Supplementary Data

The Inhibitory Effect of Protamine on Platelets is Attenuated by Heparin without Inducing Thrombocytopenia in Rodents

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Table S1. The percentage of cells of various categories in bone marrow of mice on the 35th day of the experiment.

Parameter	VEH	UFH	UFH + PS	PS
Erythroid (E)				
Proerythroblasts	1.5 (0–3)	1.5 (0–4)	1.5 (0–6)	0.5 (0–3)
Basophilic erythroblasts	6.0 (3–7)	4.5 (3–9)	**3.0 (0–4)****	4.0 (1–7)
Polychromatic erythroblasts	9.0 (7–15)	7.5 (1–14)	8.5 (4–11)	**6.5 (4–11)***
Oxyphilic erythroblasts	6.5 (4–10)	5.0 (1–10)	5.5 (5–9)	6.5 (3–9)
Total of erythroid cells	21.5 (18–30)	19.0 (7–29)	17.5 (9–24)	**15.5 (12–24)***
Myeloid (M)				
Myeloblasts	0.0 (0–2)	0.0 (0–1)	0.0 (0–2)	0.0 (0–1)
Promyelocytes	5.5 (3–11)	**3.5 (1–5)***	5.5 (3–10)	**3.5 (2–7)***
Myelocytes	21.5 (17–25)	21.5 (19–24)	20.5 (19–26)	21.5 (20–26)
Metamyelocytes	16.0 (13–18)	17.0 (15–23)	**18.0 (15–22)***	**18.0 (15–25)****
Band	19.0 (10–23)	20.0 (13–26)	19.0 (18–21)	20.5 (16–23)
Neutrophils	14.0 (6–22)	13.5 (9–25)	15.0 (11–19)	16.0 (8–31)
Eosinophils	**-**	**-**	0.0 (0–1)	-
Basophils	**-**	**-**	-	-
Total of myeloid cells	75.5 (65–83)	75.5 (68–92)	79.5 (74–88)	79.5 (73–90)
Lymphocytes	4.0 (3–7)	**2.0 (1–8)***	**2.0 (1–3)*****	3.0 (0–4)
Monocytes	0.0 (0–1)	0.5 (0–2)	0.0 (0–1)	0.5 (0–1)
M/E ratio	3:1	4:1	4:1	5:1

Abbreviations: VEH: vehicle, UFH: unfractionated heparin, PS: protamine sulfate. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs vehicle, Kruskal-Wallis ANOVA with Dunn's post hoc test. Results are shown as median with range; $n = 8$.

Table S2. Complete blood count in rats after UFH and PS administration alone or in combination during the one-hour observation and on the 35th day of the experiment.

