

Supplementary Material

Unified Methodology for the Primary Preclinical In Vivo Screening of New Anticoagulant Pharmaceutical Agents from Hematophagous Organisms

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Pharmacodynamic profile after substance administration. Time-dependance effect on clotting times and fibrinogen concentration

Table S1. Individual values of hemostasis parameters in male ICR mice, saline solution, 5 ml intravenously

Saline solution, 15 min							
Parameter							
№ mice	1	2	3	4	5	Mean	SD
APTT, sec	17.2	21.9	19.7	22.7	22.1	20.7	2.3
PTT, sec	9.9	10	10	10.6	10.6	10.2	0.3
Fibrinogen concentration, g/l	1.779	1.696	1.544	1.736	1.831	1.717	0.109
Saline solution, 30 min							
Parameter							
№ mice	6*	7	8	9	10	Mean	SD
APTT, sec	-	20.9	22.3	19.7	24.3	21.8	2.0
PTT, sec	-	9.6	10.3	10	10.4	10.1	0.4
Fibrinogen concentration, g/l	-	2.039	2.164	1.838	1.964	2.001	0.137
Saline solution, 60 min							
Parameter							
№ mice	11	12	13	14	15	Mean	SD
APTT, sec	24.7	23.5	25.4	22	26.4	24.4	1.7
PTT, sec	10.1	10.1	10.3	9.9	10.6	10.2	0.3
Fibrinogen concentration, g/l	1.646	1.518	1.838	1.736	1.634	1.674	0.120
Saline solution, 90 min							
Parameter							
№ mice	16	17	18	19	20*	Mean	SD
APTT, sec	24.8	29	26.3	21.5	-	25.4	3.1
PTT, sec	9.8	10	10	9.1	-	9.7	0.4
Fibrinogen concentration, g/l	1.904	1.933	1.893	2.226	-	1.989	0.159
Saline solution, 120 min							
Parameter							
№ mice	21	22	23	24	25	Mean	SD
APTT, sec	13.4	24.0	22.4	26.0	24.0	22.0	5.0
PTT, sec	10.3	10.5	10.9	11.5	11.2	10.9	0.5
Fibrinogen concentration, g/l	1.964	1.973	1.973	1.508	1.854	1.854	0.200
Saline solution, 180 min							
Parameter							
№ mice	26	27	28	29	30	Mean	SD
APTT, sec	24.9	23.1	25.5	21.6	26.7	24.4	2.0
PTT, sec	10.5	10	10.6	10.2	10.4	10.3	0.2
Fibrinogen concentration, g/l	1.833	1.904	1.793	2.211	1.862	1.921	0.167

& Data not received for technical reasons

* p≤0.05, ** p≤0.01 relative to the “saline” group according to the Mann-Whitney U-test

Table S2. Individual values of hemostasis parameters in male ICR mice, heparin, 20 IU/kg/5 ml intravenously

Parameter		Heparin, 20 IU/kg, 15 min					
№ mice	31	32	33	34	35	Mean	SD
APTT, sec	31.7	37.5	20.4	23.8	25.2	27.7 *	6.8
PTT, sec	11.1	11.3	10.5	10.5	9.8	10.6	0.6
Fibrinogen concentration, g/l	1.42	1.698	1.535	1.286	1.104	1.409 *	0.228

Parameter		Heparin, 20 IU/kg, 30 min					
№ mice	36	37	38&	39	40	Mean	SD
APTT, sec	21.8	32.3	-	29.9	55.2	34.8 *	14.3
PTT, sec	10.8	11.2	-	11	10.9	11.0	0.2
Fibrinogen concentration, g/l	1.49	1.433	-	1.67	1.717	1.578 *	0.137

Parameter		Heparin, 20 IU/kg, 60 min					
№ mice	41	42&	43	44	45	Mean	SD
APTT, sec	15.2	-	21	37	21.9	23.8	9.3
PTT, sec	7.4	-	9.8	9.3	10.4	9.2	1.3
Fibrinogen concentration, g/l	1.202	-	1.291	0.568	1.44	1.125 *	0.384

