MicroRNA profiling of plasma from B6C3F1 mice treated with sunitinib

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Abstract

Sunitinib is a tyrosine kinase inhibitor used to treat cancers, such as metastatic renal cell carcinoma, gastrointestinal stromal tumor, and pancreatic neuroendocrine tumors. Adverse cardiotoxic events, such as left ventricular dysfunction, hypertension, and heart failure, were observed in clinical studies despite extending cancer patient survival. Currently, there are no clinical biomarkers available to predict the early onset of sunitinib-induced cardiotoxicity during cancer therapy. Growing evidence suggests that miRNAs may play a role in susceptibility to adverse drug reactions and have emerged as promising biomarkers of cardiovascular pathologies and therapeutic response. To address the knowledge gap in cardiotoxicity susceptibility, healthy male and female B6C3F1 mice were treated with 80 mg/kg/day of sunitinib or vehicle through oral gavage daily for 21 days. The cardiac function was measured via ultrasound imaging 24 hours post-dosing on days 0, 3, 6, 9, 12, and 21, and blood samples were collected for evaluation of the myocardial injury marker cardiac troponin-I (cTnI) and microRNAs (miRNAs). There were no changes in plasma cTnI concentrations, indicating that sunitinib did not cause significant cardiac injury in either sexes. Stroke volume was significantly decreased on treatment Day 3, 6, 9, 12 and 21, particularly in males in a time-dependent manner. Similarly, a consistent decline in cardiac output was also observed. Left ventricular ejection fraction and fractional shortening were significantly decreased on Day 6 in females and Day 9 in males but were comparable to baseline values on Day 21. Preliminary results of miRNA profiling in plasma indicate that the highest number of miRNAs were differentially expressed in Day 6 female (82 miRNAs) and Day 9 male mice (66 miRNAs), wherein 39% and 42% of the differentially expressed miRNAs are associated with cardiotoxicity-related effects such as cardiac enlargement, cardiac proliferation, cardiac necrosis and cell death. Although recovery potential was observed by Day 12 in the disease-free mice, the differential expression of miRNAs may be indicative of early cardiac effects that may be associated with development of delayed sunitinib-induced cardiotoxicity. Collectively, these results may aid in identifying biomarkers that could identify patients susceptible to delayed sunitinib-induced cardiotoxicity and impact monitoring decisions.

Experimental design

Animals: B6C3F$_1$ mice (Male & Female)
Drugs: Sunitinib (oral gavage)
Vehicle (oral gavage)
Dose: 80 mg/kg body wt /day Sunitinib
Sac: 24 hours after the last dose at 0, 3, 6, 9, 12 and 21-day

Sex	Treatment	End point	D00	D03	D06	D09	D12	D21
Male/Female	Vehicle/Sunitinib	cTnI assay, miRNA profiling	N=5	N=5	N=5	N=5	N=5	N=5
		Echocardiography imaging	N=10	N=10	N=10	N=10	N=10	N=10

Objectives

To measure in sunitinib- and vehicle-treated male and female B6C3F1 mice

1. Plasma levels of myocardial injury marker cardiac Troponin-I.
2. Changes in left ventricular ejection fraction, fractional shortening, stroke volume and cardiac output (CO) using the Vevo 3100 ultrasound imaging system.
3. miRNA expression profiles in the plasma.

Materials & Methods

10-week old mice were treated with a daily dose of 80 mg/kg sunitinib or vehicle via oral gavage for 21 days. The animals were sacrificed 24 hrs after dosing on day 0, 3, 6, 9, 12, and 21. Cardiac functional parameters were measured before each sacrifice using the Vevo 3100 ultrasound imaging system (N=10 mice/group). Blood was collected for the measurement of cTnI and miRNA profiling in plasma (N=5 mice/group). The concentration of cTnI was measured in all treatment groups using the Erenna SMC cTnI Immunoassay kit (EMD Millipore, MA). miRNA profiling was performed using HTG EdgeSeq technology (Firalis, France). One-way ANOVA was used to compute the differentially expressed miRNAs (p-value < 0.05 and fold change >1.5) using Statistical Analysis System (SAS) software. Ingenuity Pathway Analysis (IPA) was used to identify the miRNAs involved in various cardiac pathologies.

