LSIM Safety Institute

S19-2. Application of the microsampling method to rodent safety studies

○Kazuaki Takahashi[*1], Shun Kawaguchi[*1], Takeshi Funakoshi[*1], Keiko Nakai[*1], Dai Yamamoto[*1], Takayuki Okamura[*1], Yoshiro Saito[*2], Munehiro Nakagawa[*1]

*1: LSIM Safety Institute, *2: National Institute of Health Sciences

Contents

Contents

What is microsampling

Method to collect a very small amount of blood to assess drug and metabolite concentrations for determination of TK parameters

Sampling volume： **50 µL or less**

Target matrix： **Blood, plasma, serum**

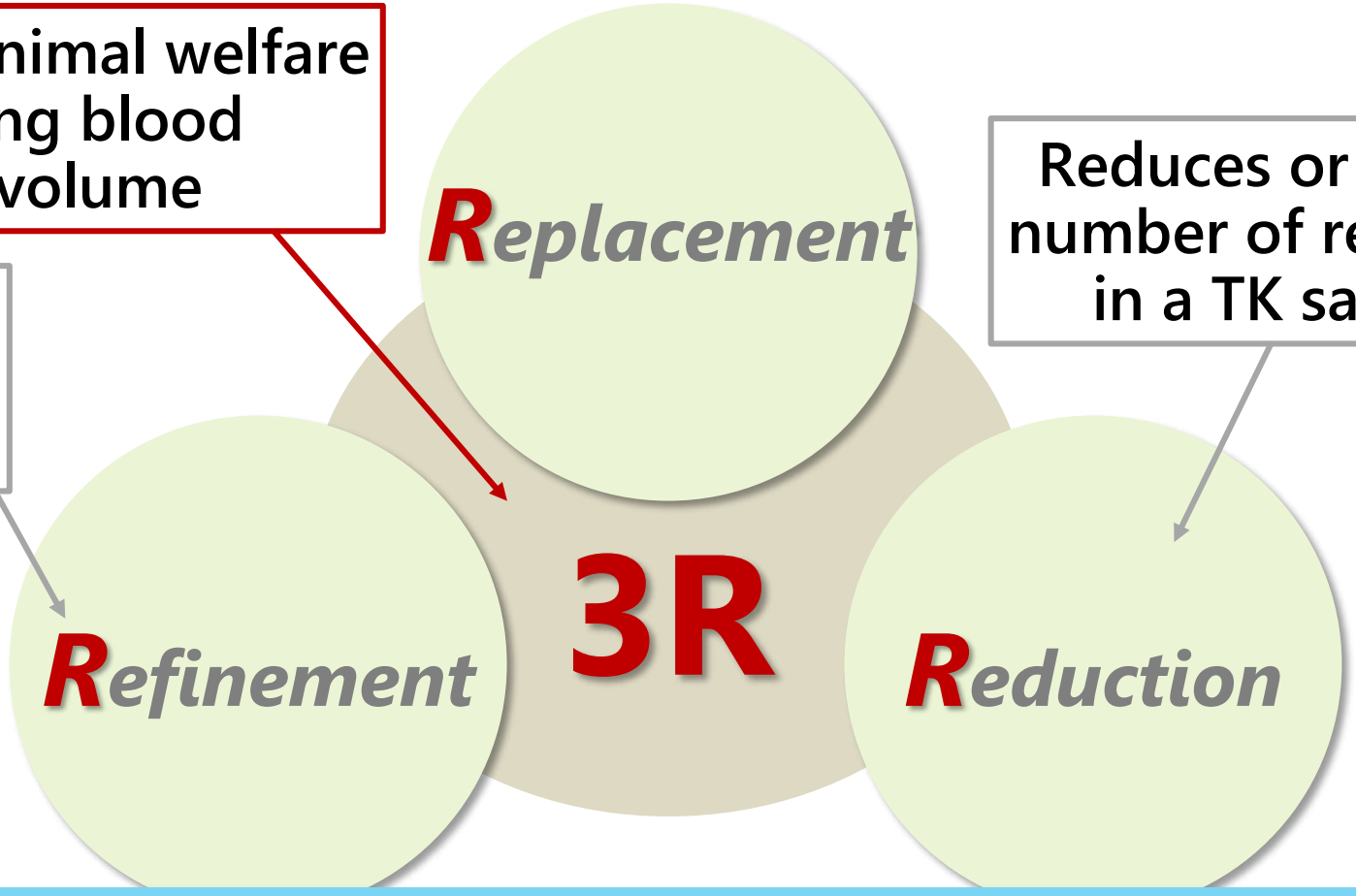
Target animal： **Rats or mice for toxicity evaluation**

Details are specified in Q & A on the use of microsampling technique in ICH S3A “Guidance on Toxicokinetics (assessment of systemic exposure in toxicity studies)” issued in 2019.

Advantages

Contributes to animal welfare by minimizing blood collection volume

Minimizes pain and distress in animals



Reduces or eliminates the number of required animals in a TK satellite group

Reduction of the amount of test substance

Advantages

Preliminary 2-week repeated dose study in rats

Dose level	Conventional method (Total 72 rats)				Microsampling (Total 40 rats)			
	Main group		TK group		Main group		TK group	
	Male	Female	Male	Female	Male	Female	Male	Female
0 mg/kg	5	5	4	4	5	5	0	0
100 mg/kg	5	5	4	4	5	5	0	0
300 mg/kg	5	5	4	4	5	5	0	0
1000 mg/kg	5	5	4	4	5	5	0	0

Amount of test substance* 73g→41g

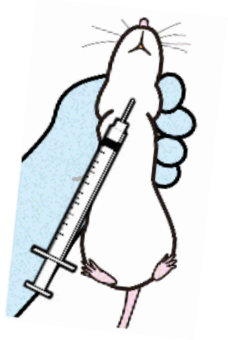
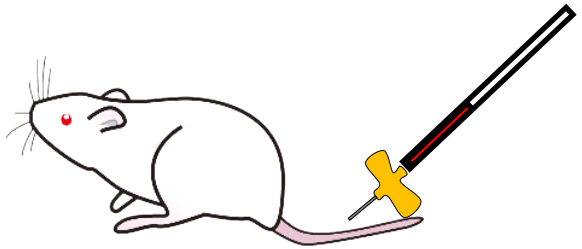
*: Theoretical value not including losses

Reduction by 44%

Contents

Preferred site for microsampling

Site for repeated sampling without anesthesia

	Advantages	Disadvantages
Subclavian vein 	Easy to collect the appropriate amount of blood	• Requires advanced blood sampling technique • Difficulty to confirm hemostasis (not visible) • Possibly have an effect on tissues due to exposure via the pectoral muscle
Tail vein 	• Easy to collect blood • Easy to arrest hemorrhage • Limited effect on tissues (minimal damage to the tail)	• Difficult to secure the appropriate amount of blood (weak blood flow) • Inappropriate for administration to the lateral vein (test substance contamination risk)