Parameter		Heparin, 20 IU/kg, 90 min					
№ mice	46	47	48	49&	50	Mean	SD
APTT, sec	23.6	27.8	20.3	-	24.9	24.2 *	3.1
PTT, sec	10.5	11.2	10.4	-	11.1	10.8	0.4
Fibrinogen concentration, g/l	1.52	1.567	1.461	-	1.482	1.508 *	0.047

& Data not received for technical reasons

* $p \leq 0.05$, relative to the “saline” group according to the Mann-Whitney U-test

Table S3. Individual values of hemostasis parameters in male ICR mice, variegin, 3 mg/kg/5 ml intravenously

Parameter		Variegin, 3 mg/kg, 15 min					
№ mice		51	52	53	54	55	Mean SD
APTT, sec		84.4	56.6	49.8	52.9	56.6	60.1 ** 13.9
PTT, sec		14.2	15.1	13.5	14.1	13.8	14.1 ** 0.6
Fibrinogen concentration, g/l		1.292	1.268	1.544	1.381	1.360	1.369 ** 0.108
Parameter		Variegin, 3 mg/kg, 30 min					
№ mice		56	57	58	59	60	Mean SD
APTT, sec		44.1	36.8	18	28.5	30.6	31.6 9.7
PTT, sec		11.7	11.1	9.7	10.3	10.5	10.7 0.8
Fibrinogen concentration, g/l		1.878	1.389	1.75	1.878	1.709	1.721 * 0.200
Parameter		Variegin, 3 mg/kg, 60 min					
№ mice		61	62	63	64	65	Mean SD
APTT, sec		23.4	38.8	18.9	31.8	33.6	29.3 8.0
PTT, sec		11	11.6	11.8	11.7	10.9	11.4 ** 0.4
Fibrinogen concentration, g/l		1.588	1.45	2.175	1.437	1.599	1.650 0.303
Parameter		Variegin, 3 mg/kg, 90 min					
№ mice		66	67&	68	69	70&	Mean SD
APTT, sec		22.8	-	25.4	27.1	-	25.1 2.2
PTT, sec		9.9	-	9.8	8.8	-	9.5 0.6
Fibrinogen concentration, g/l		1.684	-	1.862	1.937	-	1.828 0.130

* p≤0.05, ** p≤0.01 relative to the “saline” group according to the Mann-Whitney U-test

Table S4. Individual values of hemostasis parameters in male ICR mice, dabigatran etexilate (10 mg/kg/10ml oral)