Parameter	T	VEH	UFH 150 U/kg	UFH 150 U/kg + PS 1.5 mg/kg	PS 1.5 mg/kg
WBC (10^3/mm^3)	**15 min**	4.8 (1.9–6.8)	3.7 (3.6–7.8)	4.0 (3.3–6.5)	3.6(2.2–5.5)
	30 min	4.3 (2.0–6.4)	3.5 (3.2–4.1)	4.1 (1.1–10.1)	3.8 (3.2–5.1)
	60 min	2.2 (1.6–6.4)	**2.9 (2.1–3.3)^**	3.1 (2.4–9.8)	**2.3 (2.1–4.3)**#
	35 day	3.2 (2.3–3.9)	3.0 (2.4–4.1)	2.7 (1.8–3.6)	3.1 (1.9–6.1)
RBC (10^6/mm^3)	**15 min**	7.1 (6.6–8.1)	7.4 (6.7–8.1)	7.1 (6.4–7.9)	7.2 (6.9–8.3)
	30 min	7.1 (6.9–8.1)	7.2 (7.0–7.9)	7.2 (6.7–8.1)	7.1 (6.8–8.3)
	60 min	6.5 (5.8–7.9)	**7.0 (6.2–7.9)^**	**6.6 (6.3–7.9)^** ##	**6.6 (5.7–7.0)^^** ##
	35 day	8.8 (7.7–9.2)	8.5 (8.2–9.3)	8.6 (7.9–9.4)	8.8 (8.5–9.2)
HGB (g/dl)	**15 min**	14.6 (13.4–14.9)	14.0 (13.2–14.8)	14.1 (13.5–15.4)	14.0 (13.8–15.6)
	30 min	14.8 (13.2–14.9)	13.7 (12.7–13.9)	13.7 (13.4–15.7)	14.0 (13.3–15.4)
	60 min	12.5 (11.3–15.3)	13.1 (12.9–13.9)	**12.8 (12.2–15.4)^^** #	**12.7 (10.9–14.1)^^** #
	35 day	15.9 (14.5–16.8)	15.9 (15.2–16.5)	15.8 (14.6–17.1)	16.6 (15.2–17.2)
HCT (%)	**15 min**	44.2 (38.4–47.0)	43.5 (41.7–46.5)	44.2 (41.1–46.2)	43.8 (43.2–49.4)
	30 min	45.2 (41.5–46.8)	42.5 (39.9–43.5)	43.2 (41.6–48.1)	43.93 (41.7–49.2)
	60 min	38.7 (35.4–46.9)	**41.3 (40.8–43.8)^**	**39.6 (37.9–46.6)^** #	**39.7 (37.4–42.8)^^** #
	35 day	48.7 (44.3–50.9)	48.1 (45.6–51.3)	48.0 (43.8–51.6)	48.9 (46.9–52.5)
MCV (μm^3)	**15 min**	60.0 (58–63)	59.0 (57–60)	60.0 (59–62)	60.5 (59–63)
	30 min	60.0 (58–64)	59.0 (57–60)	60.0 (59–62)	60.5 (59–63)
	60 min	59.0 (57–)64	59.0 (57–60)	60.0 (59–62)	60.0 (59–63)
	35 day	56.0 (54–58)	56.0 (55–58)	56.0 (55–58)	55.0 (53–58)
MCH (pg)	**15 min**	20.0 (18.5–21.0)	**18.8 (18.2–19.0)***	19.6 (18.9–20.4)	19.3 (18.7–20.1)
	30 min	19.1 (18.3–21.0)	18.6 (18.1–19.3)	**19.3 (18.8–20.3)^**	19.3 (18.5–20.0)
	60 min	18.9 (18.3–21.6)	18.5 (18.0–19.2)	**19.3 (18.7–20.2)^**	19.3 (18.8–20.3)
	35 day	18.5 (17.6–19.0)	18.5 (17.7–19.2)	18.6 (18.2–19.0)	18.3 (17.3–19.4)
MCHC (g/dl)	**15 min**	32.9 (31.2–34.9)	31.7 (31.4–32.2)	32.2 (31.4–33.3)	31.9 (31.5–32.3)
	30 min	31.7 (31.4–33.0)	31.9 (31.5–32.7)	**32.0 (31.6–32.7)^**	31.9 (31.2–32.5)
	60 min	31.9 (31.7–35.4)	31.8 (31.3–32.6)	32.1 (31.5–33.1)	32.2 (31.4–33.0)
	35 day	33.0 (32.1–33.6)	32.9 (32.2–33.7)	33.1 (32.8–33.7)	32.8 (32.3–34.0)

Abbreviations: WBC: white blood cells, RBC: red blood cells, HGB: hemoglobin, HCT: haematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, T: time, UFH: unfractionated heparin, PS: protamine sulfate. * $p < 0.05$ vs vehicle at the corresponding time point, Kruskal-Wallis ANOVA with Dunn's post hoc test; ^ $p < 0.05$, ^^ $p < 0.001$ vs 15 min within same group; # $p < 0.05$, ## $p < 0.01$ vs 30 min within same group, Friedman ANOVA with Dunn's post hoc test. Results are shown as median with range; $n = 5–10$.

Table S3. Complete blood count in mice at 3, 15 and 60 min, and on the 35th day after UFH and PS administration alone or in combination.

