P-225

ラットを用いたアザチオプリン反復投与毒性試験における毒性評価に及ぼすマイクロサンプリング法の影響

○川口 瞬[1], 高橋 一彰[1], 船越 武[1], 中井 恵子[1], 山本 大[1], 岡村 隆之[1], 田中 庸一[2], 斎藤 嘉朗[2], 中川 宗洋[1]　[1]株式会社LSIM安全科学研究所, [2]国立医薬品食品衛生研究所

Effect of microsampling method on toxicity evaluation of azathioprine in repeated dose toxicity studies in rats

○Shun Kawaguchi[1], Kazuaki Takahashi[1], Takeshi Funakoshi[1], Keiko Nakai[1], Dai Yamamoto[1], Takayuki Okamura[1], Yoichi Tanaka[2], Yoshiaki Saitou[2], Munehiro Nakagawa[1]
[1]LSIM Safety Institute Corporation　[2]National Institute of Health Sciences

Background and Purpose

Microsampling (MS) method is an useful technique used in toxicokinetics (TK) studies in which a very small amount of blood (less than 50 μL) is collected to determine the concentrations of a drug and its metabolites. The high sensitivity of analytical instruments has made it possible to measure with small amounts of samples, allowing TK analysis in the main study group of the toxicity study. Therefore, this technique can contribute to reducing the number of animals used.
On the other hand, there have not been enough reports on the effect of MS method on toxicity evaluation. We have presented the effects of differences of the device and blood collection site on the evaluation, and the results comparing the MS method with the conventional method with drugs that induce hepatotoxicity and myelotoxicity. It has been suggested that the stress on animals caused by the MS method may affect the evaluation of immune system. Therefore, we investigated the effects of the MS method on repeated-dose toxicity evaluation using azathioprine with reports on immunotoxicity.

Materials and Methods

[Animals] Crl:CD(SD) rats (Female), 6 weeks old
[Dosing frequency] Once daily, 4 weeks
[Test article] Azathioprine (Vehicle: 0.5 w/v% Methyl Cellulose; MC)
[Microsampling method] About 50 μL/points from subclavian vein without anesthesia
Device: BD Lo-Dose™ Insulin syringe 29G (Becton, Dickinson and Company)

[Examination items]
Clinical signs, Body weight, Food consumption, Urinalysis (Clinitek advantus, TBA-2000FR), Hematology (CA-500, XT-2000iV), Blood chemistry (Epalyzer2, TBA-2000FR), Pathology: Organ weight (*), Necropsy, Histopathology (Liver*, spleen*, kidney*, heart*, lung/bronchus*, thymus*, femur/bone marrow, mandibular lymph node, mesenteric lymph node), TK analysis (Analyte: 6-mercaptopurine [azathioprine metabolite], Points: Before dosing (only final dosing), 0.5, 1, 2, 4, 8 and 24 hours after dosing)

[Statistical analysis]
The Data on body weight, food consumption, hematology, blood chemistry, urinalysis, and organ weights were statistically analyzed as follows. The safety study system (tsPharma Labsite, Fujitsu Limited) was used for the analysis.

1. The effects of azathioprine administration
They were analyzed by comparing 0 mg/kg (control) with 3 and 10 mg/kg in the MS group and the non-blood sampling group. The Dunnet or Steel test was performed.

2. The effects of the MS method
They were analyzed by comparing the same doses between the non-blood sampling group and the MS group. The t-test or Welch's t-test was performed.

[Group composition]

*: Control (0.5 w/v% MC)

Test article	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Conc. (mg/mL)	Number of animals
<Non-blood sampling Group>				
Control*	0	5	0	5
Azathioprine	3	5	0.6	5
Azathioprine	10	5	2	5
<Microsampling Group>				
Control*	0	5	0	5
Azathioprine	3	5	0.6	5
Azathioprine	10	5	2	5

