マイクロサンプリング普及のためのレギュラトリーサイエンス的解析

○高橋 一彰[1], 服部 則道[2],神野 文宏[3], 大塚 博比古[4], 中井 惠子[1], 宅見 あすか[2], 渋井 勇祐[2], 田中 庸一[5], 齊藤 公亮[5], 小林 章男[6], 斎藤 嘉朗[5]

Regulatory Scientific Analysis for Increased Use of Microsampling

○Kazuaki TAKAHASHI[1], Norimichi HATTORI[2], Fumihiro JINNO[3], Hirohiko OHTSUKA[4], Keiko NAKAI[1], Asuka TAKUMI[2], Yusuke SHIBUI[2], Yoichi TANAKA[5], Kosuke SAITO[5], Akio KOBAYASHI[6], Yoshiro SAITO[5]
1: Mediford Corporation, 2: AJINOMOTO CO., INC., 3: Axcelead Drug Discovery Partners, Inc., 4: SEIKAGAKU CORPORATION, 5: National Institute of Health Sciences, 6: INTERNATIONAL UNIVERSITY OF HEALTH AND WELFARE

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BACKGROUND/PURPOSE

Recently, animal welfare has been attracting worldwide attention, and implementation of 3Rs (replacement, reduction, and refinement) is prioritized in every way possible in drug development. Microsampling (MS), in which small amounts of blood are collected (typically no more than 50 µL per time point), is attracting attention in this context. The toxicokinetic (TK) evaluation of samples collected from the animals in the main study group by MS in the general toxicity study enables the analysis of the relationship between toxicity and drug exposure levels in the individual values and can contribute to the 3Rs by reducing or eliminating the number of satellite animals and reducing the amount of blood sampled (Fig.). However, no standard methods have been established for practical use, particularly with regard to the appropriate volume of blood to be collected over a period of time. Therefore, for increased use of MS in non-clinical studies, providing a guideline for appropriate MS blood collection volume is necessary while taking its toxic effect into account. The most important parameter to consider concerning toxicological effects of MS would be the percentage of the total blood collection volume to circulating blood volume in animals, when circulating blood volume/body weight in rats is estimated to be 64 ml/kg [1].

In previous reports on MS, the cervical and tail veins have been mainly selected as the site of TK blood collection, and it was considered that the amount of blood to be collected could vary according to the site of blood collection. Similarly, it was considered possible that the effects of MS might vary between conditions where animals are under the stress of drug treatment and conditions without drug treatment.

In this presentation, a regulatory scientific analysis based on a systematic review was conducted with the aim of obtaining a blood collection volume that would have no effect on the animals or would be generally acceptable, based on the estimated circulating blood volume.

[1] Diehl et al., J Appl Toxicol. 2001

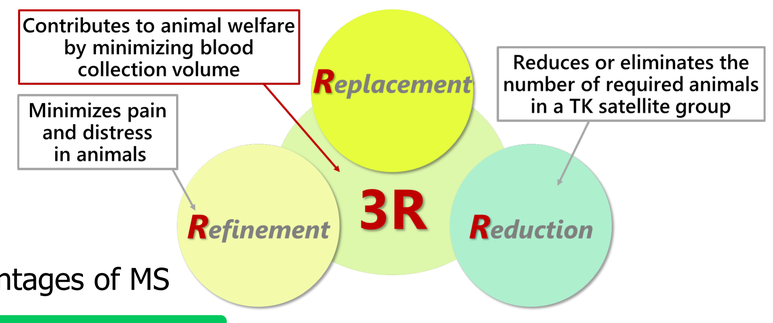


Fig. Advantages of MS

RESULTS

BASICS

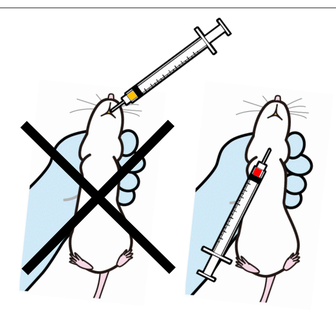
Appropriate volume of blood samples taken from the cervical vein of drug-free animals

