Comparison between administration routes in brain distribution and drug efficacy of ASO (ICV vs IT)

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Purpose

Many of ASOs targeting the central nervous system (CNS) cannot penetrate the blood-brain-barrier, and therefore these ASOs are administered intrathecally or intracerebroventricularly that allows direct exposure to CNS. In clinical, intrathecal injection (IT) is selected due to low invasiveness, whereas intracerebroventricular injection (ICV) is widely used in experimental animals, especially mice. However, there are few reports evaluating distribution, exposure and an effect of *metastasis associated lung adenocarcinoma transcript 1 (Malat1)* ASO in CNS after IT. In this study, we investigated the differences in the pharmacokinetics and knockdown (KD) efficiency of ASO for each administration routes using mice.

Results and Discussion

Our IT method was a robust procedure, as demonstrated by reproducible results.

The distribution of ASO in the brain was homogeneous after ICV, whereas that was inhomogeneous after IT. The striatum and the hippocampus were low distribution, and the spinal cord was high distribution compared with the cortex and the cerebellum.

ICV and IT are administration routes that enable direct exposure of the ASOs to CNS.

On the other hand, since there is a difference in the distribution of ASO in the brain between IT and ICV, it is necessary to carefully select the administration route depending on the target site. Therefore, the verification using IT may be necessary in Non-clinical study.

Material method

▪ASO

Mouse *Malat1* ASO[1)]

5'-GGGTmCAGCTGCCAATGmCTAG-3'

ASO has fully phosphorothioate backbone.

Underlining; 2'-O-methoxyethyl modified base ASO

mC; 5-methyl cytosine

All ASOs were purchased from GENEDESIGN, Ajinomoto Bio-Pharma Services.

▪Animal study

C57BL/6J mice at 8 weeks of age were administered *Malat1* ASO at 37.5 µg/animal (approximately 1.5 mg/kg, ICV and IT) , 50 mg/kg (subcutaneous injection, SC) or saline (n = 3-5/group). Mice were euthanized 1, 4, 24 hours and 14 days after dose and tissues were collected.

All animal experiment protocols were approved by the Institutional Animal Care and Use Committee of Shonan Health Innovation Park.

▪Pharmacokinetics (PK)

ASO concentration was measured by hybridization ELISA.

▪Gene expression analysis

Malat1 mRNA expression were quantified by quantitative real-time polymerase chain reaction using GAPDH as a housekeeping gene.

▪Histology analysis

Collected tissues were fixed by 10% neutral buffered formalin and embedded in paraffin. Sections were stained by *in situ* hybridization (ISH) with the probe for mouse *Malat1* ASO.

Methods of ISH

- Probe: miRNAscope™ LS Probe -SR-ASO-Mm-Malat1-S1 (ACD bio, Catalog No. 1153028-S1)
- Staining kit: miRNAscope LS Reagent Kit - RED (ACD bio, Catalog No. 324600) and BOND Polymer Refine Red Detection Kit (ACD bio, Catalog No. DS9390)

Reference

1) G. Hung, et. Al. 2013. Nucleic Acid Ther 23:6, 369-378.

COI disclosure information

We have no financial relationship to disclose for our presentation contents.

Evaluation of distribution in tissue and mRNA knockdown

1. Establishment of IT method in conscious mice

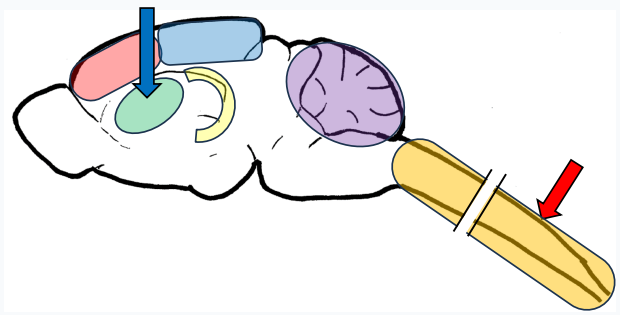

Fig.1 The administration sight and the regions of the brain for evaluation of ASO distribution.
Blue arrow; administration point of ICV,
red arrow; administration point of IT,
red region; cortex (front), blue region; cortex (rear),
green region; striatum, yellow region; hippocampus,
purple region; cerebellum and orange region; spinal cord.

