ラットにおける気管内投与による反復投与毒性試験のための背景データ

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Collection of background data for repeated dose toxicity studies by intratracheal instillation in rats

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Objective

Intratracheal instillation is useful when evaluating toxicity of various substances by an airway route. However, information is limited regarding repeated dose toxicity studies. The present study was conducted to obtain additional data to supplement background data.

Materials and Methods

· Test system

Crl:CD(SD), male, 8 weeks old (start of dosing)

· Study group

Anesthetic	Dosing solution	Number of animals
None[1]	None	6
Sevoflurane (Sevo)	None[2]	6
	DW	6
	PBS	6
Isoflurane (Iso)	None[2]	6
	DW	6
	PBS	6

1, non-treatment control
(none of dosing/anesthetization)
2, Anesthetization only

· Dosing (intratracheal instillation)

Equipment	Solution
Indwelling needle (18G × 64 mm, Terumo) Syringe pump (1 mL, Nipro)	Distilled water (DW, water for injection, Otsuka) Phosphate-buffered saline (PBS, Takara Bio)
Volume	Anesthetic
1 mL/kg^{-BW}	Sevoflurane (Sevofrane, Maruishi) Isoflurane (Pfizer, Mylan)
Frequency, duration	Anesthetization
1 dose/day, 5 days/week, 4 weeks	Sevoflurane (induction and maintenance: 5%) Isoflurane (induction: 5%, maintenance: 2.5%)

· Observation and measurements

Clinical sign, body weight, food consumption, hematology, blood chemistry, bronchoalveolar lavage fluid (BALF: cytology, chemistry), organ weight (lung, liver), gross necropsy, histopathology (lung)

· Statistical analysis

Bartlett (5%), Tukey/Steel Dwass (5%, two-sided)
Non-treatment control vs. Sevoflurane/Isoflurane (non-dosing, DW, PBS)
F-test, t-test/Aspin-Welch (5%, two-sided)
Sevoflurane vs. Isoflurane

Results

· Clinical sign

Death　2 males Sevo + DW group
2 males Iso + DW group

Note:
Respiration occasionally stopped after DW dosing, artificial respiration was effective.

· BALF Cytology (Mean ± S.D.)

Group	Total cell (10^3 Cells)	Lymph (%)	Neu (%)	Neu (%)	Eos (%)	Mac (%)
Non-treatment	1373.8 ± 872.0	3.38 ± 1.64	9.45 ± 5.53	9.45 ± 5.53	2.55 ± 1.43	84.62 ± 8.16
Sevo + None	1802.7 ± 330.8	2.20 ± 0.32	8.03 ± 1.30	8.03 ± 1.30	1.70 ± 1.00	88.07 ± 2.22
Sevo + DW	3097.3 ± 1213.1	3.35 ± 1.90	5.00 ± 0.65	5.00 ± 0.65	4.78 ± 2.45	86.88 ± 2.83
Sevo + PBS	3512.7 ± 1967.3	2.13 ± 1.16	8.23 ± 6.19	8.23 ± 6.19	2.68 ± 2.60	86.95 ± 9.52
Iso + None	1887.6 ± 668.1	2.24 ± 0.80	9.68 ± 3.03	9.68 ± 3.03	2.82 ± 2.64	85.26 ± 5.84
Iso + DW	2138.0 ± 1319.8	2.35 ± 0.68	8.10 ± 2.74	8.10 ± 2.74	3.43 ± 1.32	86.13 ± 3.79
Iso + PBS	1828.6 ± 918.7	3.16 ± 2.86	8.50 ± 6.09	8.50 ± 6.09	1.04 ± 0.44	87.30 ± 9.15

No significant difference among the groups

· Lung weight

Group	Absolute (g)		BW ratio (%)	
Non-treatment	1.761 ± 0.077		0.386 ± 0.027	
Sevo + None	1.570 ± 0.061	a	0.355 ± 0.014	
Sevo + DW	1.940 ± 0.084	a	0.452 ± 0.024	b
Sevo + PBS	1.781 ± 0.159		0.398 ± 0.032	
Iso + None	1.625 ± 0.135		0.370 ± 0.019	
Iso + DW	1.861 ± 0.102	a	0.451 ± 0.021	
Iso + PBS	1.732 ± 0.117		0.397 ± 0.023	

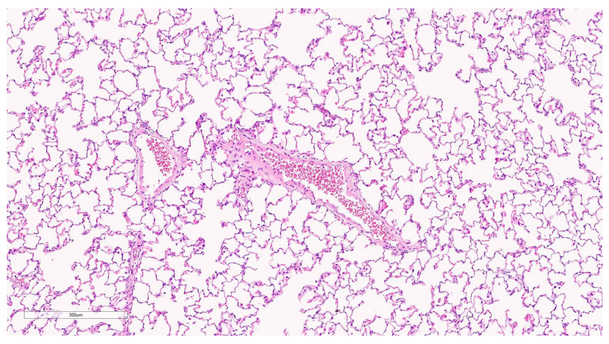
Significant difference
a: non-treatment group, b: only anesthetic group

· Other measurements

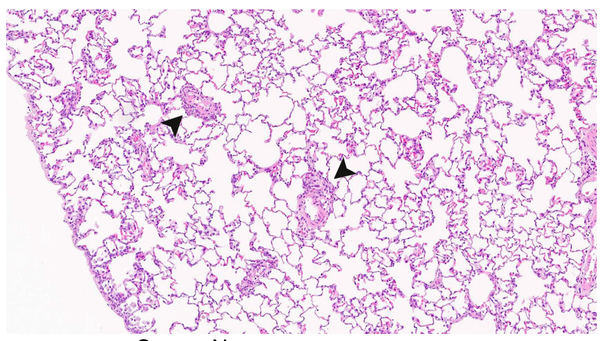
Item	Results
Body weight	No significant difference among the groups
Food consumption	No apparent changes among the groups
Hematology	No apparent changes among the groups, data within the background range
Blood chemistry	No apparent changes among the groups, data within the background range
Gross necropsy	No gross changes