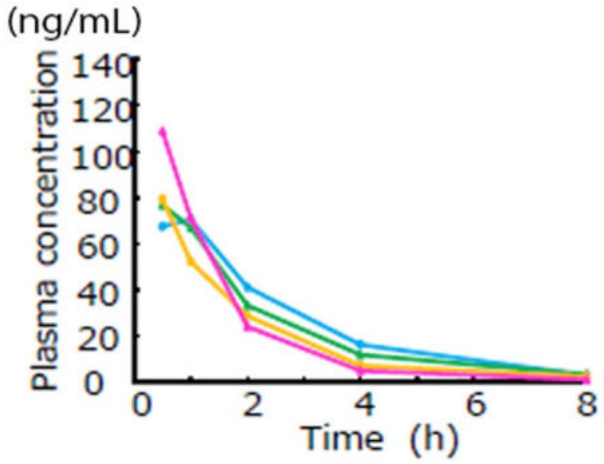
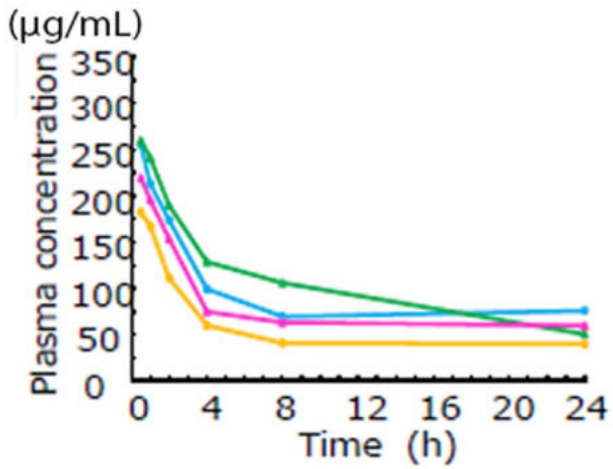
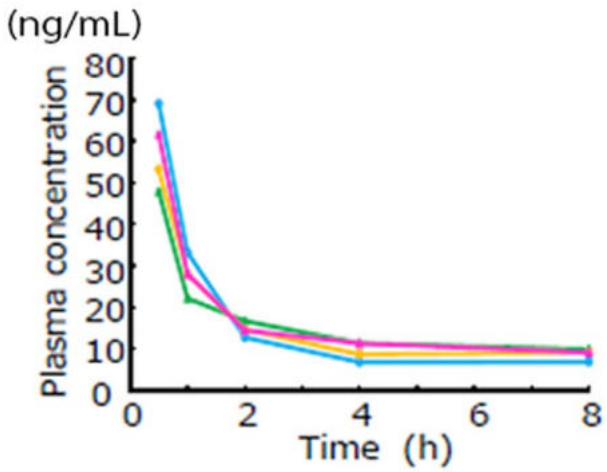
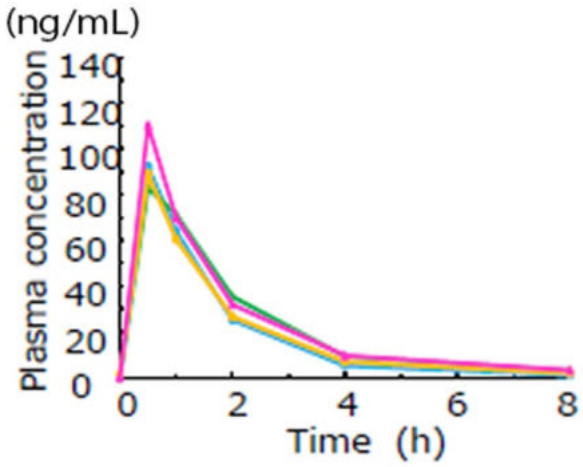
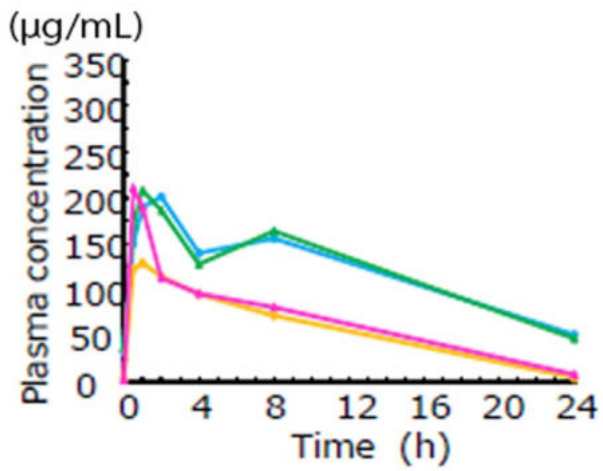
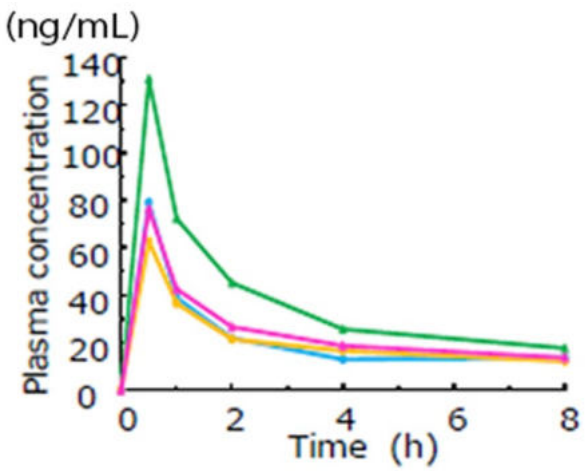
Effect on TK results by blood sampling site

Method: 7-week-old Crl:CD(SD) rats, 3 or 5/animals/sex/group

Test substance	Methotrexate **MTX**	Acetaminophen **APAP**	Doxorubicin **DOX**
Route	**Oral dose**		**Intraperitoneal dose**
Dose level	0.2 mg/kg/day	1000 mg/kg/day	2.5 mg/kg/week
Frequency	Once/day, 7 times/week, 4 weeks (total 28 times)		Once/day, once/week, 4 weeks (total 5 times)

Sampling site	**Tail vein**	**Subclavian vein**
Volume	Approx. 50 µL/time point (amount of plasma: approx. 20 µL/time point)	
Time point	**First**: 0.5, 1, 2, 4, 8, and 24 hr (6 time points) **Final**: Pre, 0.5, 1, 2, 4, 8, and 24 hr (7 time points, 28th or 5th)	
Device	Glass capillary + 25G winged needle	27G FN Syringe
Anticoagulant	Applied to glass capillary in advance (air dried)	Treated with flushing with liquid