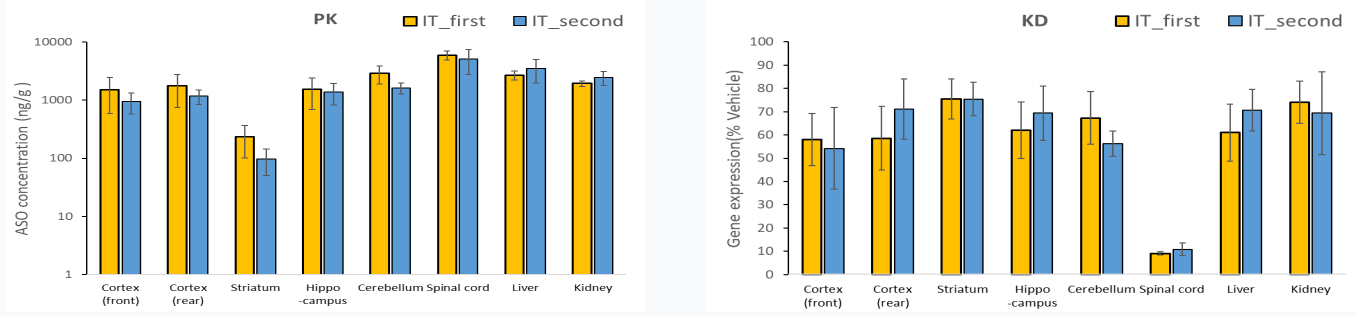

Fig.2 ASO concentration and *Malat1* mRNA expression after IT were evaluated twice for the reproducibility of IT method.
Data were expressed as Mean ± SD, IT_first; n=5, IT_second; n=3.

2. Comparison between three administration routes in CNS distribution and drug efficacy of ASO at 14 days after dosing

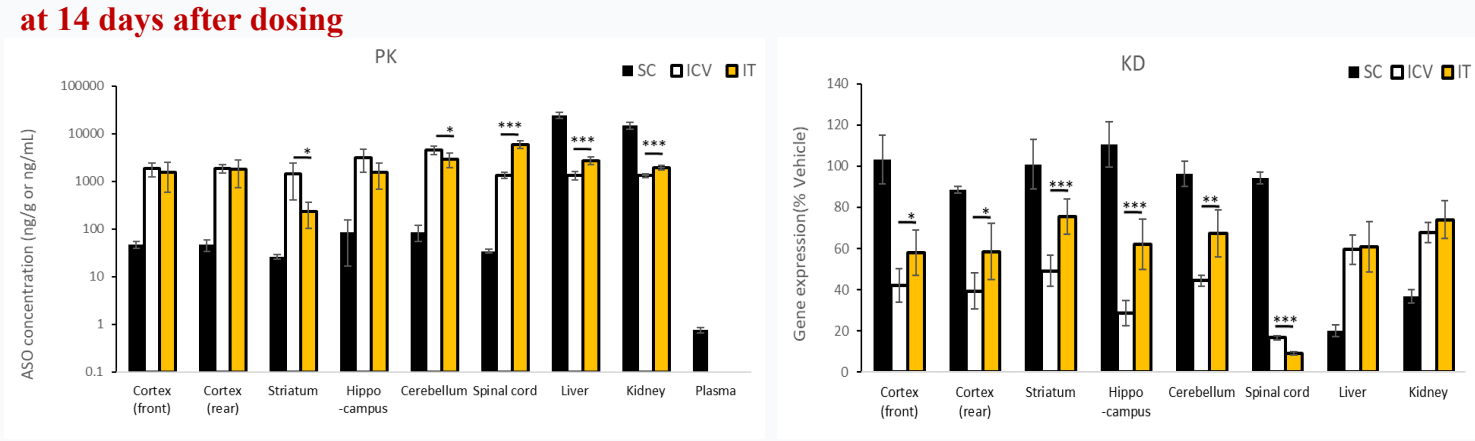

Fig.3 ASO concentration and *Malat1* mRNA expression were evaluated at 14 days after SC, ICV and IT. Statistical analysis was performed to compare between ICV and IT that enable direct exposure to CNS. Data were expressed as Mean ± SD, n=5. *p < 0.05, **p < 0.01, ***p < 0.001 by Student's t-test.

