aragen.com

Targeted CNS Drug Delivery: Stereotaxic Animal Model and ICV Administration

Executive Summary

The development of effective drugs for Central Nervous System (CNS) disorders remains a significant challenge due to the complexities of the human brain and the limitations of traditional drug delivery methods. Stereotaxic animal models combined with intracerebroventricular (ICV) drug administration have emerged as an essential tool for understanding the pharmacokinetics and pharmacodynamics of potential CNS drug candidates.

This white paper outlines the application of a stereotaxic animal model with ICV delivery to enhance CNS drug delivery, focusing on methotrexate (MTX), a widely used immunosuppressive and chemotherapy agent, for the treatment of rheumatoid arthritis and neoplastic diseases, such as Acute Lymphoblastic Leukemia (ALL) and non-Hodgkin lymphoma. By establishing this model, we have gained valuable insights into methotrexate's distribution across various brain regions and plasma, highlighting the potential of ICV delivery to optimize drug penetration, reduce systemic toxicity, and improve therapeutic outcomes for low-permeability CNS drugs.

Background

Overview of CNS Drug Development

Developing therapeutics for CNS disorders is hindered by multiple challenges, primarily due to:

- Incomplete understanding of the biology of multifaceted CNS disorders such as Alzheimer's disease.
- The presence of the Blood-Brain Barrier (BBB), which restricts penetration of drugs into the brain.
- Lack of clinically relevant animal models in which to test new drugs.

Importance of Animal Models

Animal models play a crucial role in evaluating the efficacy, pharmacokinetics, and pharmacodynamics of CNS drugs. Among these, stereotaxic models enable precise drug administration, thereby facilitating accurate assessments of drug distribution in the brain.

Methotrexate (MTX) and its CNS Relevance

Overview of Methotrexate

- While MTX is primarily used for the treatment of rheumatoid arthritis and neoplastic diseases, such as ALL and non-Hodgkin lymphoma, it has shown to be effective for certain cancers that have spread to the brain. High-dose methotrexate is used to treat CNS metastases including those from breast cancer and Primary Central Nervous System Lymphoma (PCNSL).
- It has low brain penetration when administered systemically, and to be effective in CNS metastases, higher doses are required, which can lead to increased side effects.

Relevance in CNS Drug Development

- MTX serves as a model compound to explore enhanced CNS drug delivery strategies.
- Understanding MTX's CNS pharmacokinetics can aid in optimizing drug formulations for neurological disorders.

Challenges in CNS Drug Delivery

- **Blood-Brain Barrier (BBB) Permeability:** The BBB serves as a major obstacle in CNS drug delivery due to its structural features (Figure 1), significantly reducing drug entry into brain tissues.
- **Physicochemical Properties and BBB Penetration:** Drugs with unfavorable physicochemical characteristics (e.g., high molecular weight, poor lipophilicity) exhibit limited passive diffusion across the BBB, impeding their effective CNS delivery.
- **Targeting and Selectivity:** Achieving precise drug delivery to specific brain regions is challenging, resulting in suboptimal drug localization and potential off-target effects.