Effect on TK results by blood sampling site

	MTX	APAP	DOX
First			
Final			

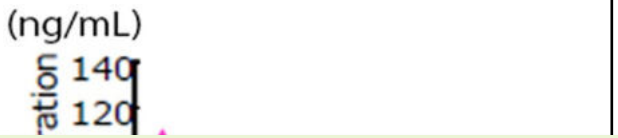
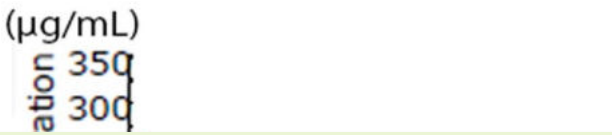
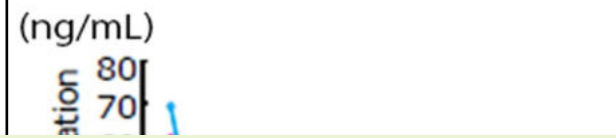
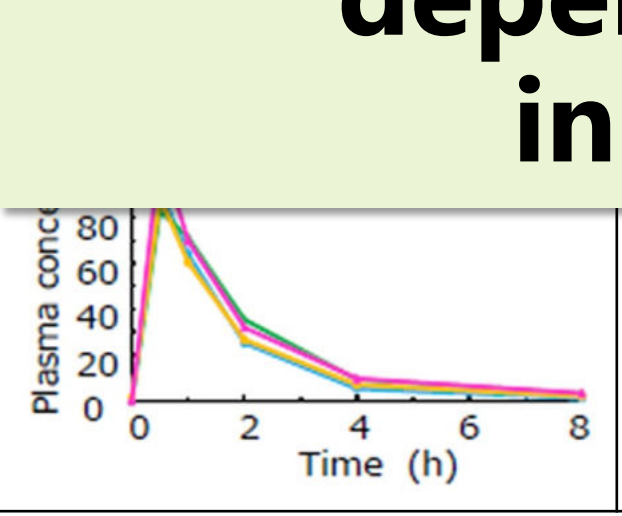
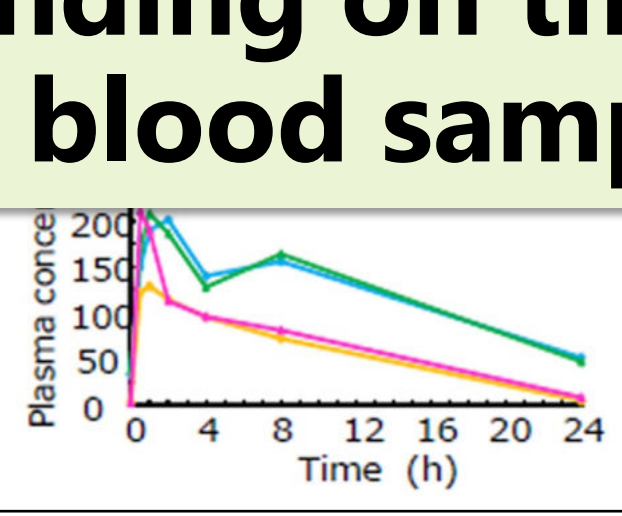
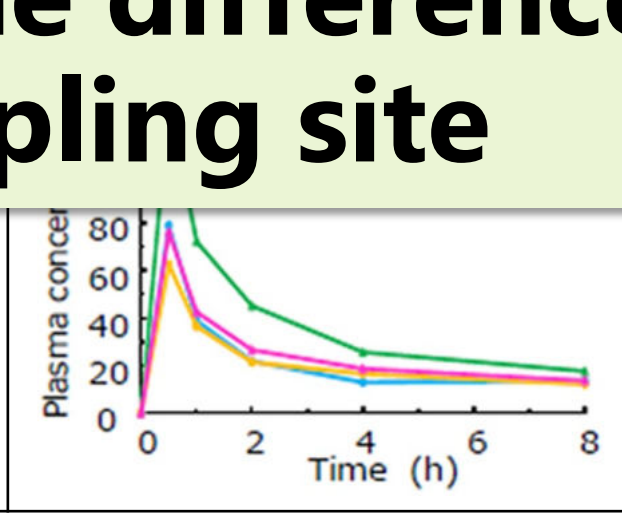
: Tail vein, male

: Subclavian vein, male

: Tail vein, female

: Subclavian vein, female

Effect on TK results by blood sampling site

	MTX	APAP	DOX
First			
Final			

No clear difference was noted in plasma drug concentration depending on the difference in blood sampling site

Comparison of blood sampling devices

Effect on TK results by differences in devices

After a single oral dose of acetazolamide (60 or 200 mg/kg) to male rats (6-week-old, n=3 each group), blood was collected by microsampling using various devices.

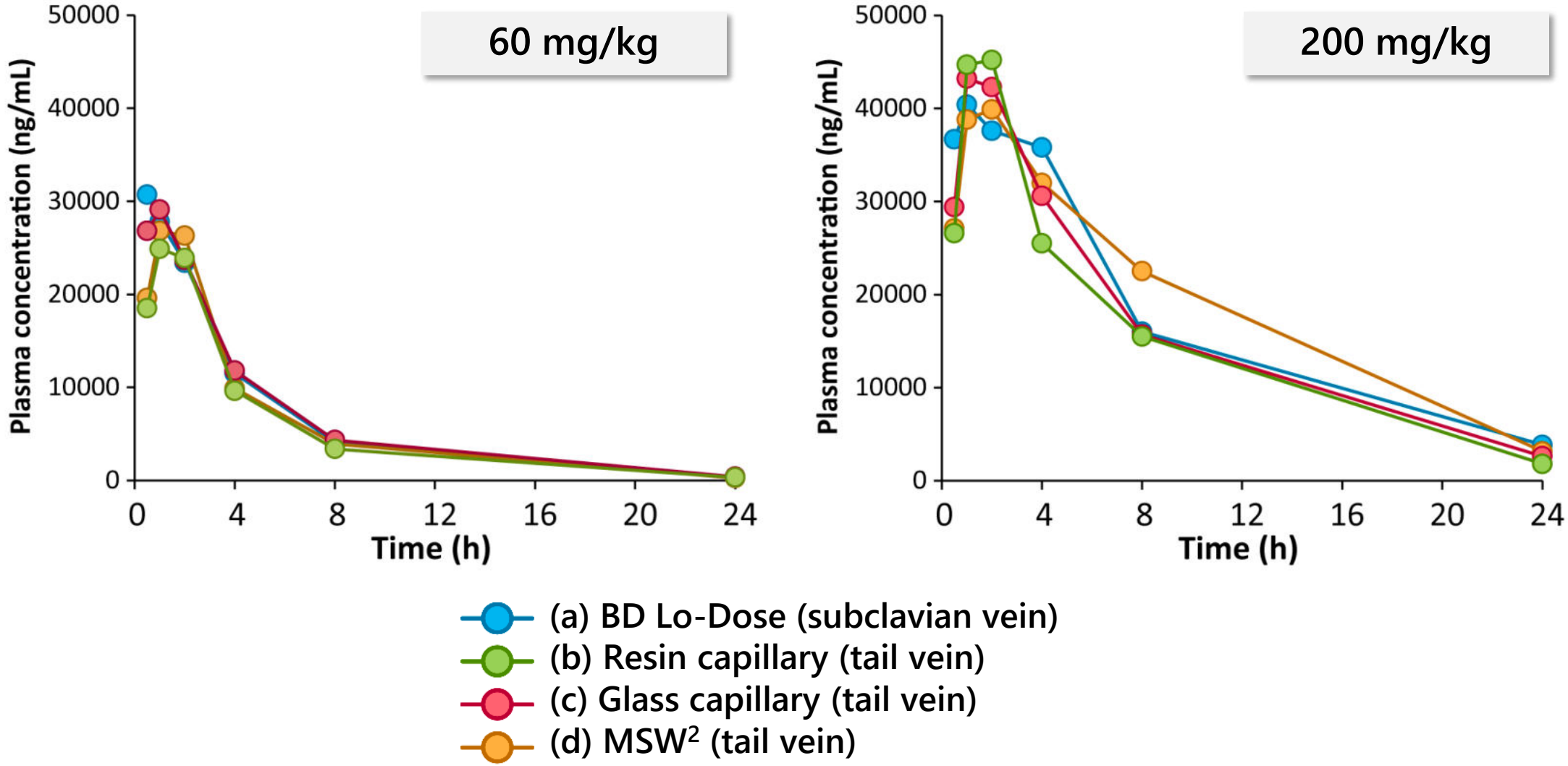
The plasma drug concentration was determined by the LC-MS/MS method and compared.