Parameter		Dabigatran etexilate, 10 mg/kg, 15 min					
№ mice		71	72	73	74	75	Mean SD
APTT, sec		31.5	23.9	56.4	39.9	49.5	40.2 ** 13.1
PTT, sec		13.9	11.8	15	14.6	16.4	14.3 ** 1.7
Fibrinogen concentration, g/l		1.767	1.841	1.698	1.777	1.482	1.713 0.139
Parameter		Dabigatran etexilate, 10 mg/kg, 30 min					
№ mice		76	77	78	79	80	Mean SD
APTT, sec		49.6	36.5	39.9	43	58.5	45.5 ** 8.7
PTT, sec		14.2	13.1	11.9	13.3	15.1	13.5 ** 1.2
Fibrinogen concentration, g/l		1.652	1.248	1.777	1.583	1.433	1.539 ** 0.204
Parameter		Dabigatran etexilate, 10 mg/kg, 60 min					
№ mice		81	82	83	84	85	Mean SD
APTT, sec		41.3	31.9	42.3	43.8	55.9	43.0 ** 8.6
PTT, sec		12	12.3	12.1	12.7	14	12.6 ** 0.8
Fibrinogen concentration, g/l		1.505	1.374	1.863	1.583	1.468	1.559 0.186
Parameter		Dabigatran etexilate, 10 mg/kg, 90 min					
№ mice		86	87	88	89*	90	Mean SD
APTT, sec		35.1	38	31.9	-	35.5	35.1 * 2.5
PTT, sec		12	12.9	12	-	12.2	12.3 * 0.4
Fibrinogen concentration, g/l		1.727	1.355	1.543	-	1.591	1.554 0.154
Parameter		Dabigatran etexilate, 10 mg/kg, 120 min					
№ mice		91	92	93	94	95	Mean SD
APTT, sec		21.2	30.6	23	37	26.2	27.6 6.4
PTT, sec		20.1	10.7	10.3	10.8	22.6	14.9 6.0
Fibrinogen concentration, g/l		2.377	1.652	1.875	1.600	2.078	1.916 0.320
Parameter		Dabigatran etexilate, 10 mg/kg, 180 min					
№ mice		96	97	98	99	100	Mean SD
APTT, sec		31.2	24.2	28	26.2	31.2	28.2 3.1
PTT, sec		11.9	10.9	11.1	22.6	12.4	13.8 5.0
Fibrinogen concentration, g/l		2.022	1.634	1.583	2.078	1.426	1.749 0.286

& Data not received for technical reasons

* p≤0.05, ** p≤0.01 relative to the “saline” group according to the Mann-Whitney U-test

Table S5. Individual values of hemostasis parameters in male ICR mice, dabigatran etexilate (25 mg/kg/10ml oral)

Parameter		Dabigatran etexilate, 25 mg/kg, 15 min					
№ mice		101	102	103	104	105	Mean SD
APTT, sec		78.8	85.9	71.5	69.8	67.7	74.7 ** 7.5
PTT, sec		24	27.2	24	20.8	19.7	23.1 ** 3.0
Fibrinogen concentration, g/l		0.469	0.374	0.81	0.197	1.264	0.623 ** 0.422
Parameter		Dabigatran etexilate, 25 mg/kg, 30 min					
№ mice		106	107	108	109	110	Mean SD
APTT, sec		47.8	56.9	37.3	50	56.3	49.7 ** 8.0
PTT, sec		14.5	17.6	13.6	13.5	15.9	15.0 ** 1.7
Fibrinogen concentration, g/l		1.667	1.421	1.97	1.521	1.514	1.619 0.215
Parameter		Dabigatran etexilate, 25 mg/kg, 60 min					
№ mice		111	112	113	114	115	Mean SD
APTT, sec		45.9	47.7	47.5	55.2	75.8	54.4 ** 12.5
PTT, sec		14.5	14.1	14.1	15.2	19.4	15.5 ** 2.2
Fibrinogen concentration, g/l		1.842	1.915	1.684	1.579	1.254	1.655 0.260
Parameter		Dabigatran etexilate, 25 mg/kg, 90 min					
№ mice		116	117	118	119	120	Mean SD
APTT, sec		30.9	46.1	41.1	43.8	65.4	45.5 ** 12.6
PTT, sec		13.7	13.1	13.1	13.2	16.9	14.0 ** 1.6
Fibrinogen concentration, g/l		1.659	1.684	1.793	1.618	1.507	1.652 0.104
Parameter		Dabigatran etexilate, 25 mg/kg, 120 min					
№ mice		121	122	123	124	125	Mean SD
APTT, sec		38.1	37.5	35.2	44.2	16.7	34.3 10.4
PTT, sec		11.9	11.9	12.1	13	20.5	13.9 ** 3.7
Fibrinogen concentration, g/l		1.243	1.505	1.626	1.512	2.299	1.637 0.396
Parameter		Dabigatran etexilate, 25 mg/kg, 180 min					
№ mice		126	127	128	129	130	Mean SD
APTT, sec		35.7	33.3	41.4	56.7	47.8	43.0 ** 9.5
PTT, sec		11.7	11.2	12.8	14.5	14	12.8 ** 1.4
Fibrinogen concentration, g/l		1.757	1.679	1.661	1.461	1.591	1.630 ** 0.111

