Pharmacological and Toxicological Profiling and Efficacy Predictability of an Antisense Oligonucleotide Following Repeated Intrathecal Administration

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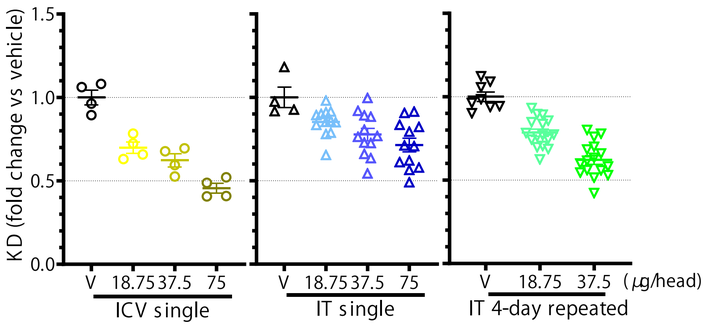
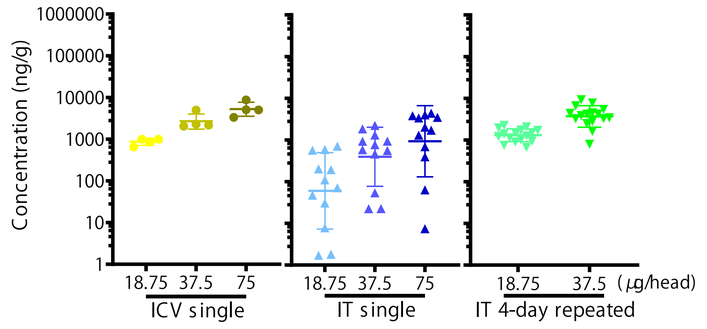
Background and Objective

Direct CNS administration is a promising approach for achieving target engagement in the brain. Optimization of dosing regimens requires balancing efficacy and tolerability.
In our previous study, single IT injection resulted in lower cortical target engagement than ICV injection. However, repeated IT dosing achieved comparable efficacy through accumulation of ASO in cortical tissue. Notably, repeated dosing did not increase acute post-dose toxicity (Previously presented at The 99th Annual Meeting of the Japanese Pharmacological Society, 2026).

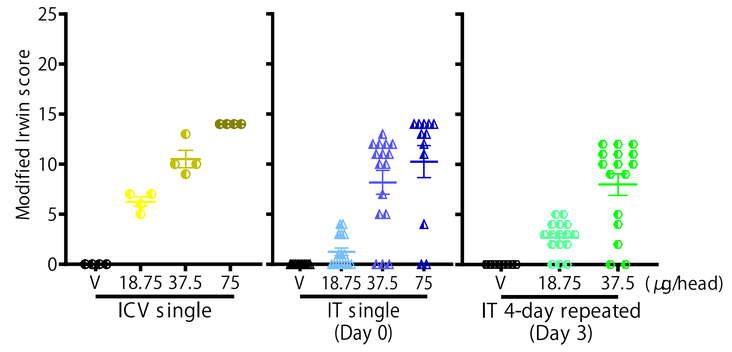
KD (Cortex)



ASO concentration (Cortex)



Modified Irwin score



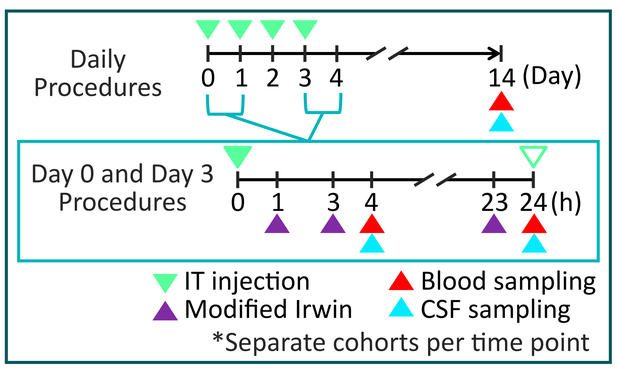
These findings suggested that efficacy and toxicity may be driven by different exposure compartments.
We hypothesized that efficacy is associated with brain exposure, whereas toxicity is more closely associated with CSF exposure. To test this hypothesis, temporal profiles of CSF and plasma concentrations were evaluated in relation to toxicity following repeated IT injection.

Materials and Methods

ASO Mouse MALAT1 ASO [1)]: 5'-GGGTmCAGCTGCCAATGmCTAG-3'; ASO has fully phosphorothioate backbone. Underlining: 2'-O-methoxyethyl modified base ASO; mC: methylcytosine. ASO was purchased from GENEDESIGN, Ajinomoto Bio-Pharma Services. 1) G. Hung, et.al. 2013. *Nucleic Acid Ther* **23**:6, 369-378.

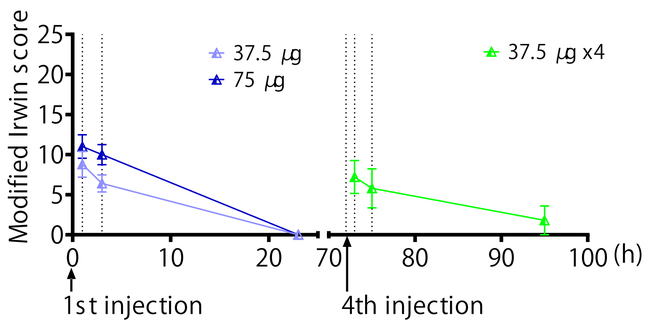
Animal study C57BL/6J mice (7 weeks old; Jackson Laboratory Japan, Yokohama) received ASO or saline (10 µL/head) via IT injection. In the repeated-dose study, mice received the same dose once daily for 4 consecutive days. Separate cohorts were assigned to each plasma/CSF sampling time point. Plasma and CSF samples were collected terminally at designated time points, with CSF obtained following euthanasia. Modified Irwin assessments were conducted at designated post-dose time points after the 1st or 4th injection.
All animal experiment protocols were approved by the IACUC of Shonan Health Innovation Park.