3. Distribution analysis of ASO by ISH after ICV and IT

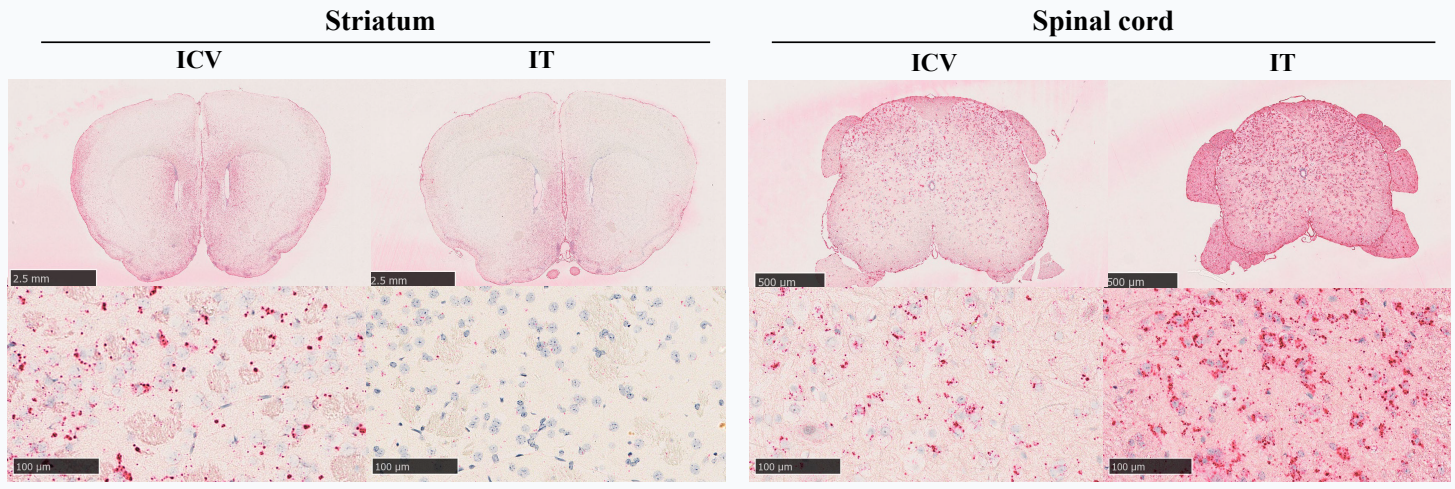

Fig. 4 ISH of *Malat1* ASO uptake by striatum and spinal cord at 14 days after ICV or IT.
ASO signals (red) were observed almost exclusively observed in the cytoplasm of neuron cells.
The upper 4 images are at low magnification and the lower 4 images are at high magnification.
Bar =2.5 mm for Striatum, 500 um for Spinal cord (upper) and 100 um (lower)

4. Time-course of ASO concentration and gene expression after ICV and IT on brain and spinal cord

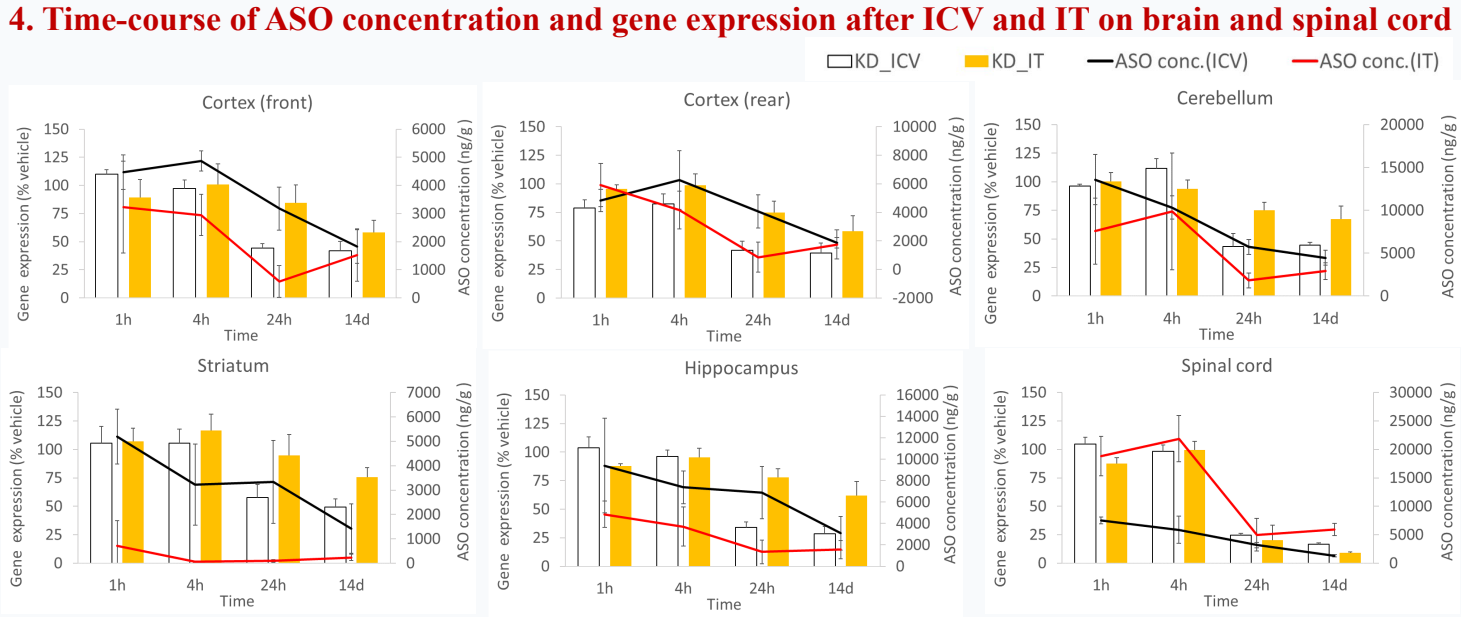

Fig.5 ASO concentration and *Malat1* mRNA expression were shown versus time after ICV or IT.
Data were expressed as Mean ± SD, n=3-5.