Oral Gavage Cuprizone Model: A Translational Tool for Acute Demyelination and Early-State MS Drug Development.

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BACKGROUND

Cuprizone (CPZ) intoxication is a well-established non-immune model of central nervous system (CNS) demyelination widely used to investigate the mechanisms underlying multiple sclerosis (MS). Traditionally, CPZ is administered through the diet, but this approach often introduces variability due to inconsistent intake, metabolic differences, and diet-related factors, resulting in poor reproducibility across studies. To overcome these limitations, we established a controlled oral gavage-based CPZ model that ensures precise dosing and uniform exposure, thereby improving reproducibility and experimental reliability. This approach provides a more standardized platform to study demyelination, neuroinflammation, and potential neuroprotective interventions in preclinical MS research. A treatment group receiving dimethyl fumarate (DMF) was included as a therapeutic reference.

METHODS

Animals and experimental design

- Male C57BL/6 mice (7 weeks old) were used to establish an acute cuprizone (CPZ)-induced demyelination model. After one week of acclimatization, mice were randomly assigned into three groups (n=10 per group): (G1) Control, (G2) CPZ + Vehicle, and (G3) CPZ + DMF (15 mg/kg).

Cuprizone and drug administration

- CPZ was administered by oral gavage at a dose of 200 mg/kg twice daily (BID) for 5 weeks to induce demyelination. DMF was co-administered orally (15 mg/kg, BID) in the treatment group during the same period. Vehicles consisted of 0.5% carboxymethyl cellulose (CMC) for CPZ and 0.8% hydroxypropyl methylcellulose (HPMC) for DMF.

Behavioral assessment

- Motor coordination and functional performance were evaluated using the rotarod test. Disease severity was assessed using a composite clinical disease score at regular intervals throughout the study.

Sample collection

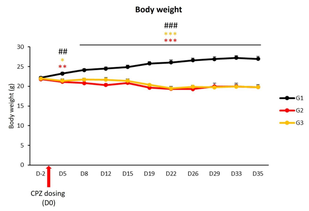
- At the end of the treatment period (Week 13), mice were euthanized for tissue collection. Brain regions including the corpus callosum, hippocampus, and cortex were harvested and processed for immunohistochemical analysis to assess demyelination and neuroinflammatory changes.

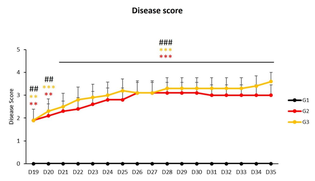
Histological and Immunohistochemical analysis

- Coronal brain sections were subjected to Luxol Fast Blue staining to evaluate demyelination. Immunohistochemistry was performed using antibodies against MBP for myelin integrity, GFAP for astrocytosis, Iba1 for microgliosis, and Olig2 for oligodendrocyte lineage assessment.

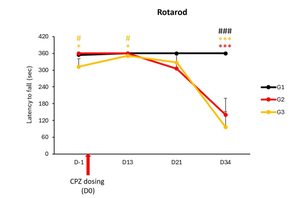
RESULTS

1. Body weight and disease score



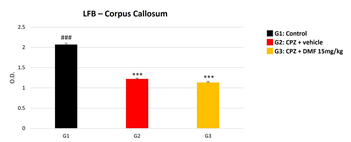


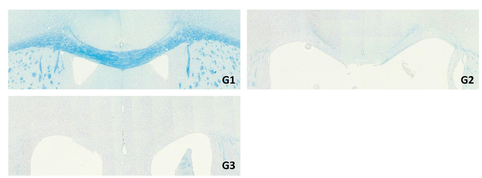
2. Motor coordination and functional performance



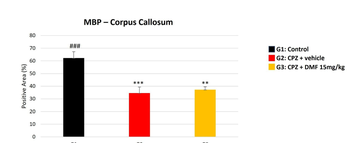
3. Histologial and immunohistochemical analysis

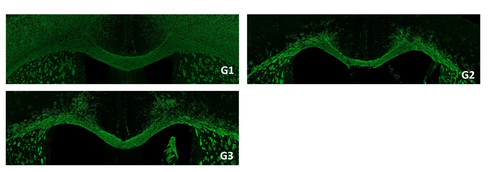
A. Demyelination - LFB

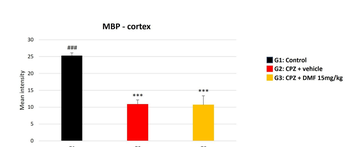


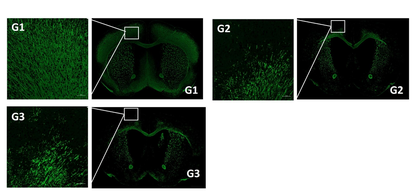


B. Myelin Integrity - MBP

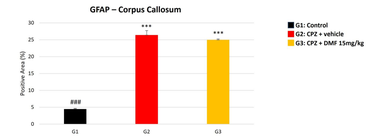


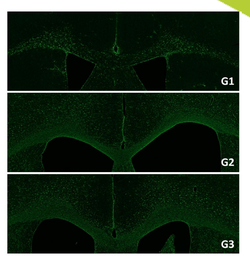




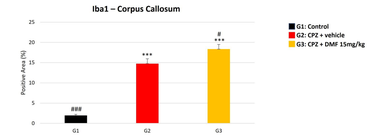


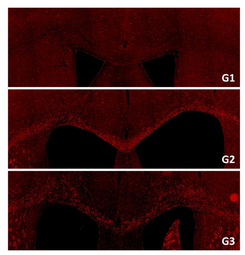
C. Astrocytosis - GFAP



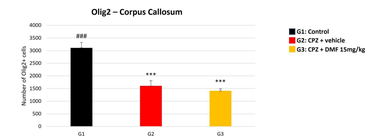


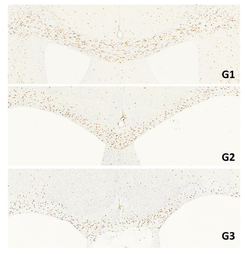
D. Microgliosis – Iba1





E. Oligodendrocyte lineage – Olig2





All values are presented as mean ± standard error (SEM)
All values were analyzed using one-way ANOVA with LSD post-hoc analysis
* : 0.05>p, ** : 0.01>p, *** : 0.001>p ; G1 vs All group. # : 0.05>p, ## : 0.01>p, ###: 0.001>p ; G2 vs All group.

CONCLUSIONS

1. **Oral gavage administration of cuprizone (CPZ) produces robust and reproducible acute demyelination in C57BL/6 mice, potentially minimizing variability associated with diet-based CPZ models.**
2. **CPZ-treated mice exhibit significant functional impairments, reflected by increased disease scores and reduced motor coordination on the rotarod, directly correlating with histological evidence of demyelination.**
3. **This paradigm provides multiple complementary endpoints—including body weight, disease score, LFB staining, and IHC markers (MBP, GFAP, Iba1, Olig2)—offering a reliable and translational platform for early-stage evaluation of remyelinating and neuroprotective therapies.**
4. **DMF has shown mixed efficacy in preclinical MS models, and no therapeutic effect was evident under the current study conditions.**