Measurement of ASO concentration ASO concentration in plasma and tissue was measured by Hybridization ELISA.

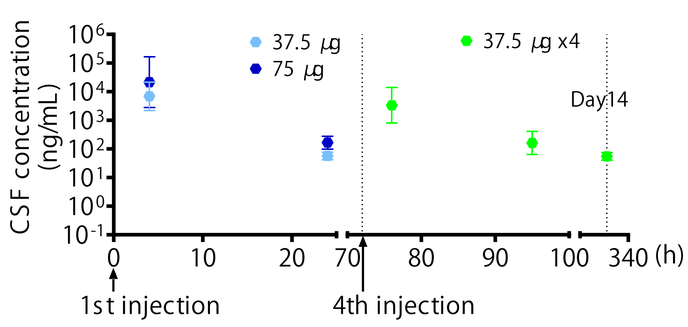


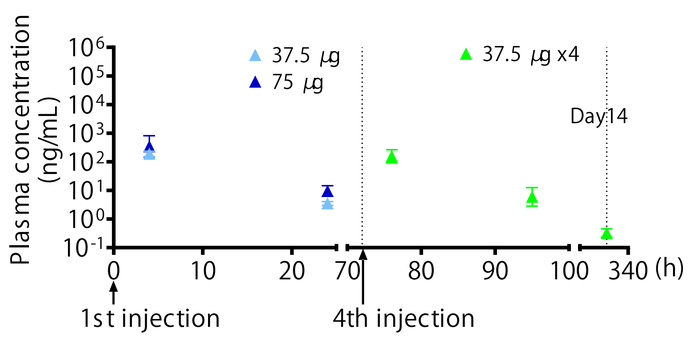
Results

(A) Time course of acute toxicity

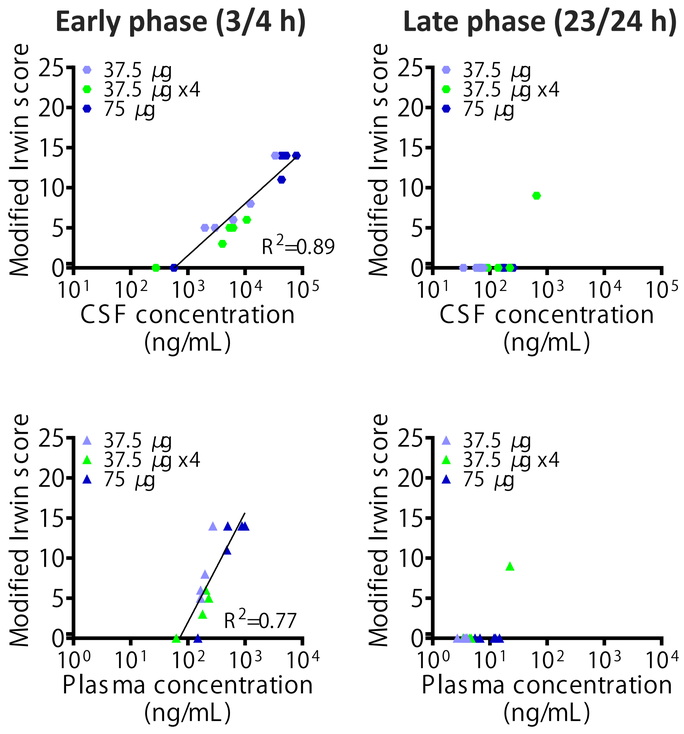


(B) Time course of CSF and plasma concentrations

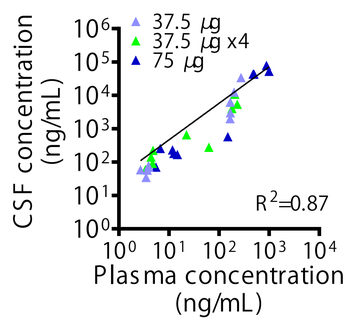




(C) Exposure–toxicity relationship



(D) Correlation between CSF and plasma concentrations



(A) Acute toxicity was transient, with elevated Modified Irwin scores at 1-3 h post-dose that returned to near baseline by 24 h. Data are presented as mean ± SEM.
(B) CSF and plasma concentrations declined rapidly after dosing and were markedly reduced by 24 h. Data are presented as geometric mean ± geometric SD.
(C) Modified Irwin scores correlated with both CSF and plasma concentrations ($R^2 \approx 0.8$).
(D) Plasma concentrations were strongly correlated with CSF concentrations.

Conclusion

To investigate exposure metrics associated with acute toxicity following IT injection of ASO, temporal profiles of CSF and plasma concentrations were evaluated and compared with Modified Irwin scores.

▪ Acute toxicity was transient and largely resolved by 24 h post-dose. ▪ Modified Irwin scores correlated with both CSF and plasma concentrations. ▪ CSF and plasma concentrations were strongly correlated. ▪ Plasma concentrations may serve as a surrogate marker for predicting acute toxicity following IT administration.

These findings suggest that acute toxicity following IT injection is driven by transient exposure in the CSF compartment rather than sustained tissue accumulation. Plasma monitoring may provide a practical approach for predicting and managing acute toxicity during dose optimization and clinical translation.

Conflict of Interest Disclosure

The presenter has no conflicts of interest to disclose related to this presentation within the past three years.

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ADDP's Oligonucleotide Drug Discovery