Results

1. Measurement of cardiac Troponin-I

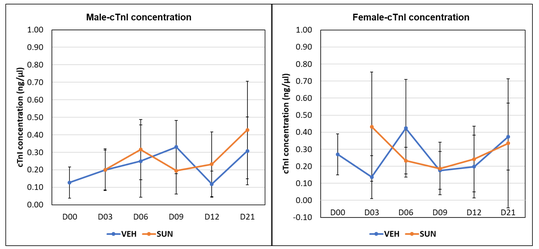
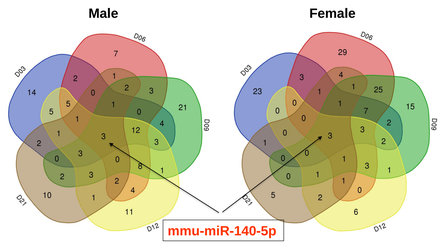


Fig 1: Plasma concentrations of cardiac troponin-I in male and female B6C3F1 mice treated with vehicle or sunitinib. Values are mean ± SD (N=5 mice/group). No significant difference in cTnI concentration was observed in both male and female mice after sunitinib treatment compared to control group indicating that sunitinib did not cause any overt cardiac injury with 21 days of treatment.

3. miRNA profiling in plasma



		D03	D06	D09	D12	D21
Male	Upregulated	27	33	34	33	24
	Downregulated	29	18	32	30	8
	Total	56	51	66	63	32
Female	Upregulated	34	16	24	12	11
	Downregulated	12	66	41	11	10
	Total	46	82	65	23	21

Fig. 3 Venn diagram of differentially expressed miRNAs in plasma from sunitinib- vs vehicle-treated male and female mice at Day 03, 06, 09, 12, and 21 (p-value<0.05). Differential expression of miRNAs was observed at each cumulative dose. miR-140-5p which plays a role in cardiomyocyte apoptosis was consistently downregulated throughout treatment in both male and female mice. Highest number of miRNAs were differentially expressed in male (66 miRNAs) and female mice (82 miRNAs) at D09 and D06 after sunitinib treatment, respectively when their LVEF and FS were the lowest. Table.1 shows the number of miRNAs differentially expressed during each treatment day (p-value<0.05, FC>1.5).