Acetazolamide:
Due to its high transfer rate to blood cells, it may be affected by repeated blood sampling with microsampling devices.

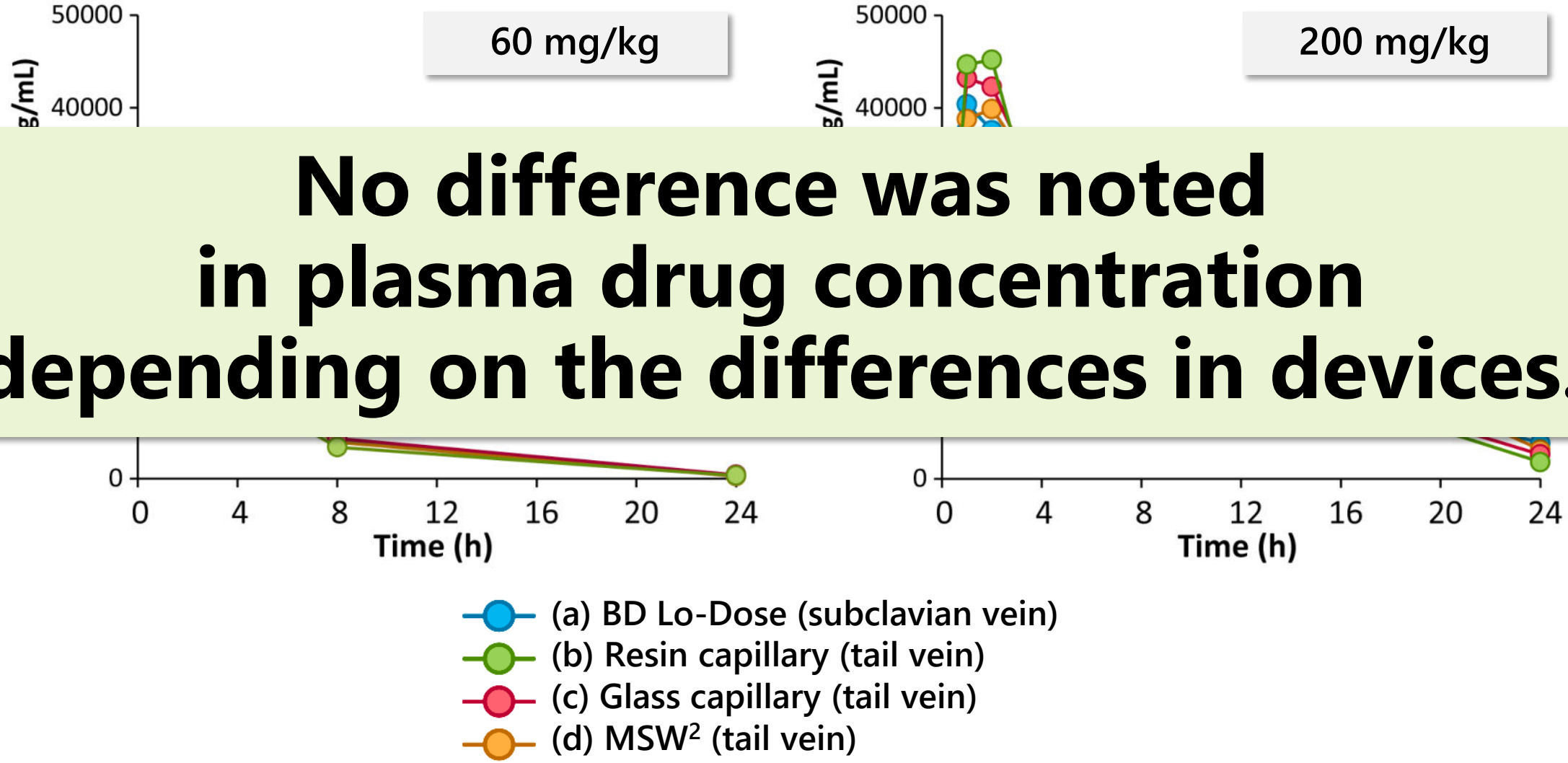
Comparison of blood sampling devices

Blood sampling device	(a) BD Lo-Dose™ Insulin Syringe with 29G Needle	(b) Resin capillary	(c) Glass capillary	(d) MSW2™ Type Udck™
				 Microsampling Wing™
Site	Subclavian vein	Tail vein＋25G winged needle for test animals		
Volume	Approx. 50 µL/time point			Approx. 23 µL/time point
Time point	0.5, 1, 2, 4, 8, and 24 hr (6 time points)			

