
	GnRH-III(Dau=Aoa-YRRL) (8)	-0.001	-0.002	-0.004*
	{GnRH-III(Dau=Aoa-YRRL-C)} ₂ (11)	-0.001	-0.002	-0.004*
	{GnRH-III(Dau=Aoa-C)} ₂ (9)	-0.002	-0.001	-0.001
	[⁴ N-MeSer]-GnRH-III(Dau=Aoa) (6)	-0.002	-0.002	0.001
	{[⁴ N-MeSer]-GnRH-III(Dau=Aoa-C)} ₂ (10)	-0.001	-0.003*	-0.005**

Table S5: Short- and long-term cytotoxic effect of GnRH-III conjugates containing methotrexate and daunorubicin on (a) HUVEC and (b) HCM cell lines.

(a) HUVEC		10 ⁻⁸ mol/L	10 ⁻⁷ mol/L	10 ⁻⁶ mol/L
Short- term	Daunorubicin	0.005	0.016*	0.009
	Methotrexate	-0.003	0.003	0.005**
	GnRH-III(Mtx-K(Dau=Aoa)) (13)	0.001	0.002	0.013*
	[⁴ Lys(Mtx)]-GnRH-III(Dau=Aoa) (15)	0.006	-0.002	0.006
Long- term	Daunorubicin	0.006	0.004	-0.058***
	Methotrexate	-0.008	-0.006	0.001
	GnRH-III(Mtx-K(Dau=Aoa)) (13)	-0.001	0.001	0.001
	[⁴ Lys(Mtx)]-GnRH-III(Dau=Aoa) (15)	0.003	-0.001	-0.001
(b) HCM		10 ⁻⁸ mol/L	10 ⁻⁷ mol/L	10 ⁻⁶ mol/L
Short term	Daunorubicin	-0.001	-0.001	0.028***
	Methotrexate	0.001	0.001	0.001
	GnRH-III(Mtx-K(Dau=Aoa)) (13)	-0.002	0.002	-0.003
	[⁴ Lys(Mtx)]-GnRH-III(Dau=Aoa) (15)	-0.003	-0.002	-0.001
Long term	Daunorubicin	-0.001	-0.006***	-0.021***
	Methotrexate	0.002	-0.001	0.001
	GnRH-III(Mtx-K(Dau=Aoa)) (13)	-0.001	-0.001	-0.001
	[⁴ Lys(Mtx)]-GnRH-III(Dau=Aoa) (15)	-0.001	0.001	-0.001