Results: continued

2. Measurement of cardiac functional parameters

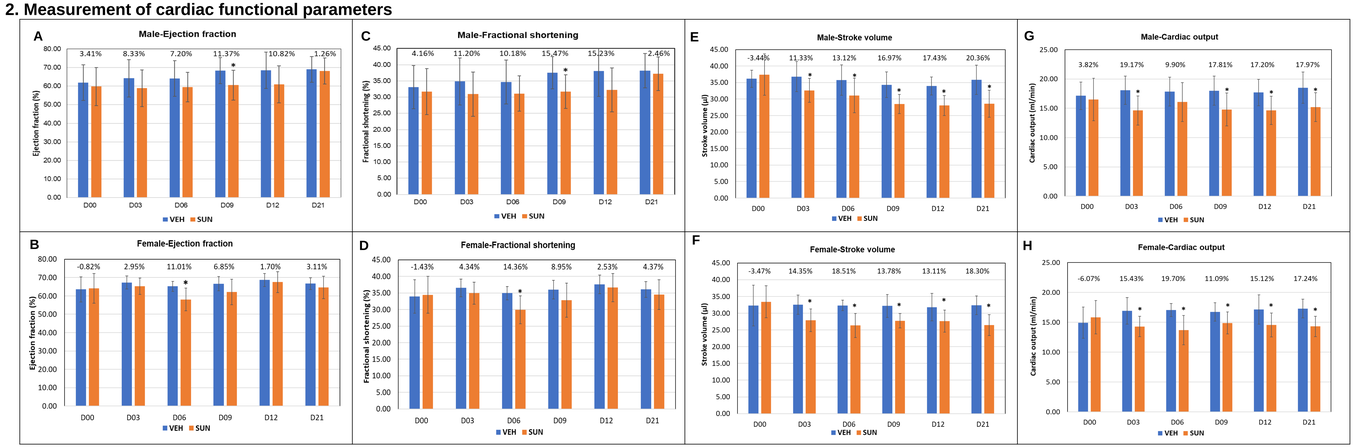


Fig. 2 Measurement of cardiac parameters in sunitinib vs vehicle-treated male and female mice at Day 00, 03, 06, 09, 12, and 21. Values are mean ±SD (N=10 mice/group;*, p<0.05). Values above the bar represent the percentage decline after sunitinib treatment compared to vehicle group. LVEF and FS were decreased significantly (p<0.05) in female mice at D06 after sunitinib treatment with the highest decline in LVEF (~11%) and FS (~15%) compared to vehicle group (Fig. 2B & 2D). In comparison, stroke volume was significantly decreased on treatment Day 03, 06, 09, 12 and 21, particularly in males in a time-dependent manner and with increases in number of doses (Fig.2E & 2F). Similar effects were also observed in the overall cardiac output with consistent decreases in both male and female mice compared to vehicle treated control mice (Fig.2G & 2H).

3. miRNA profiling in plasma (continued)

A Cardiovascular system development and function related miRNAs

Category	Male					Female				
	D03	D06	D09	D12	D21	D03	D06	D09	D12	D21
Development of vasculature	28	27	29	30	16	27	34	31	10	12
Vasculogenesis	28	26	29	30	15	27	33	30	9	11
Angiogenesis	28	26	29	30	15	27	33	30	9	11
Morphology of cardiovascular system	26	24	28	25	16	26	31	29	9	11
Cardiogenesis	23	22	25	23	15	25	30	27	6	8
Development of cardiovascular tissue	24	20	24	23	-	26	27	24	-	8
Development of endothelial tissue	24	20	24	23	11	26	27	24	7	8
Endothelial cell development	24	20	24	23	11	26	27	24	-	8
Abnormal morphology of cardiovascular system	25	24	28	25	16	25	30	28	8	10
Morphology of heart	24	23	27	24	15	25	30	27	7	9
Abnormal morphology of heart	24	23	27	24	15	25	30	27	7	9
Proliferation of endothelial cells	23	18	-	22	10	26	26	-	7	7
Morphology of vasculature	23	-	-	20	-	25	-	-	-	-
Cell movement of endothelial cells	25	21	-	23	-	26	-	24	-	9
Morphology of vessel	-	-	-	19	-	24	-	26	-	8
Abnormal morphology of vasculature	-	-	-	-	-	24	-	-	-	-
Migration of endothelial cells	-	-	-	-	-	26	-	-	7	9
Growth of blood vessel	-	21	26	21	13	24	25	25	5	6
Morphology of blood vessel	-	-	-	-	-	23	-	-	-	-
Vascularization	20	-	-	-	-	21	-	20	-	-
Enlargement of heart	-	-	-	24	-	-	28	-	-	9
Proliferation of pericytes	-	-	-	-	-	-	-	-	5	-
Morphology of heart ventricle	-	18	-	19	11	-	-	-	6	-
Proliferation of hepatic stellate cells	-	-	-	-	-	-	-	-	5	-
Hypertrophy of heart	-	20	-	22	-	-	-	-	6	-
Proliferation of vascular smooth muscle cells	-	20	-	20	12	-	-	-	-	6
Morphogenesis of cardiovascular system	-	18	-	20	-	-	-	-	-	6
Proliferation of heart cells	-	-	-	-	-	-	-	-	-	8
Morphology of cardiac muscle	-	-	-	20	-	-	-	-	-	8