Plasma drug concentrations per device



Plasma drug concentrations per device

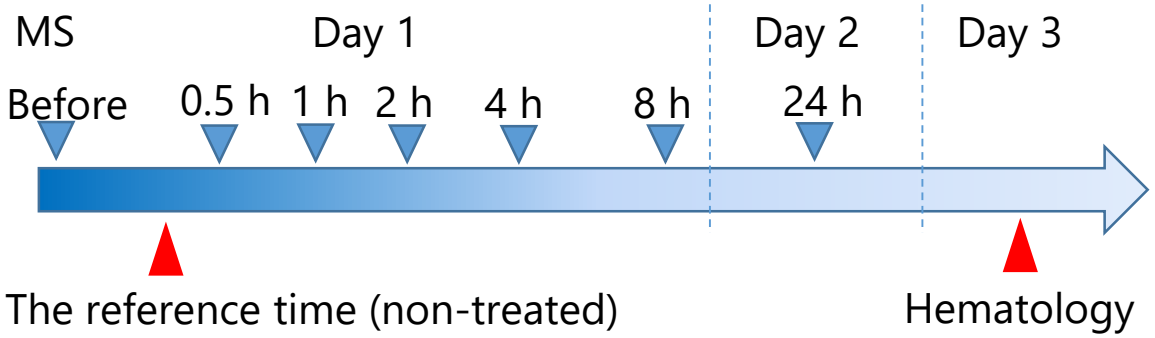


No difference was noted in plasma drug concentration depending on the differences in devices.

Contents

Confirmation of reversibility in blood collection over time

In mice



Animals: Crl:CD1(ICR) mice (Charles River Laboratories Japan, Inc.), 7 weeks old

Tail vein: Disposable winged injection needle (25 G, CLEA Japan, Inc.)
Hematocrit tube (Paul Marienfeld GmbH & Co. KG)

Subclavian vein: BD Lo-Dose™ Insulin Syringes (25 G, Becton, Dickinson and Company)

MS volume: About 50 µL/sampling time point/animal
Circulating blood volume of mice: 72 mL/kg*

Blood sampling volume vs. circulating blood volume (%)
= (Blood sampling volume)×100/72 mL/kg×(Group mean body weight)

Group	1	2	3	4	5	6	7
Number of animals (Male: Female)	3:3	3:3	3:3	3:3	3:3	3:3	3:3
Treatment	-	-	-	-	-	-	-
MS (points)	NON-MS	3 points (Before, 2, 24 h)	2 points (0.5, 4 h)	2 points (1, 8 h)	3 points (Before, 2, 24 h)	2 points (0.5, 4 h)	2 points (1, 8 h)
Blood collection site	-	Tv	Tv	Tv	Sv	Sv	Sv

Tv: Tail vein, Sv: Subclavian vein

*: EFPIA, ECVAM. Guide for administration of test article (route of administration, volume) and blood collection in experimental animals, 2000.

Confirmation of reversibility in blood collection over time

In mice

Group	1		2		3		4		5		6		7	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Number of animals	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Sampling site	Non-MS		Tv		Tv		Tv		Sv		Sv		Sv	
Number of blood sampling	0	0	3	3	2	2	2	2	3	3	2	2	2	2
Blood sampling volume vs. circulating blood volume (%)	-	-	6.4	8.7	4.1	5.8	4.1	5.7	6.3	8.7	4.2	5.8	4.2	5.5
Red Blood Cell Count (10^6/μL)	8.210	8.433	7.670	7.417*	7.673	7.947	7.997	7.610*	7.880	7.890	7.960	7.453*	8.337	7.997
Rate of change vs. Non-MS (%)	-	-	-6.6	-12.0	-6.5	-5.8	-2.6	-9.8	-4.0	-6.4	-3.0	-11.6	1.5	-5.2
Hemoglobin Conc. (g/dL)	13.63	14.43	12.97	12.50*	13.27	13.53*	12.97	12.93*	13.10	13.27	13.37	12.90*	13.37	13.67
Rate of change vs. Non-MS (%)	-	-	-4.8	-13.4	-2.6	-6.2	-4.8	-10.4	-3.9	-8.0	-1.9	-10.6	-1.9	-5.3
Hematocrit (%)	42.23	42.90	40.50	38.77*	40.40	41.40	40.50	39.03*	39.57	40.03	40.93	38.67*	41.67	41.47
Rate of change vs. Non-MS (%)	-	-	-4.1	-9.6	-4.3	-3.5	-4.1	-9.0	-6.3	-6.7	-3.1	-9.9	-1.3	-3.3

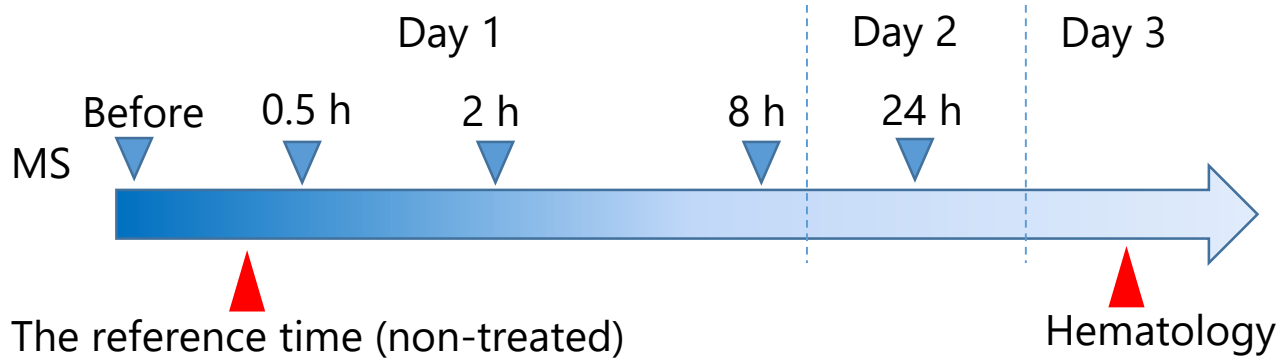
M: Male, F: Female, *p<0.05 vs. Group 1, Tv: Tail vein, Sv: Subclavian vein

Confirmation of reversibility in blood collection over time

In mice

Group	1	2	3	4	5	6	7	8	9	10	11
Number of animals (M:F)	3:3	3:3	3:3	3:3	3:3	3:3	3:3	3:3	3:3	3:3	3:3
Treatment	-	-	-	-	-	-	-	-	-	-	-
MS	NON-MS	Before	0.5 h	2 h	8 h	24 h	Before	0.5 h	2 h	8 h	24 h
Blood collection site	-	Tv	Tv	Tv	Tv	Tv	Sv	Sv	Sv	Sv	Sv