Reference	Animal	Sex	Drug	Blood sampling points	Blood sampling volume	Percentage of blood collection volume to circulating blood volume[*1]	Number of evaluated organizations	Blood sampling site	Evaluation points	Toxicological effect
Yokoyama *et al.*, 2020	Rats Crl:CD(SD)	Males/ females	None	Days 1-2: 0.5, 1, 2, 4, 8, and 24 hrs Days 27-28: 0[*2], 0.5, 1, 2, 4, 8, and 24 hrs	50 µL/point	Days 1-2: 2.1%-2.6% in males and 2.9%-3.3% in females Days 27-28: 1.3%-1.5% in males and 2.2%-2.5% in females	4	Cervical vein	Day 29	No or minimal influences on body weight gain, food consumption, hematological and blood clinical chemistry parameters, and organ weights.
Hattori *et al.*, 2020	Rats Crl:CD(SD)	Males/ females	None	Days 1-2: 0.5, 1, 2, 4, 8, and 24 hrs Days 27-28: 0[*3], 0.5, 1, 2, 4, 8, and 24 hrs	0 µL/point or 100 µL/point[*4]	Days 27-28 100 µL/point group: 2.8% in males and 4.5% in females	1	Cervical vein	Day 29	Decreases in red blood cells, hemoglobin concentration, and hematocrit in females and an increase in fibrinogen in males.

*1: Mean circulating blood volume is regarded as 64 mL/kg for rats (Diehl et al., 2001).
*2: Time 0 was set in the morning.
*3: Time 0 refers to the point before dosing.
*4: The 100 µL/point do not fall into the MS category.

Red square: There were toxicological effects due to blood collection.
Blue square: Maximum percentage in which there were no toxicological effects

When blood samples are taken from the cervical vein of drug-free animals, it is recommended that 3% of the circulating blood volume should be the upper limit of the total blood collection within 24 hours, with a recovery period of at least 1 day between the end of the transit blood collection and the evaluation point.



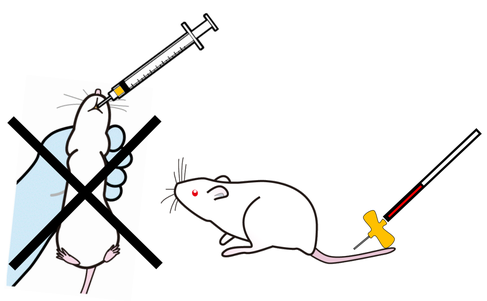
Appropriate volume of blood samples taken from the tail vein of drug-free animals

Reference	Animal	Sex	Drug	Blood sampling points	Blood sampling volume	Percentage of blood collection volume to circulating blood volume[*1]	Number of evaluated organizations	Blood sampling site	Evaluation points	Toxicological effect
Hattori *et al.*, 2020	Rats Crl:CD(SD)	Male/ females	None	Days 1-2: 0.5, 1, 2, 4, 8, and 24 hrs Days 29-30: 0[*2], 0.5, 1, 2, 4, 8, and 24 hrs	Exp 2: 0 µL/point, 50 µL/point, or 100 µL/point[*3]	Days 29-30 50 µL/point group: 1.5% in males and 2.5% in females 100 µL/point group: 2.9% in males and 4.7% in females	1	Tail vein	Day 31	No parameters were affected after sampling of up to 100 µL.
Powles-Glover *et al.*, 2014	Rats Crl:WI(HAN)	Males/ females	None	Days 1-2: 0.5, 1, 2, 4, 8, and 24 hrs Days 14-15: 0.5, 1, 2, 4, 8, and 24 hrs	Group 1: Sham Group 2: 200 µL/point[*3] Group 3: 32 µL/point	Group 2 Males: 5.7% on day 1 and 5.5% on day 15 Group 2 Females: 8.5% on day 1 and 8.2% on day 15 Group 3 Males: 0.9% on day 1 and 0.8% on day 15 Group 3 Females: 1.4% on day 1 and 1.3% on day 15[*4]	1	Tail vein	Day 15	Group 2: Decreases in hemoglobin, hematocrit, and red blood cell count and increases in reticulocytes, monocytes, neutrophils, AST, and GLDH Group 3: A small increase in monocyte and a slight decrease in hemoglobin concentration.