B Cardiovascular disease-related miRNAs

Category	Male					Female				
	D03	D06	D09	D12	D21	D03	D06	D09	D12	D21
Abnormal morphology of cardiovascular system	25	24	28	25	16	25	30	28	8	10
Abnormal morphology of heart	24	23	27	24	15	25	30	27	7	9
Abnormality of heart ventricle	23	19	23	19	11	25	25	24	6	7
Vascular tumor	23	-	25	-	15	24	24	-	-	-
Vascular lesion	-	20	24	23	-	24	-	25	6	8
Abnormal morphology of vasculature	-	-	-	-	-	24	-	-	-	-
Vaso-occlusion	-	22	-	26	-	26	-	-	-	-
Angioma	-	-	-	-	14	23	-	-	-	-
Occlusion of artery	-	22	-	26	-	26	-	-	-	-
Occlusion of blood vessel	-	22	-	26	-	26	-	-	-	-
Hemangioma	23	-	25	-	14	23	-	-	-	-
Anemia	-	25	-	26	15	-	30	28	7	9
Cardiovascular neoplasm	23	-	25	-	-	-	24	25	-	-
Enlargement of heart	-	-	-	24	-	-	28	-	-	9
Cell death of cardiomyocytes	-	-	-	-	-	-	23	-	-	-
Familial cardiovascular disease	-	-	-	-	11	-	-	-	8	9
Aneurysm	-	-	-	-	-	-	-	-	4	-
Familial vascular disease	-	-	-	-	9	-	-	-	7	7
Peripheral vascular disease	-	-	-	-	-	-	-	-	9	-
Familial aortic disorder	-	-	-	-	-	-	-	-	5	-
Aortic dilatation	-	-	-	-	-	-	-	-	4	-
Familial aortic aneurysm	-	-	-	-	-	-	-	-	3	-
Loeys-Dietz syndrome	-	-	-	-	-	-	-	-	3	-
Aortic aneurysm	-	-	-	-	-	-	-	-	4	-
Thoracic aortic aneurysms and dissections	-	-	-	-	-	-	-	-	3	-
Familial thoracic aortic aneurysms and dissections	-	-	-	-	-	-	-	-	3	-
Apoptosis of cardiomyocytes	-	-	-	-	-	-	-	-	5	-
Hypertrophy of heart	-	20	-	22	-	-	-	-	6	-
Microangiopathy	-	-	22	-	12	-	-	-	-	7
Arteriosclerosis	-	-	-	25	-	-	-	-	-	-
Atherosclerosis	-	-	-	25	-	-	-	-	-	-

C Cardiotoxicity-related miRNAs

Category	Male					Female				
	D03	D06	D09	D12	D21	D03	D06	D09	D12	D21
Cardiac Necrosis/Cell Death	20	17	22	19	12	20	24	20	6	8
Cardiac Enlargement	24	23	26	24	15	24	28	26	6	9
Heart Failure	23	19	22	21	13	23	21	22	5	8
Cardiac Proliferation	20	18	20	19	10	20	24	22	5	8
Cardiac Dysfunction	21	17	21	20	11	23	19	21	6	7
Cardiac Fibrosis	20	17	19	19	12	21	20	21	5	7
Cardiac Infarction	22	17	22	20	11	22	22	22	6	7
Congenital Heart Anomaly	19	15	20	16	8	22	18	21	4	8
Cardiac Damage	18	14	15	16	-	14	16	16	5	5
Cardiac Arrythmia	21	17	19	19	9	23	19	19	4	7
Cardiac Arteriopathy	22	19	22	19	11	23	21	21	4	6
Cardiac Stenosis	11	11	12	11	5	11	10	12	2	4
Cardiac Dilation	19	18	17	18	11	20	20	17	4	8
Congestive Cardiac Failure	14	12	13	13	7	16	14	15	-	-
Cardiac Hemorrhaging	5	2	2	2	2	5	4	4	1	2
Pulmonary Hypertension	15	16	17	15	10	14	19	19	4	4
Cardiac Inflammation	11	8	11	10	4	9	8	11	4	4
Cardiac Hypoplasia	7	4	6	6	-	9	5	6	-	-
Cardiac Regeneration	-	4	4	5	2	6	4	-	1	3
Cardiac Degeneration	2	2	4	2	1	-	-	4	1	1
Cardiac Pulmonary Embolism	-	7	9	7	3	9	7	7	3	2
Tachycardia	-	6	-	6	-	8	6	-	2	2
Cardiac Thrombosis	-	3	-	4	1	-	-	6	1	1
Cardiac Hyperplasia	-	-	4	-	1	4	4	4	-	2
Cardiac Stress Response	#N/A	-	-	-	-	-	-	-	1	-