Parameter	T	VEH	UFH 150 U/kg	UFH 150 U/kg + PS 1.5 mg/kg	PS 1.5 mg/kg
WBC (10^3/mm^3)	**3 min**	0.6 (0.4–1.0)	0.8 (0.2–1.3)	0.5 (0.2–0.6)	0.3 (0.2–1.7)
	15 min		**0.4 (0.2–0.5)***	0.5 (0.2–0.8)	1.5 (0.4–6.4)
	60 min	1.5 (1.4–2.2)	1.8 (1.2–2.7)	1.7 (1.2–2.3)	1.7 (1.3–2.9)
	35 day	1.8 (0.8–4.5)	1.4 (0.9–2.2)	1.6 (0.7–5.5)	1.4 (0.4–2.1)
RBC (10^6/mm^3)	**3 min**	7.4 (7.1–7.6)	**7.1 (5.9–7.9)***	**7.0 (6.6–7.1)****	7.2 (6.2–7.8)
	15 min		7.1 (6.6–8.1)	**7.0 (6.5–7.4)***	7.4 (6.6–7.8)
	60 min	7.2 (6.9–8.1)	7.6 (6.9–7.9)	7.6 (7.5–7.6)	7.6 (6.9–7.9)
	35 day	4.3 (4.0–4.6)	4.2 (3.9–4.5)	4.2 (4.0–4.5)	4.2 (4.0–4.3)
HGB (g/dl)	**3 min**	12.7 (12.4–13.0)	12.3 (10.6–14.1)	**11.8 (11.2–12.1)****	12.1 (10.5–13.4)
	15 min		12.2 (11.0–14.1)	**11.9 (11.1–12.8)***	12.4 (11.3–13.5)
	60 min	11.9 (11.4–13.1)	12.2 (10.8–13.2)	12.4 (12.1–12.9)	12.4 (11.6–13.0)
	35 day	12.7 (12.3–13.1)	12.4 (11.0–12.8)	**12.0 (11.5–13.2)***	12.3 (11.7–12.8)
HCT (%)	**3 min**	41.4 (39.5–41.9)	40.0 (33.9–44.1)	**38.6 (38.1–40.9)***	40.1 (34.4–43.3)
	15 min		40.2 (36.3–44.8)	39.1 (36.8–42.2)	41.8 (36.5–44.3)
	60 min	37.9 (36.2–43.6)	39.7 (35.2–43.2)	40.7 (40.1–41.5)	40.1 (36.9–42.3)
	35 day	33.0 (31.3–35.7)	32.6 (30.5–35.0)	32.6 (31.1–34.8)	32.1 (31.0–33.0)
MCV (μm^3)	**3 min**	55.0 (54–56)	**57.0 (54–58)****	**57.0 (55–58)***	56.0 (55–57)
	15 min		56.0 (55–58)	**56.5 (55–57)***	**56.0 (55–57)***
	60 min	53.0 (53.0–54.0)	52.0 (51.0–55.0)	54.0 (53.0–55.0)	53.5 (52.0–54.0)
	35 day	78.0 (76–79)	78.0 (77–78)	77.0 (76–78)	77.0 (76–78)
MCH (pg)	**3 min**	17.4 (16.9–17.5)	17.6 (17.1–17.9)	16.9 (16.1–17.7)	17.2 (16.8–17.7)
	15 min		17.2 (16.8–17.8)	17.1 (16.4–17.8)	17.1 (16.5–17.5)
	60 min	16.6 (16.2–16.6)	16.6 (15.8–16.8)	16.4 (16.1–17.0)	16.4 (16.2–16.9)
	35 day	29.4 (28.2–31.1)	28.3 (28.1–29.5)	29.0 (27.4–29.4)	29.8 (28.8–30.2)
MCHC (g/dl)	**3 min**	31.2 (30.6–31.9)	30.7 (30.1–32.4)	**29.2 (29.1–31.6)***	30.7 (29.6–31.7)
	15 min		30.8 (29.3–31.9)	**30.4 (29.0–31.9)***	30.8 (28.8–31.2)
	60 min	31.4 (30.0–31.5)	30.9 (30.6–31.7)	30.6 (29.8–31.0)	30.9 (30.7–31.5)
	35 day	38.2 (36.6–39.4)	**36.7 (36.0–38.0)***	37.5 (35.8–38.0)	38.8 (37.0–39.2)

Abbreviations: WBC: white blood cells, RBC: red blood cells, HGB: hemoglobin, HCT: haematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, T: time, UFH: unfractionated heparin, PS: protamine sulfate. *$p < 0.05$, **$p < 0.01$ vs vehicle, Kruskal-Wallis ANOVA with Dunn's post hoc test. Results are shown as median with range; n = 5–8.