*1: Mean circulating blood volume is regarded as 64 mL/kg for rats (Diehl et al., 2001).
*2: Time 0 was set in the morning.
*3: The 100 µL/point and 200 µL/point do not fall into the MS category.
*4: The body weight range for day 1 and day 15 was regarded as about 330 g and 340 g for Group 2 males, 340 g and 360 g for Group 3 males, and 220 g and 230 g for Group 2 and 3 females, respectively, from the figures and tables in the article.

Red square: There were toxicological effects due to blood collection.
Blue square: Maximum percentage in which there were no toxicological effects

When blood samples are taken from the tail vein of drug-free animals, it is recommended that 5% of the circulating blood volume should be the upper limit of the total blood collection within 24 hours, with a recovery period of at least 1 day between the end of the transit blood collection and the evaluation point.



APPLICATIONS

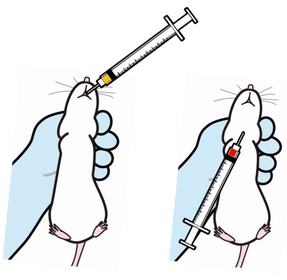
Appropriate volume of blood samples taken from the cervical vein of drug-treated animals

Reference	Animal	Sex	Drug	Blood sampling points	Blood sampling volume	Percentage of blood collection volume to circulating blood volume[*1]	Number of evaluated organizations	Blood sampling site	Evaluation points	Toxicological effect
Hattori *et al.*, 2023	Rats Crl:CD(SD)	Female	Methapyrilene	Days 1-2: 0.5, 1, 2, 4, 8, and 24 hrs Days 27-28: 0[*2], 0.5, 1, 2, 4, 8, and 24 hrs	50 µL/point	Days 1-2: 2.8%-2.9% in females Days 27-28: 2.1%-2.3% in females	2	Cervical vein	Day 29	The changes observed at each of the two facilities were not reproducible.
Ohtsuka *et al.*, 2022	Rats Crl:CD(SD)	Female	Phenacetin	Days 1-2: 0.5, 1, 2, 4, 8, and 24 hrs Days 27-28: 0[*2], 0.5, 1, 2, 4, 8, and 24 hrs	50 µL/point	Days 1-2: 2.8% in females Days 27-28: 2.3%-2.7% in females	2	Cervical vein	Day 29	Minimal effects on a few hematological parameters.
Tanaka *et al.*, 2023	Rats Crl:CD(SD)	Female	Azathioprine	Days 1-2: 0.5, 1, 2, 4, 8, and 24 hrs Days 27-28: 0[*2], 0.5, 1, 2, 4, 8, and 24 hrs	50 µL/point	Days 1-2: 2.7%- 2.9% in females Days 27-28: 2.2%-2.4% in females	3	Cervical vein	Day 29	Minimal or no effects on almost all parameters except for leukocytic parameters.

*1: Mean circulating blood volume is regarded as 64 mL/kg for rats and 72 mL/kg for mice(Diehl et al., 2001).
*2: Time 0 refers to the point before dosing.

Blue square: Maximum percentage in which there were no toxicological effects

When blood samples are taken from the cervical vein of drug-treated animals, it is recommended that 3% of the circulating blood volume should be the upper limit of the total blood collection within 24 hours, with a recovery period of at least 1 day between the end of the transit blood collection and the evaluation point.



CONCLUSIONS

1. Appropriate volume of blood samples from the cervical vein or tail vein of drug-free animals was ≤3% or ≤5%, respectively, of the circulating blood volume within 24 hours.

Blood sampling from the cervical vein is a common and rapid method in Japan. However, it is speculated that blood leakage from the sampling site may explain why toxic effects due to MS are more frequently observed compared to blood sampling from the tail vein of drug-free animals. Therefore, continuous improvement of blood sampling techniques, especially from the cervical vein, is considered crucial.

2. Appropriate volume of blood samples taken from the cervical vein of drug-treated animals was ≤3% of the circulating blood volume within 24 hours.

Although the effects of MS on the azathioprine induced immunotoxicity on leukocytic parameters could not be completely ruled out, other studies conducted under drug treatment conditions have shown that MS has minimal toxicological effects.

With the blood sampling volumes described above, the application of MS in non-clinical toxicity studies in rats is deemed feasible.