M: Mail, F: Female, Tv: Tail vein, Sv: Subclavian vein



Confirmation of reversibility in blood collection over time

In mice

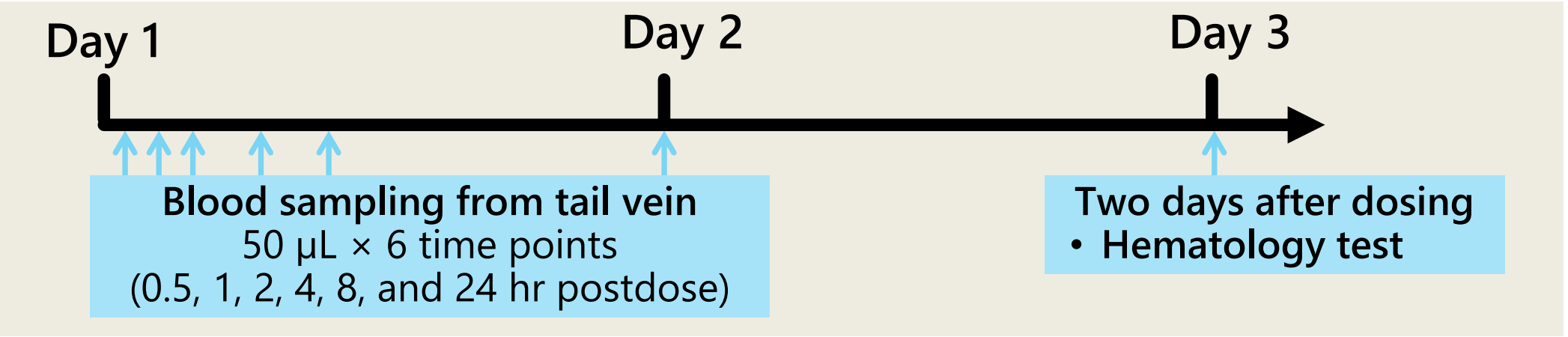
M: Male, F: Female, Tv: Tail vein, Sv: Subclavian vein

Group	1		2		3		4		5		6		7		8		9		10		11	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Number of animals	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Sampling site	Non-MS		Tv		Tv		Tv		Tv		Tv		Sv		Sv		Sv		Sv		Sv	
Number of blood collection	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Blood sampling volume vs. circulating blood volume (%)	-	-	2.1	2.6	2.1	2.7	2.1	2.7	2.1	2.7	2.1	2.6	2.1	2.8	2.1	2.7	2.2	2.7	2.1	2.8	2.1	2.7
Red Blood Cell Count (10^6/μL)	8.427	8.570	7.777	8.667	8.320	8.933	8.203	9.030	8.557	8.630	8.057	8.763	8.680	8.037	9.050	8.070	8.020	8.673	8.430	8.263	8.610	8.317
Rate of change vs. Non-MS (%)	-	-	-7.7	1.1	-1.3	4.2	-2.7	5.4	1.5	0.7	-4.4	2.3	3.0	-6.2	7.4	-5.8	-4.8	1.2	0.0	-3.6	2.2	-3.0
Hemoglobin Conc. (g/dL)	13.80	14.07	12.87	14.33	13.60	14.50	13.67	14.70	14.07	14.33	13.23	14.20	14.13	13.67	14.87	13.40	13.40	14.37	13.70	13.93	14.07	13.77
Rate of change vs. Non-MS (%)	-	-	-6.7	1.8	-1.4	3.1	-0.9	4.5	2	1.8	-4.1	0.9	2.4	-2.8	7.8	-4.8	-2.9	2.1	-0.7	-1	2	-2.1
Hematocrit (%)	43.13	42.27	40.47	43.27	42.30	43.63	43.77	43.67	44.20	42.77	41.70	43.50	43.67	41.80	45.63	41.70	42.50	43.30	42.53	41.87	43.77	42.70
Rate of change vs. Non-MS (%)	-	-	-6.2	2.4	-1.9	3.2	1.5	3.3	2.5	1.2	-3.3	2.9	1.3	-1.1	5.8	-1.3	-1.5	2.4	-1.4	-0.9	1.5	1

Confirmation of reversibility in blood collection over time

In rats

Method: Single oral dose of water for injection, 6-week-old Crl:CD(SD) rats,
2 animals/sex, **Male: 2.1%, Female: 2.9% (vs. circulating blood volume (%))**



Results:

	♂			♀		
	RBC (10^6/µL)	HGB (g/dL)	HCT (%)	RBC (10^6/µL)	HGB (g/dL)	HCT (%)
1. Test result	6.43	13.9	42.0	6.41	13.7	40.4
2. Background value (Mean±2S.D.)	6.68 ±0.30	14.21 ±0.68	43.37 ±2.07	6.76 ±0.32	14.25 ±0.54	42.48 ±1.76

（Erythrocytic parameters are shown.）

Confirmation of reversibility in blood collection over time

In rats

Method: Single oral dose of water for injection, 6-week-old Crl:CD(SD) rats,
2 animals/sex, **Male: 2.1%, Female: 2.9% (vs. circulating blood volume (%))**

Day 1 Day 2 Day 3

The effect of MS on hematological results was slight, if total amount of blood collection was less than 3% of circulating blood volume.

Re

	(10⁶/μL)	(g/dL)	(%)	(10⁶/μL)	(g/dL)	(%)
1. Test result	6.43	13.9	42.0	6.41	13.7	40.4
2. Background value (Mean±2S.D.)	6.68 ±0.30	14.21 ±0.68	43.37 ±2.07	6.76 ±0.32	14.25 ±0.54	42.48 ±1.76

（Erythrocytic parameters are shown.）

Contents

Influence on the toxicological evaluation of a test compound

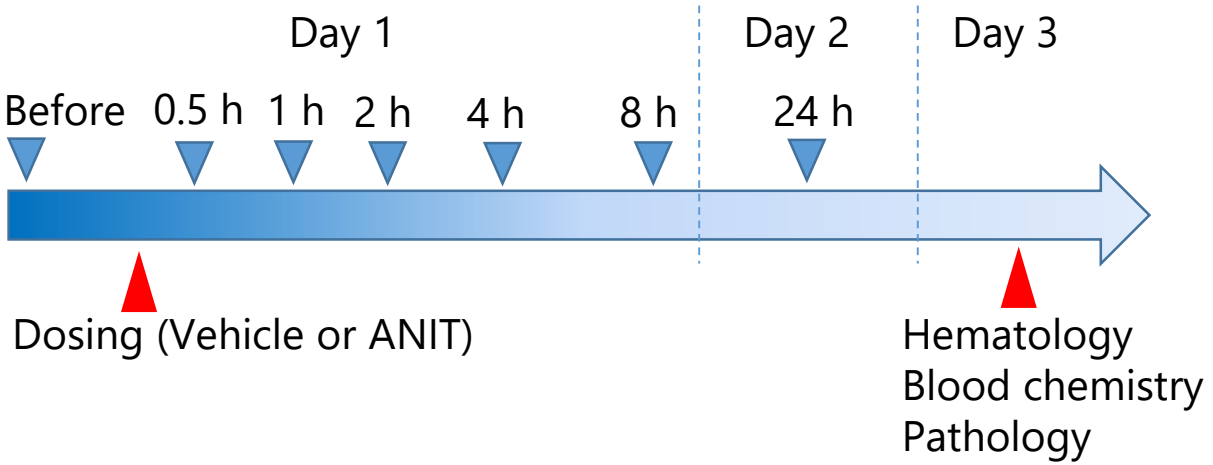
Animals: Crl:CD1(ICR) mice (Charles River Laboratories Japan, Inc.), 7 weeks old

Test article: 1-naphthyl isothiocyanate (Abbr.: ANIT, Tokyo Chemical Industry Co., Ltd.)