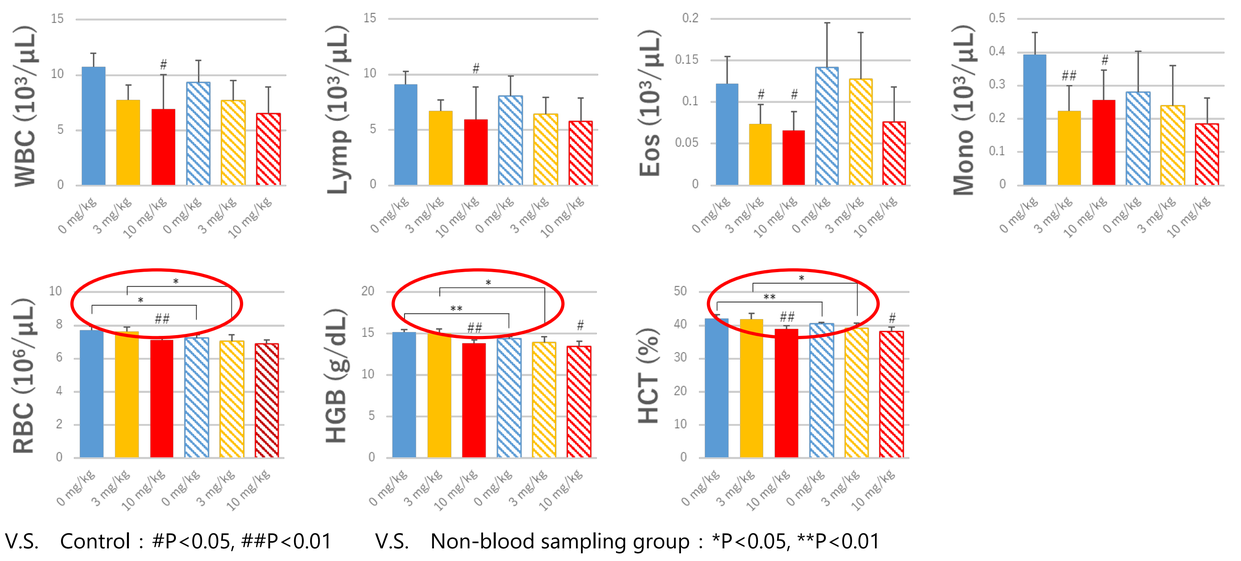
Result

Clinical signs, Body weight, Food consumption, Urinalysis

No effects on toxicity evaluation by the MS method were observed.

Hematology

Non-blood sampling group：, MS group：



V.S. Control：#P<0.05, ##P<0.01　V.S. Non-blood sampling group：*P<0.05, **P<0.01

(V.S. Control) ↓: Decrease

10 mg/kg: RBC↓, HGB↓, HCT↓, WBC↓, Lymphocyte↓, Eosinophil↓, and Monocyte↓
3 mg/kg: Eosinophil↓ and Monocyte↓

➡The effects of azathioprine administration were seen in the MS group as well as in the non-blood sampling group. Therefore, no effects on the toxicity evaluation by the MS method were noted.

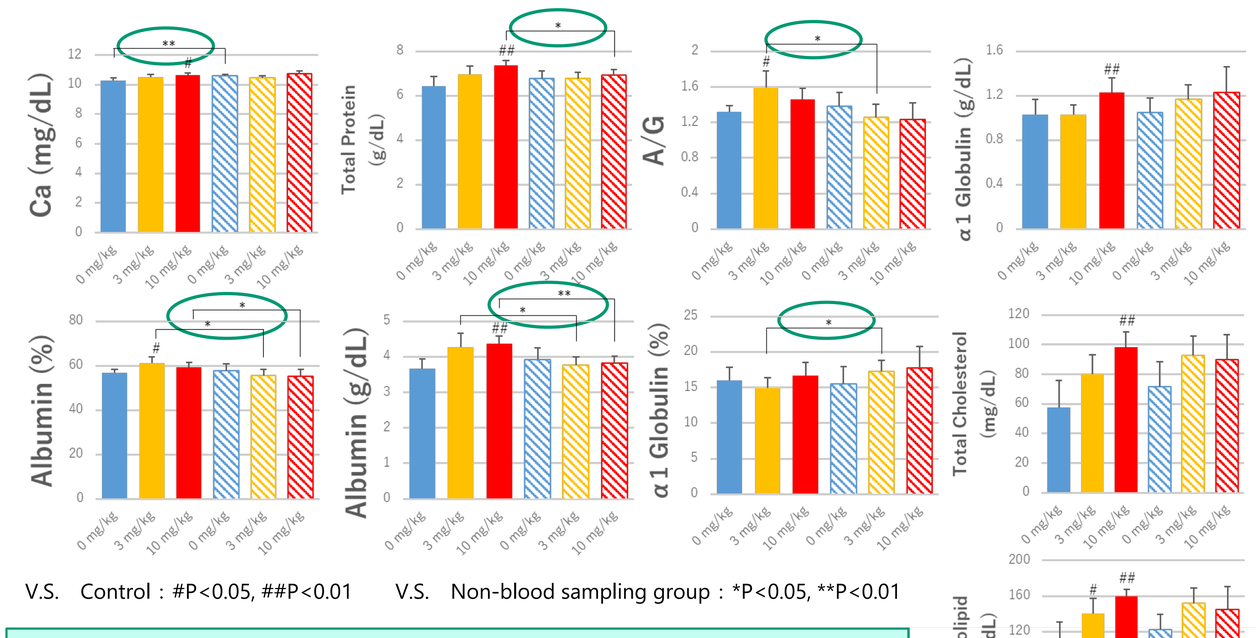
(V.S. Non-blood sampling group) ↓: Decrease