** p<0.01 relative to the “saline” group according to the Mann-Whitney U-test

Table S6. Tail-cut bleeding time after substance administration in male SD rats (individual values)

Number of animals in the group	Saline	Heparin, 20 IU/kg, I.V.	Heparin, 100 IU/kg, I.V.	Dabigatran etexilate, 12.5 mg/kg P.O.	Dabigatran etexilate, 25 mg/kg P.O.	Variegin, 3.75 mg/kg, I.V.	Variegin, 7.5 mg/kg, I.V.	Variegin, 30 mg/kg, I.V.
1	252	1101	1800	1095	1800	1511	1800	1800
2	586	953	1800	959	1800	1338	1800	1800
3	376	1164	1800	691	1800	1217	1800	1800
4	343	1185	1800	950	1800	1113	1800	1800
5	268	1331	1800	1299	1800	1247	1800	1800
6	366	1203	1800	1230	1800	1409	1800	1800
Mean	365	1156 **	1800 **	1037 **	1800 **	1306 **	1800 **	1800 **
SD	120	125	0	220	0	143	0	0

** p≤0.01 relative to the “saline” group according to the Mann-Whitney U-test

Table S7. Individual values of APTT after substance administration in male SD rats in tail-cut bleeding time (individual values)

Number of animals in the group	Saline	Heparin, 20 IU/kg, I.V.	Heparin, 100 IU/kg, I.V.	Dabigatran etexilate, 12.5 mg/kg P.O.	Dabigatran etexilate, 25 mg/kg P.O.	Variegin, 3.75 mg/kg, I.V.	Variegin, 7.5 mg/kg, I.V.	Variegin, 30 mg/kg, I.V.	Intact animal
1	15.1	22.3	14.6	37.3	43.7	39	49	145.4	17.8
2	17	25.3	16	27.2	40.4	39.9	47.2	184.8	17.1
3	20.3	22.3	111.7	27.6	37.3	38.5	42.6	106.5	17.1
4	20.8	27.5	100.4	36.4	44.9	37.1	ND	146	ND
5	15.3	22.5	20.2	35.6	34.2	39	ND	214.9	ND
6	21.2	23.6	160.5	34.8	36.7	38.7	ND	211.4	ND
Mean	18.3	23.9 **	70.6	33.2 **	39.5 **	38.7 **	46.3 *	168.2 **	17.3
SD	2.8	2.1	62.2	4.5	4.2	0.9	3.3	42.8	0.4

* p≤0.05, ** p≤0.01 relative to the “saline” group according to the Mann-Whitney U-test

Table S8. Individual values of PTT after substance administration in male SD rats in tail-cut bleeding time (individual values)

Number of animals in the group	Saline	Heparin, 20 IU/kg, I.V.	Heparin, 100 IU/kg, I.V.	Dabigatran etexilate, 12.5 mg/kg P.O.	Dabigatran etexilate, 25 mg/kg P.O.	Variegin, 3.75 mg/kg, I.V.	Variegin, 7.5 mg/kg, I.V.	Variegin, 30 mg/kg, I.V.	Intact animal
1	19.5	21.3	18.9	27.2	38.7	29.4	31.5	101.2	22.4
2	20.2	21.4	19.9	25.0	34.5	31.0	35.7	161.9	23.3
3	19.9	20.5	24.1	24.8	30.4	30.9	30.3	79.1	21.0
4	20.4	23.4	24.3	28.0	34.7	29.2	ND	100.9	ND
5	19.3	22.0	20.0	28.3	27.1	28.8	ND	212.4	ND
6	20.1	23.3	29.6	28.8	34.8	29.4	ND	208.8	ND
Mean	19.9	22.0 **	22.8	27.0 **	33.4 **	29.8 **	32.5 *	144.1 **	22.2 *
SD	0.4	1.2	4.0	1.7	4.0	0.9	2.8	58.5	1.2