Tail vein: Disposable winged injection needle (25 G, CLEA Japan, Inc.) Hematocrit tube (Paul Marienfeld GmbH & Co. KG)

Subclavian vein: BD Lo-Dose™ Insulin Syringes (25 G, Becton, Dickinson and Company)

MS volume: About 50 µL/sampling time point/animal



Group	1	2	3	4	5	6
Number of animals (Male: Female)	3:3	3:3	3:3	3:3	3:3	3:3
Treatment	Vehicle	Vehicle	ANIT	ANIT	ANIT	ANIT
Dose level (mg/kg)	0	0	75	75	75	75
MS (points)	NON-MS	3 points (Before, 2, 24 h)	NON-MS	3 points (Before, 2, 24 h)	2 points (0.5, 4 h)	2 points (1, 8 h)
Blood collection site	-	Tv	-	Tv	Tv	Tv

Tv: Tail vein, Sv: Subclavian vein

Influence on the toxicological evaluation of a test compound

Group	1		2		3		4		5		6	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Number of animals	3	3	3	3	3	3	3	3	3	3	3	3
Treatment	Vehicle		Vehicle		ANIT		ANIT		ANIT		ANIT	
Sampling site	Non-MS		Tv		Non-MS		Tv		Tv		Tv	
Number of blood collection	0	0	3	3	0	0	3	3	2	2	2	2
ASAT (U/L)	32.7	44.7	37.3	38.0	88.0	538.0	358.3	559.0	140.0	437.7	332.0	759.3
ALAT (U/L)	22.0	23.0	20.3	16.3	41.0	295.3	307.7	357.7	86.3	148.0	174.0	374.7
GLDH (U/L)	20.7	8.7	15.0	10.0	48.0	501.3	413.3	753.3	151.3	247.7	239.7	590.3
γGT (U/L)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.3	0.3	0.7
ALP (U/L)	310.0	370.0	300.7	384.3	230.7	1019.3	257.0	560.3	238.3	538.0	340.7	860.7
Total Bilirubin (mg/dL)	0.10	0.07	0.10	0.10	0.10	1.87	0.13	1.87	0.10	0.90	0.10	0.43
Total Bile Acid (μmol/L)	1.20	3.70	1.17	2.93	4.70	433.77	35.30	401.37	18.53	170.27	32.13	193.27
Urea Nitrogen (mg/dL)	14.10	11.07	13.83	10.10	12.50	8.53	11.10	8.30	11.63	9.50	16.37	12.40
Creatinine (mg/dL)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Glucose (mg/dL)	262.0	225.3	228.3	235.3	163.7$	169.3$	164.7	176.0	219.0	179.7	132.7#	178.0
Total Cholesterol (mg/dL)	129.7	101.7	165.7$	108.0	145.7	311.7	176.7	196.3	176.3	169.3	224.7	180.7
Triglyceride (mg/dL)	66.0	63.0	78.0	68.3	37.0	33.0	64.7	34.3#	31.0	45.3	20.3	25.3#

M: Male, F: Female, Tv: Tail vein, Sv: Subclavian vein, $ $p<0.05$ vs. Group 1, # $p<0.05$ vs. Group 2,

Group 3 vs Group 4, 5, or 6: There were no statistically significant changes.

Blue text: Changes considered to be due to ANIT administration. Statistically significant differences and deviations of individual values from background data of the test facility were taken into account.

Influence on the toxicological evaluation of a test compound

Group	1		2		3		4		5		6	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Number of animals	3	3	3	3	3	3	3	3	3	3	3	3
Treatment	Vehicle		Vehicle		ANIT		ANIT		ANIT		ANIT	
Sampling site	Non-MS		Tv		Non-MS		Tv		Tv		Tv	
Number of blood collection	0	0	3	3	0	0	3	3	2	2	2	2
Total Protein (g/dL)	5.67	6.03	5.87	6.03	5.83	6.03	6.03	5.67	5.67	6.10	6.20	6.07
A/G Ratio	1.430	1.847	1.520	1.857	0.887$	1.007$	1.090#	1.233#	1.100#	1.233#	0.840#	1.240#
Albumin (g/dL)	3.33	3.92	3.54	3.92	2.69$	3.01$	3.13	3.12	2.96	3.36	2.83	3.32
α1 Globulin (g/dL)	0.42	0.29	0.40	0.27	0.39	0.29	0.46	0.27	0.37	0.30	0.42	0.30
α2 Globulin (g/dL)	0.59	0.49	0.62	0.49	0.90	0.88$	0.80#	0.67	0.77#	0.71	0.95#	0.70
β Globulin (g/dL)	0.68	0.69	0.63$	0.67	0.82	0.80	0.75#	0.71	0.72	0.75	0.83#	0.77
γ Globulin (g/dL)	0.66	0.65	0.67	0.69	1.04$	1.06$	0.89	0.90	0.85	0.98	1.18#	0.97
Ca (mg/dL)	9.73	9.40	9.63	9.80	9.87	9.90	9.63	9.57	9.57	9.93	9.67	10.23
Inorganic Phosphorus (mg/dL)	7.47	6.43	6.93$	7.20	7.17	7.50	7.47	6.93	6.80	7.17	6.90	7.93
Na (mmol/L)	146.7	143.0	148.3	146.7$	149.7$	145.7	149.7	146.7	148.0	149.0	149.0	147.3
K (mmol/L)	5.10	4.53	4.13$	4.53	4.43	4.50	4.50	4.27	4.20	4.17	4.90#	4.63
Cl (mmol/L)	109.0	109.7	109.0	111.7	112.7	109.3	110.0	111.7	111.7	112.7	111.7	109.3

M: Male, F: Female, Tv: Tail vein, Sv: Subclavian vein, $ $p<0.05$ vs. Group 1, # $p<0.05$ vs. Group 2,
Group 3 vs Group 4, 5, or 6: There were no statistically significant changes.
Blue text: Changes considered to be due to ANIT administration.