0, 3 and 10 mg/kg: RBC↓, HGB↓, and HCT↓

➡Significant differences in erythrocyte system parameters caused by the MS method were noted; however, these changes were within the range of the background data and considered to have minor effects on the evaluation of azathioprine toxicity. (Red circle)

Blood chemistry

Non-blood sampling group：, MS group：



V.S. Control：#P<0.05, ##P<0.01　V.S. Non-blood sampling group：*P<0.05, **P<0.01

(V.S. Control) ↓: Decrease, ↑: Increase

10 mg/kg: Total Cholesterol↑, Phospholipid↑, Ca↑, Total protein↑, Albumin↑, and α1 Globulin↑
3 mg/kg: Phospholipid↑, A/G↑, and Albumin↑

➡The effects of azathioprine administration were seen in the MS group as well as in the non-blood sampling group. Therefore, no effects on the toxicity evaluation by the MS method were noted.

(V.S. Non-blood sampling group) ↓: Decrease, ↑: Increase

10 mg/kg: Total protein↓, Albumin↓, 3 mg/kg: A/G↓, Albumin↓, α1 Globulin↑, 0 mg/kg: Ca↑

➡Attenuation of the effects of azathioprine caused by the MS method was noted; however, it was within the range of the background data and considered to have no effects on the evaluation of azathioprine toxicity. (Green circle)

Pathological examination

		Non-blood sampling group			MS group		
Dose level (mg/kg)		0	3	10	0	3	10
Organ Weight-Body Weight Ratio							
Thymus	(mg)	416.0	380.1	206.3##	484.9	438.6	266.1##
	(10⁻³%)	190.93	175.44	97.08##	224.17*	199.26	129.89#
Macroscopic Findings							
Thymus	Small	0/5	0/5	3/5	0/5	0/5	3/5
Microscopic Findings							
Thymus	Decrease, Cortex, Lymphocyte,	0/5	0/5	3/5	0/5	0/5	3/5

V.S. Control：#P<0.05, ##P<0.01

(V.S. Control)

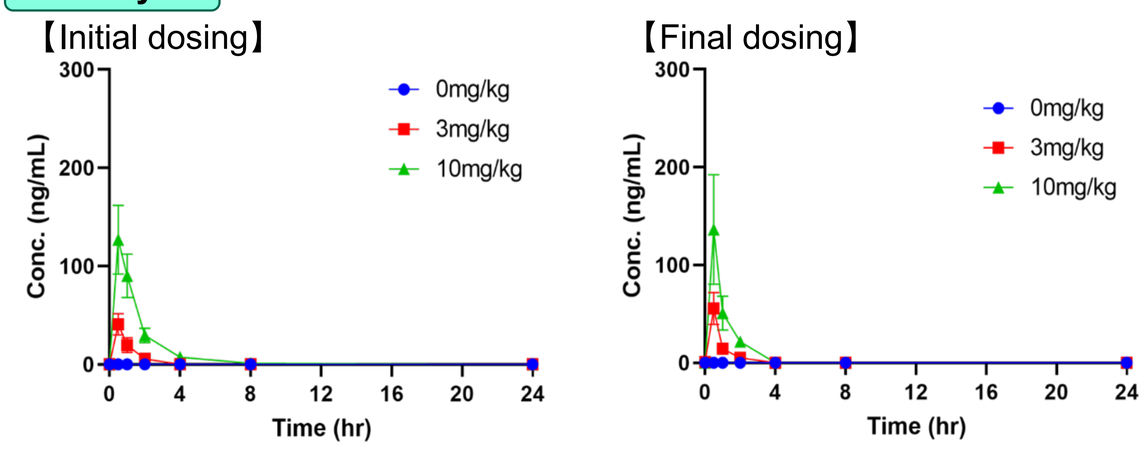
Low thymus weight, macroscopic smallness, and decreased lymphocytes in thymic cortex were noted.

➡The effects of azathioprine administration were seen in the MS group as well as in the non-blood sampling group. Therefore, no effects on the toxicity evaluation by the MS method were noted.

(V.S. Non-blood sampling group)

➡No effects on toxicity evaluation by the MS method were noted.

TK analysis



Plasma 6-mercaptopurine concentration increased with increasing dose. Moreover, no effects on the exposure level were observed after repeated administration.

Conclusion

1. The results of this study suggest that the effect of the MS method in the evaluation of azathioprine toxicity is limited to minor changes within the background data and that the MS method can be used for evaluation of immune system parameters.

2. The MS method was found to affect erythrocyte parameters as a previous report[1]. It was deemed necessary to take into account toxicological characteristics of test articles when using the MS method for repeated-dose toxicity studies.

Reference

1) Yui Akagawa, Toxicity Evaluation in Repeated-Dose Toxicity Studies in Rats and Effects of Microsampling Methods on Pharmacokinetics. Poster presented at: The 45th Annual Meeting of the Japanese Society of Toxicology, July 18, 2018; Osaka.