* p≤0.05, ** p≤0.01 relative to the “saline” group according to the Mann-Whitney U-test

Table S9. Individual values of fibrinogen concentration after substance administration in male SD rats in tail-cut bleeding time (individual values)

Number of animals in the group	Saline	Heparin, 20 IU/kg, I.V.	Heparin, 100 IU/kg, I.V.	Dabigatran etexilate, 12.5 mg/kg P.O.	Dabigatran etexilate, 25 mg/kg P.O.	Variegin, 3.75 mg/kg, I.V.	Variegin, 7.5 mg/kg, I.V.	Variegin, 30 mg/kg, I.V.	Intact animal
1	2.377	1.933	1.646	1.97	1.626	1.868	1.619	0.967	1.922
2	2.197	1.9	1.683	2.091	2.078	1.777	1.740	0.739	1.994
3	2.053	1.991	1.816	2.299	2.254	3.001	1.603	1.053	2.003
4	2.299	1.596	1.665	2.078	1.701	1.956	ND	0.912	ND
5	2.531	1.956	1.665	2.041	2.029	1.900	ND	0.486	ND
6	2.377	2.003	1.588	2.053	1.701	1.816	ND	0.5	ND
Mean	2.306	1.897 **	1.677 **	2.089 *	1.898 *	2.053 *	1.654 *	0.776 **	1.973 *
SD	0.165	0.152	0.076	0.111	0.256	0.469	0.075	0.242	0.044

* p≤0.05, ** p≤0.01 relative to the “saline” group according to the Mann-Whitney U-test

Table S10. Results of hematological analysis in male SD rats in tail-cut bleeding time