Influence on the toxicological evaluation of a test compound

	Group	1		2		3		4		5		6	
	Sex	M	F	M	F	M	F	M	F	M	F	M	F
	Number of animals	3	3	3	3	3	3	3	3	3	3	3	3
	Treatment	Vehicle		Vehicle		ANIT		ANIT		ANIT		ANIT	
	Sampling site	Non-MS		Tv		Non-MS		Tv		Tv		Tv	
	Number of blood collection	0	0	3	3	0	0	3	3	2	2	2	2
Blood sampling volume vs. circulating blood volume (%)		-	-	6.1	8.1	-	-	6.4	8.0	4.0	5.4	4.0	5.4
Clinical signs*	Bite wound, Anogenital region	1	0	0	0	0	0	0	0	0	0	0	0
Body weights		-	-	-	-	-	-	-	-	-	-	-	-
Food consumption		-	-	-	-	-	-	-	-	-	-	-	-
Hematology	Red Blood Cell Count (10^6/μL)	8.560	8.550	7.673$	7.503$	8.713	8.250	7.993	7.183	7.663#	7.273	7.647#	8.027
	Hemoglobin Conc. (g/dL)	14.50	14.57	12.87$	12.97$	14.50	14.10	13.60	12.17	12.63	12.57	12.90	13.50
	Hematocrit (%)	44.83	43.53	39.87$	39.33$	43.30	41.40	40.53	35.60#	38.60#	37.40	39.43#	39.50
	Reticulocyte (%)	4.650	3.147	4.937	5.657	4.413	3.333	4.963	6.103#	4.107	4.603	4.403	3.970

M: Male, F: Female, Tv: Tail vein, Sv: Subclavian vein, $: vs Group 1, #: vs Group 3, *: Number of animals affected, -: No noteworthy findings
Red text: Changes considered to be caused by blood collection by MS

Influence on the toxicological evaluation of a test compound

	Group	1		2		3		4		5		6	
	Sex	M	F	M	F	M	F	M	F	M	F	M	F
	Number of animals	3	3	3	3	3	3	3	3	3	3	3	3
	Treatment	Vehicle		Vehicle		ANIT		ANIT		ANIT		ANIT	
	Sampling site	Non-MS		Tv		Non-MS		Tv		Tv		Tv	
	Number of blood collection	0	0	3	3	0	0	3	3	2	2	2	2
Blood sampling volume vs. circulating blood volume (%)		-	-	6.1	8.1	-	-	6.4	8.0	4.0	5.4	4.0	5.4
Organ weight	Thymus (mg)	57.83	69.17	42.57	60.93	26.60	54.10	29.97	45.97	32.23	51.43	35.50	59.83
	Thymus ($x10^{-3}$%)	171.46	280.89	128.30	242.80	85.67	220.12	94.53	185.42	101.89	205.98	121.88	243.47
Necropsy		-	-	-	-	-	-	-	-	-	-	-	-
Histopathology*													
Spleen	Increase, Extramedullary hematopoiesis	0	0	3	3	0	2	2	3	2	3	1	3
Thymus	Atrophy	0	0	0	0	2	0	0	0	0	0	1	0
Liver	Necrosis, Hepatocyte, Focal	0	0	0	0	1	2	3	3	3	1	2	2
	Necrosis, Bile ductal epithelium	0	0	0	0	0	2	1	2	0	1	1	2
	Regeneration, Bile ductal epithelium	0	0	0	0	1	2	2	2	2	2	1	3
	Infiltrate, inflammatory cell, Glisson sheath	0	0	0	0	0	3	3	3	2	2	1	3
Gallbladder	Edema, Mucosa	0	0	0	0	1	0	1	0	1	0	0	0
	Necrosis, Mucosal epithelium	0	0	0	0	1	1	3	1	2	0	1	2
	Regeneration, Mucosal epithelium	0	0	0	0	2	2	2	3	3	1	3	3
	Infiltrate, inflammatory cell, Mucosa	0	0	0	0	1	0	2	1	1	0	1	1

M: Male, F: Female, Tv: Tail vein, Sv: Subclavian vein, *: Number of animals affected, -: No noteworthy findings

Blue text: Changes considered to be caused by ANIT administration, Red text: Changes considered to be caused by blood collection by MS

Influence on the toxicological evaluation of a test compound

	Group	1		2		3		4		5		6	
	Sex	M	F	M	F	M	F	M	F	M	F	M	F
	Number of animals	3	3	3	3	3	3	3	3	3	3	3	3
	Treatment	Vehicle		Vehicle		ANIT		ANIT		ANIT		ANIT	
	Sampling site	Non-MS		Tv		Non-MS		Tv		Tv		Tv	
	Number of blood collection	0	0	3	3	0	0	3	3	2	2	2	2
Blood sampling volume vs. circulating blood volume (%)		-	-	6.1	8.1	-	-	6.4	8.0	4.0	5.4	4.0	5.4
Organ weight	Thymus (mg)	57.83	69.17	42.57	60.93	26.60	54.10	29.97	45.97	32.23	51.43	35.50	59.83
	Thymus ($x10^{-3}$%)	171.46	280.89	128.30	242.80	85.67	220.12	94.53	185.42	101.89	205.98	121.88	243.47
Necro													-
Histop													
Splee													3
Thym													0
Liver													2
													2
	Regeneration, Bile ductal epithelium	0	0	0	0	1	2	2	2	2	2	1	3
	Infiltrate, inflammatory cell, Glisson sheath	0	0	0	0	0	3	3	3	2	2	1	3
Gallbladder	Edema, Mucosa	0	0	0	0	1	0	1	0	1	0	0	0
	Necrosis, Mucosal epithelium	0	0	0	0	1	1	3	1	2	0	1	2
	Regeneration, Mucosal epithelium	0	0	0	0	2	2	2	3	3	1	3	3
	Infiltrate, inflammatory cell, Mucosa	0	0	0	0	1	0	2	1	1	0	1	1

ANIT-induced toxic changes were not influenced by blood sampling with MS.

M: Male, F: Female, Tv: Tail vein, Sv: Subclavian vein, *: Number of animals affected, -: No noteworthy findings

Blue text: Changes considered to be caused by ANIT administration, Red text: Changes considered to be caused by blood collection by MS

Contents

Conclusion

- Microsampling is an innovative technology that promotes Refinement and Reduction of 3Rs and enables cost reduction by reducing the amount of test substance.
- We compared two blood collection sites and four types of blood collection devices, and concluded there were no practical problems on TK results.
- The effect of MS on hematological results was slight, if total amount of blood collection was less than 3% of circulating blood volume.
- ANIT-induced toxic changes in blood biochemistry, organ weights and histopathology were not influenced by blood sampling with MS.

Future issues

Microsampling is considered to have a negligible effect on the onset of toxicity considering the procedure and blood sampling volume, but when applied to the safety evaluation of new substances, careful examination and consideration should be given to the possibility that microsampling may have affected the onset of toxicity.