· Histopathological findings (Lung and bronchus, Incidence and severity)

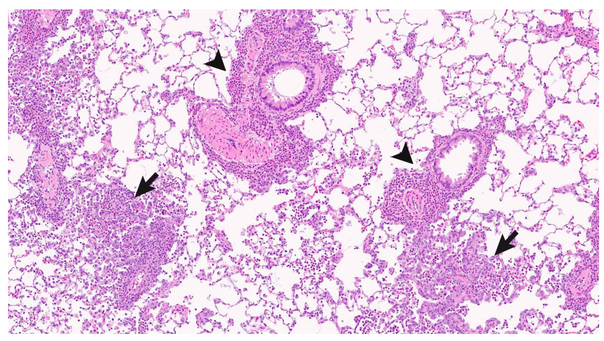
Findings	Grade	Non-treatment	Sevo + None	Sevo + DW	Sevo + PBS	Iso + None	Iso + DW	Iso + PBS
(number of animals examined)		(6)	(6)	(4)	(6)	(6)	(4)	(6)
Eosinophil infiltration, Perivascular/Peribronchiolar	minimal		3		4	4		6
	mild			3	2		4	
	moderate			1				
Infiltrate, inflammatory cell, focal	minimal		1	1	1		3	1
	mild			1				



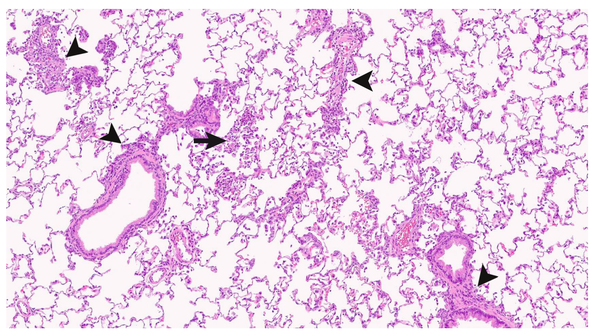
Non-treatment: No remarkable change



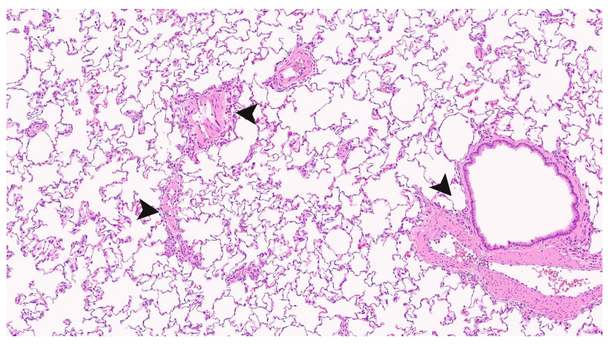
Sevo + None:
Arrow head: Eosinophil infiltration, Perivascular/Peribronchiolar



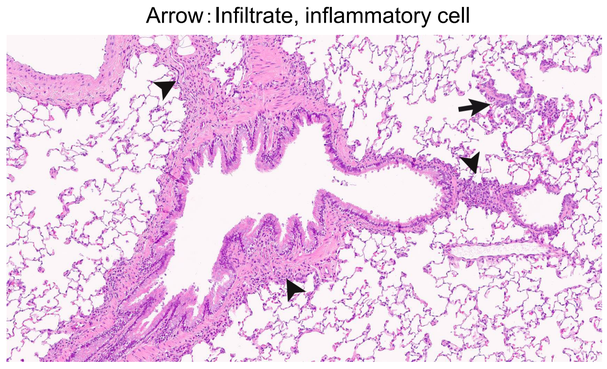
Sevo + DW:
Arrow head : Eosinophil infiltration, Perivascular/Peribronchiolar.
Arrow : Infiltrate, inflammatory cell



Sevo + PBS:
Arrow head : Eosinophil infiltration, Perivascular/Peribronchiolar.
Arrow : Infiltrate, inflammatory cell



Iso + None:
Arrow head : Eosinophil infiltration, Perivascular/Peribronchiolar.



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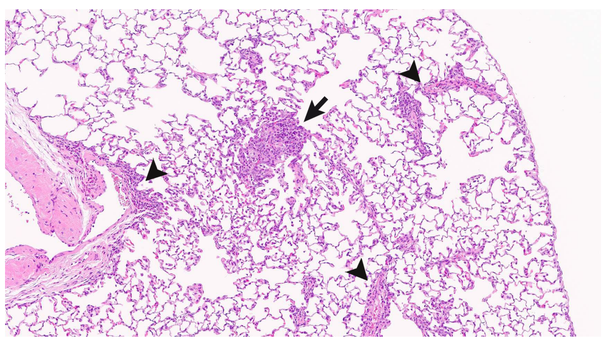

Iso + PBS:
Arrow head : Eosinophil infiltration, Perivascular/Peribronchiolar.
Arrow : Infiltrate, inflammatory cell

Conclusion

The results of this study indicated that the anesthetics for repeated intratracheal instillation induced eosinophil infiltration in the lungs, which was accelerated by DW droplet instillation. It was also suggested that DW droplets instillation possibly cause respiratory disorder. This study presented useful background data for repeated dose toxicity studies by intratracheal instillation in rats.

Name of presenting author: WAKO Kiyoshi
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The author has no COI regarding this presentation.