Parameters	Saline	Heparin, 20 IU/kg, I.V.	Dabigatran etexilate, 12.5 mg/kg P.O.	Dabigatran etexilate, 25 mg/kg P.O.	Variegin, 3.75 mg/kg, I.V.	Variegin, 7.5 mg/kg, I.V.	Intact animal
	n=6	n=6	n=6	n=6	n=3	n=3	n=3
Leucocyte, 10 ⁹ /l	10.6 ± 1.6	13.4 ± 3.2	9.4 ± 0.6	9.8 ± 1.8	9.7 ± 1.8	8.5 ± 1.3	10.8 ± 1.9
Lymphocyte, 10 ⁹ /l	9.2 ± 1.5	12.1 ± 2.7	8.3 ± 0.6	8.6 ± 1.6	8.3 ± 1.7	7.5 ± 1.1	9.7 ± 1.6
Monocyte, 10 ⁹ /l	0.5 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1 *	0.4 ± 0.1
Granulocyte, 10 ⁹ /l	0.9 ± 0.1	0.9 ± 0.5	0.8 ± 0.1	0.9 ± 0.2	1.0 ± 0.2	0.7 ± 0.1	0.8 ± 0.2
Lymphocyte, %	87.0 ± 1.5	90.4 ± 2.0	88.5 ± 0.4	87.5 ± 1.4	85.4 ± 2.9	88.3 ± 0.8	89.2 ± 0.7
Monocyte, %	4.5 ± 0.3	3.2 ± 0.5	3.4 ± 0.4	3.1 ± 0.4	4.0 ± 0.8	4.0 ± 0.4	3.3 ± 0.4
Granulocyte, %	8.5 ± 1.3	6.4 ± 1.5	8.1 ± 0.6	9.4 ± 1.6	10.6 ± 2.6	7.7 ± 0.5	7.5 ± 0.3
Red blood cell, 10 ¹² /l	8.69 ± 0.32	8.90 ± 0.26	9.18 ± 0.12	8.83 ± 0.19	8.65 ± 0.36	8.63 ± 0.66	9.21 ± 0.33
Hemoglobin, g/l	165 ± 4	159 ± 7	161 ± 5 *	159 ± 2 *	159 ± 9	157 ± 13 *	166 ± 6
Hematocrit, l/l	0.472 ± 0.017	0.483 ± 0.023 **	0.494 ± 0.016	0.479 ± 0.004	0.472 ± 0.027	0.463 ± 0.041 **	0.504 ± 0.016
Mean cell volume, fl	54.3 ± 0.5	54.2 ± 1.2 **	53.8 ± 1.8 *	54.3 ± 1.0 *	54.6 ± 1.1	53.6 ± 1.3 *	54.7 ± 0.4 *
Mean cell hemoglobin, pg	19.0 ± 0.4	17.8 ± 0.3 *	17.6 ± 0.4	18.0 ± 0.3	18.3 ± 0.4	18.2 ± 0.5	18.0 ± 0.3
Mean corpuscular hemoglobin concentration, g/l	350 ± 6	329 ± 6	327 ± 5 *	331 ± 2	336 ± 3	340 ± 6	329 ± 3
RBC distribution width, %	11.3 ± 0.2	12.9 ± 0.5	12.8 ± 0.5	12.5 ± 0.0	11.9 ± 0.3	11.8 ± 0.4	12.6 ± 0.5
RDW standart deviation, fl	28.0 ± 1.0	31.6 ± 2.1	32.3 ± 0.0	30.8 ± 1.4	29.6 ± 1.0	29.2 ± 1.3	31.6 ± 1.4 *
Platelets, 10 ⁹ /l	668 ± 25	736 ± 72	703 ± 42	735 ± 33	706 ± 29	671 ± 31	667 ± 20
Mean platelet volume, fl	5.3 ± 0.1	5.5 ± 0.2 **	5.5 ± 0.1 *	5.4 ± 0.1 *	5.4 ± 0.2 *	5.5 ± 0.2 *	5.5 ± 0.1 *
Plateletcrit, cl/l	0.354 ± 0.016	0.406 ± 0.041 **	0.389 ± 0.027 *	0.399 ± 0.016 *	0.379 ± 0.022 **	0.368 ± 0.028 *	0.369 ± 0.013 *
Platelet distribution width, %	14.7 ± 1.2	15.3 ± 1.1 **	15.9 ± 1.3 *	15.2 ± 0.6 *	14.1 ± 0.7 **	15.4 ± 0.9	16.0 ± 0.3 *

Data are presented as MEAN ± SD, * p ≤ 0.05, ** p ≤ 0.01 relative to saline group in accordance with Mann-Whitney U-test

Table S11. Inferior vena cava (IVC) stenosis model of venous thrombosis associated with hypercoagulation. Thrombus weights after substance administration in male SD rats (individual values)

Number of animals in the group	Saline		Heparin, 2 IU/kg, I.V.		Dabigatran etexilate, 5 mg/kg P.O.		Variegin, 0.175 mg/kg, I.V.	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
1	84.4	43.2	1.0	0.0	15.0	4.5	6.2	1.7
2	35.5	12.5	6.5	2.6	0.0	0.0	5.0	1.9
3	57.0	0.0	7.8	3.7	3.0	0.5	14.7	6.1
4	15.7	25.6	21.3	7.9	0.0	0.0	6.5	1.9
5	0.0	4.1	31.4	11.1	3.0	1.4	14.0	5.0
Mean	48.2	21.4	13.6 *	5.1 *	4.2 **	1.3 *	9.3 **	3.3 *
SD	29.5	17.0	12.4	4.4	6.2	1.9	4.7	2.1
SEM	14.7	8.5	5.6	2.0	2.8	0.8	2.1	0.9

* $p \leq 0.05$, ** $p \leq 0.01$ relative to the "saline" group according to the Mann-Whitney U-test