D Comparison of D06 Female Vs D09 Male unique miRNAs-Cardiotoxicity

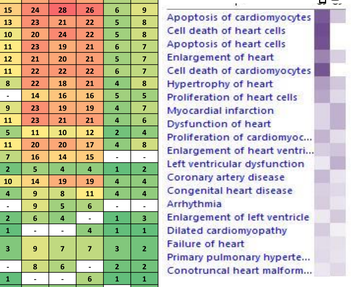


Table. 2 Expression of miRNAs related to cardiovascular development & function (A), cardiovascular diseases (B) and cardiotoxicity-related miRNAs (C) in plasma of sunitinib-treated male and female mice. The numbers in each column indicate differentially expressed miRNAs (p<0.05). The color gradient represents the expression level ranging from high (red) to low (green) in plasma. Results indicate that miRNAs associated with cardiovascular development & function, cardiovascular diseases and cardiotoxicity were highly expressed as early as at D03 in both sexes, whereas the expression of these miRNAs decreased at D12 and D21 in female mice, and at D21 in male mice. These results correlated with the recovery of EF and FS observed in female mice at later timepoints. Comparison analysis of unique differentially expressed miRNAs at D06 in female and at D09 in male mice revealed differential expression of miRNAs related to apoptosis of cardiomyocytes in the female mice, whereas male mice showed differential expression of miRNAs related to myocardial infarction and left ventricular dysfunction (D). Altogether, results from miRNA profiling in plasma may indicate a differential response of sunitinib in heart between male and female mice.

Conclusions

- No significant differences in plasma cTnI concentrations between sunitinib- and vehicle-treated male and female mice suggests the absence of cardiac injury.
- Cardiac function parameters such as EF and FS were reduced in females after 1 week of treatment followed by return to baseline with continued dosing, which may suggest temporal susceptibility to sunitinib cardiac effects followed by recovery. However, SV and CO cardiac function parameters were significantly reduced in both male and female mice throughout the treatment.
- Plasma miRNA profiling showed that highest number of miRNAs were differentially expressed in male (66 miRNAs) and female mice (82 miRNAs) at day 9 and 6 after sunitinib treatment, respectively when their LVEF and FS decreased significantly. This data imply that miRNAs might play a role in reducing the LVEF and FS upon sunitinib treatment.
- Among the differentially expressed miRNAs, miR-140-5p, which has been associated with drug-induced oxidative stress, myocardial apoptosis and cardiotoxicity was the only miRNA consistently downregulated throughout treatment in both male and female mice. The down regulation of miR140-5p may play a role in decreasing the oxidative stress to counter the cardiac effects caused by sunitinib treatment.
- Sunitinib treatment caused a differential expression of miRNAs in plasma related to cardiovascular development and function, cardiovascular disease, and cardiotoxicity in both male and female mice. The differential expression of these miRNAs might be associated with development of delayed sunitinib-induced cardiotoxicity.
- These findings provide important insights into miRNA expression changes and potential molecular mechanisms related to cardiac effects in B6C3F1 mice treated with sunitinib.

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