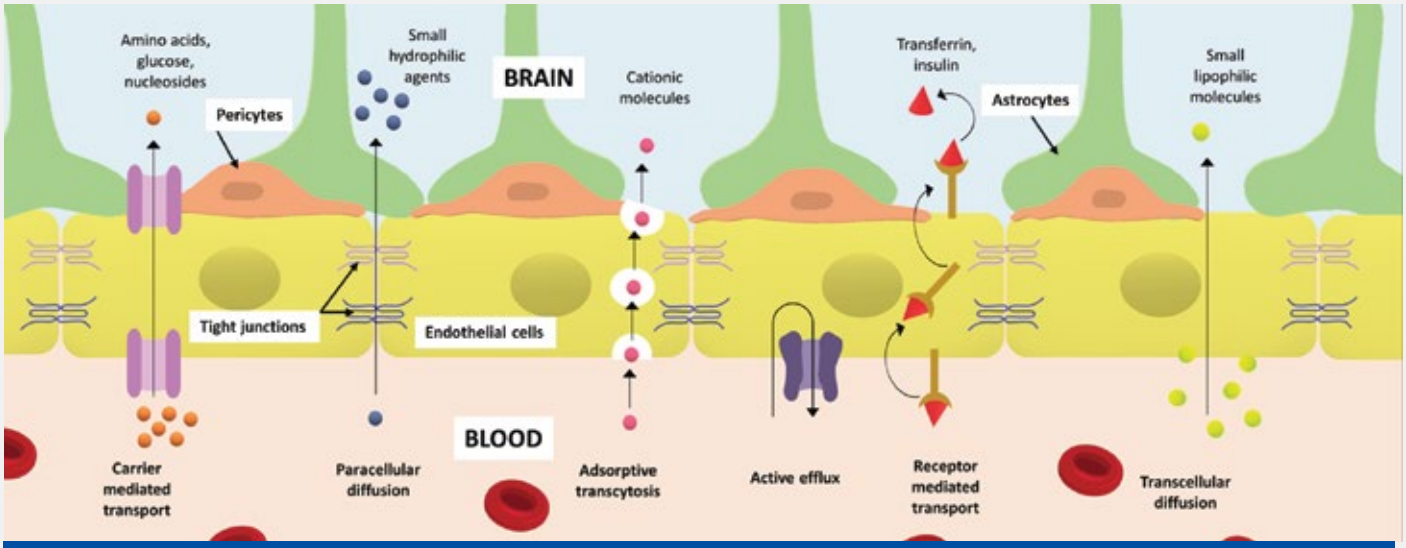

Figure 1: Blood brain barrier (BBB) depicting various types of transport systems across it.

Mechanism of MTX in the CNS

- **Mechanism of Action:** MTX exerts its effects on cell proliferation by inhibiting the enzyme, Dihydrofolate Reductase (DHFR) necessary for synthesizing purines and thymidylate, leading to a reduction in DNA synthesis and cell proliferation of rapidly dividing cancer cells that have metastasized to CNS.
- **Pharmacokinetics & Pharmacodynamics:** MTX has low permeability across the BBB when administered systemically, making ICV administration a preferred method for achieving effective drug concentrations in the brain.

Stereotaxic Surgery and ICV Injection Technique

Stereotaxic Surgery for CNS Drug Delivery

- A precise and controlled method for delivering drugs directly into specific brain regions, bypassing the BBB.
- Ensures reproducibility and targeted drug administration.

Intracerebroventricular (ICV) Administration:

- Direct ICV injection bypasses the BBB, enabling higher drug concentrations in target brain regions.

Case Study

Objective

To assess the effectiveness of combining ICV administration with stereotaxic surgery for targeted delivery of methotrexate to specific brain regions, improving drug distribution and therapeutic outcomes for CNS lymphoma treatment.

Hypothesis

The combination of ICV administration and stereotaxic surgery will enhance MTX delivery to the brain, improving bioavailability and reducing systemic toxicity.

Materials and Methods

- **Animal Model Selection:** Male Sprague-Dawley (SD) rats (200-250g), prepared according to ethical guidelines.

ICV Injection Procedure

1. **Preparation:**
   - SD rats were prepared in compliance with ethical standards.
   - The surgical area was sterilized using povidone-iodine.
2. **Positioning:**
   - Ear bars were used to stabilize the animals in the stereotaxic device (Figure 2).
   - The mouth was fixed using an anterior mount, and the head position was verified.
3. **Surgical Procedure:**
   - A midline incision was made along the skull.
   - A small burr hole was drilled at the designated coordinates for ICV injection. MTX was injected using a Hamilton syringe into the lateral ventricle.
   - **Dosage:** 1mg/kg body weight of MTX, 0.05 mL/kg dose volume.
   - **Drug Measurement:** Plasma and brain region samples (cerebral cortex, cerebrum, medulla, cerebellum, and hypothalamus) were collected 0.25 hours post-dosing, and MTX concentrations were then measured using Liquid Chromatography-Mass Spectrometry (LC-MS).

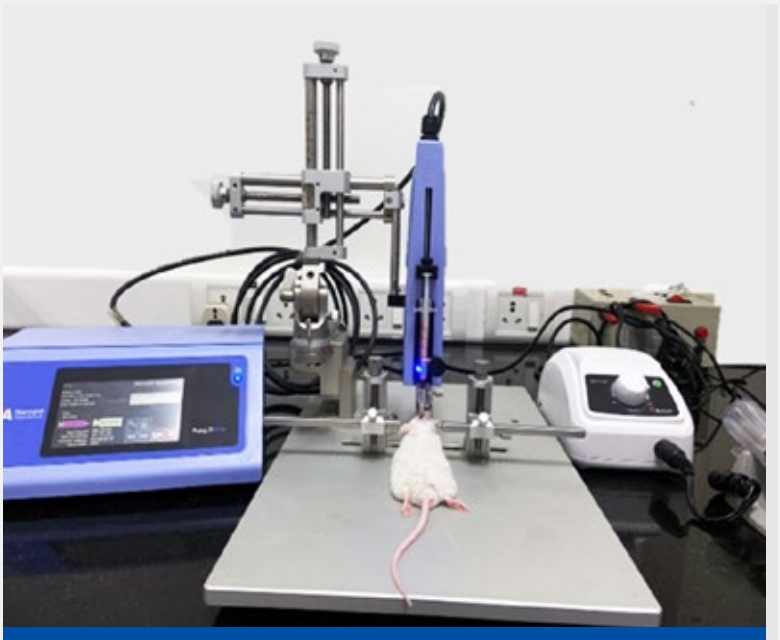

Figure 2: Stereotaxic apparatus.

