Sex-related differential susceptibility to ponatinib-induced cardiotoxicity and its relationship to modulation of Notch signaling in a murine model

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Background: Ponatinib (PON), a tyrosine kinase inhibitor approved in chronic myeloid leukemia, has proven cardiovascular toxicity. Although sex is a risk factor for PON-induced cardiotoxicity in humans, little is known about its mechanisms in general, and sex-related mechanisms in particular.

Objectives: To determine the mechanisms of sex-related PON-induced cardiotoxicity and identify potential rescue strategies in a murine model.

Methods: 24-months-old male and female C57B5 mice were treated with 3 mg/kg/day of PON or vehicle via oral gavage for 28 days, with/without siRNA-Notch1 or siRNA-scrambled via tail vein every 3 days.

Results: PON + scrambled siRNA-treated male mice had a higher number of TUNEL-positive cells, a higher percentage of senescence-associated β-galactosidase positive senescent cardiac areas, as well as a lower reactivity degree for the survival marker Bmi1 than female counterparts. Proteomics analysis of cardiac tissue showed upstream activation of nitric oxide synthase (NOS) type 2, downstream activation of cell death and production of reactive oxygen species in PON + scrambled siRNA- compared to vehicle or PON + Notch1 siRNA-treated male mice. Upstream analysis showed beta-estradiol activation, while downstream analysis showed activation of cell survival and inhibition of cell death in PON + scrambled siRNA compared to vehicle-treated female mice. PON + scrambled siRNA-treated mice also showed a downregulation of cardiac actin, which was more marked in male; as well as vessel density, which was more marked in female mice. Female hearts showed a greater extent of cardiac fibrosis than male counterparts at baseline, with no significant changes after PON treatment. In contrast, PON + scrambled siRNA-treated mice had less fibrosis than vehicle or PON + Notch1-siRNA-treated mice. Left ventricular systolic dysfunction shown in PON + scrambled siRNA-treated male mice and - to a lesser extent - by female mice was similarly reversed in both PON + Notch1-siRNA-treated male and female mice (Table 1).

Conclusions: We found a sex-related differential susceptibility and Notch1 modulation in PON-induced cardiotoxicity. This can improve our understanding of sex-related differences and help identify biomarkers in PON cardiotoxicity.

Table 1

	P<0.05* P<0.01**	P<0.05° P<0.01°°	P<0.05# P<0.01##	P<0.05§ P<0.01§§
	Male (M)		Female (F)	
	Vehicle	PON+siRNA-scr	PON+siRNA-Notch1	Vehicle	PON+siRNA-scr	PON+siRNA-Notch1
% SA-β-gal+	0.37±0.1	1.41±0.6**	0.56±0.3°°	0.61±0.2	0.77±0.2	0.55±0.2°
% Tdt+	1.31±0.02	6.12±0.17**	3.11±0.15°°	0.62±0.17#	3.75±0.35**	0.75±0.35°°
Bmi1 reactivity degree	2873±1523	5000±703*	4978±155	2968±0.731#	8567±2173**	2715±313°
Cardiac actin/GAPDH	1.97±0.25	1.01±0.16**	3.29±0.23°°	1.37±0.18#	0.97±0.01*	1.82±0.31°
% Vessel density	2.01±0.25	0.97±0.32**	3.41±0.21°°	1.72±0.32	0.49±0.52*	3,05±0.28°°
Flt1/GAPDH	1.77±0.45	0.19±0.37*	2.81±0.23°°	0.23±0.12	0.26±0.12	0.47±0.14
MMP-9/GAPDH	2±0.29	1.1±0.34*	2.98±0.58°°	0.36±0.38	0.63±0.11*	1.17±0.19°°
AQP-1/GAPDH	0.88±0.19	0.49±0.1*	1.05±0.01°°	0.7±0.13	0.35±0.07**	0.64±0.13°
% Fibrosis	0.1±0.04	0.03±0.03*	0.08±0.04	0.21±0.1#	0.19±0.12#	0.15±0.09
Coll type I/GAPDH	1±0.32	0.5±0.03*	0.71±0.17	2.2±0.43#	1.4±0.38#	1.3±0.23
% EF	65±6	27±8**	53±9**	51±13#	36±7**	52±7°°
E/A	1.77±0.07	2.09±53	2.3±0.39	0.48±0.13##	0.89±0.17##	0.84±0.9*

** vs vehicle, p < 0.01; * vs vehicle, p< 0.05; °° vs PON, p<0.01; ° vs PON, p<0.01, # vs M vehicle, p<0.05.

N=6 per each treatment group