Results & Discussion

MTX Distribution in Brain Regions

- Varying concentrations were observed across different brain regions (Figure 3).
- Highest concentrations in the cerebral cortex (18897.00 ng/g) and cerebrum (7533.25 ng/g) (Figure 3), with corresponding high tissue/plasma ratios (cerebral cortex: 588, cerebrum: 234), indicated effective drug uptake and retention (Table 1).
- Moderate MTX concentrations in the medulla (2771.83 ng/g), cerebellum (2150.42 ng/g), and hypothalamus (1195.83 ng/g) (Figure 3), with tissue/plasma ratios suggesting balanced drug distribution in all the three brain regions (Table 1).

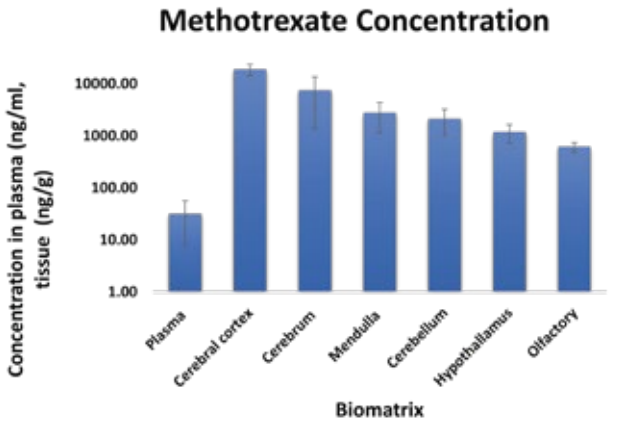

Figure 3: Methotrexate concentration in plasma and brain parenchyma.

MTX Plasma Concentrations

- MTX concentration in plasma was 32.15 ng/mL, confirming minimal systemic exposure with ICV administration.

Drug Distribution

- ICV administration enabled efficient distribution, with higher concentrations near ventricles and decreasing with distance, ensuring broad therapeutic exposure.

Stereotaxic Surgery

- Precise targeting via stereotaxic surgery enhanced MTX delivery to specific brain regions, optimizing therapeutic efficacy.

Table 1: Brain tissue and plasma ratio

Brain tissue/ Plasma in (mL/g)	Ratio
Cerebellum/Plasma	67
Cerebral cortex/Plasma	588
Cerebrum/ Plasma	234
Hypothalamus/Plasma	37
Medulla/Plasma	86
Olfactory/Plasma	19

Conclusion and Future Research Directions

Key Findings

- ICV administration with stereotaxic surgery enhances MTX bioavailability across brain regions with minimal plasma exposure.
- Demonstrates the benefits of leveraging stereotaxic animal models combined with ICV administration for delivering potential low-permeability drugs to the CNS.

Implications for Drug Development

- Findings support optimized CNS-targeted drug delivery strategies.

Limitations & Recommendations

- Need for comparative studies using alternative administration routes.
- Further investigation into long-term pharmacokinetics and potential neurotoxicity.

References

1. Gerstner E, Carson K, Grossman S, Batchelor T. Long-term outcomes in PCNSL patients treated with high-dose methotrexate and deferred radiation. Neurology. 2008;70:401-402.

2. Landeck, N., Mazza, M.C., Duffy, M., Bishop, C., Sortwell, C.E. and Cookson, M.R., 2021. Stereotaxic intracranial delivery of chemicals, proteins, or viral vectors to study Parkinson's disease. Journal of visualized experiments: JoVE, (168), pp.10-3791. doi: 10.3791/62128

3. Perkins, R.S., Davis, A., Campagne, O., Owens, T.S. and Stewart, C.F., 2023. CNS penetration of methotrexate and its metabolite 7-hydroxymethotrexate in mice bearing orthotopic Group 3 medulloblastoma tumors and model-based simulations for children. Drug Metabolism and Pharmacokinetics, 48, p.100471. https://doi.org/10.1016/j.dmpk.2022.100471

4. Pesenti, G., Foppoli, M. & Manca, D. A minimal physiologically based pharmacokinetic model for high-dose methotrexate. Cancer Chemother Pharmacol 88, 595–606 (2021). https://doi.org/10.1007/s00280-021-04305-2

5. Taylor ZV, Khand B, Porgador A, Monsonego A, Eremenko E. An optimized intracerebroventricular injection of CD4+ T cells into mice. STAR Protoc. 2021 Aug 6;2(3):100725. doi: 10.1016/j.xpro.2021.100725. PMID: 34401790; PMCID: PMC8353483.