Evaluation of Longevity of Intracerebroventricle Cannulation (IVC) in Rats and Mice

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Abstract

Purpose: Pharmacokinetic studies and research applications include the direct dosing of compounds to the intracerebroventricle of the brain, which can be achieved via intracerebroventricle cannulation (IVC). IVC patency longevity directly affects study outcomes. In this study, we investigated the patency longevity of the IVC model in rats and mice.

Methods: 22 adult male 225-275 g CD® IGS rats [Crl:CD(SD)] were randomly allocated into 2 groups of 11 each (GROUP1 and GROUP2). 22 adult male 25-28 g CD-1® IGS mice [Crl:CD1(ICR)] were randomly allocated into 2 groups of 11 each (GROUP3 and GROUP4). The animals were anesthetized by intraperitoneal injection of Ketamine (75 mg/kg for rats, 71.5 mg/kg for mice) and Xylazine (6 mg/kg for rats, 14.5 mg/kg for mice). Perioperative analgesia of Burprenorphine (0.02 mg/kg for rats, 0.05 mg/kg for mice) and Carprofen (5.0 mg/kg) were given subcutaneously. Animals were placed in a stereotaxic apparatus and a guide cannula was implanted in the left lateral ventricle of the brain. A layer of cranioplastic powder and liquid was used to affix the cannula to the exposed portion of the skull. Carprofen (5.0 mg/kg) was continued for 2 days postoperatively. IVC patency was checked weekly in GROUP1 and GROUP3 animals and biweekly in GROUP2 and GROUP4 animals for 13 weeks. The IVC was considered patent if artificial CSF (5-6 μL for rats, 2-3 μL for mice) could be injected into the intracerebroventricle without any resistance.

Results: 2 mice in GROUP3 and 1 mouse in GROUP4 failed to recover from anesthesia. The number of animals that entered the study in GROUP1, GROUP2, GROUP3 and GROUP4 was 11, 11, 9 and 10, respectively. All animals remained clinically healthy throughout the study and showed similar weight gains postoperatively. During the 13-week study, 7 of 41 animals were removed at various time points due to the IVC becoming dislodged. In GROUP1, 10 of 11 rats (91%) remained at week 4 and decreased to 8 of 11 (73%) at week 13. In GROUP2, 10 of 11 rats (91%) remained at week 7 and decreased to 8 of 11 (73%) at week 13. In GROUP3, 9 of 9 mice (100%) remained at week 13. In GROUP4, 9 of 10 mice (90%) remained at week 5 and continued until study end. Animals that completed the study were patent through study end at 13 weeks.

Conclusions: Greater than 70% of rats and 90% of mice completed the 13-week study, maintaining IVC patency throughout. The cause of IVC dislodgement was not determined, but this observation highlights that failures unrelated to catheter patency may occur during longer-term studies and animal numbers should be adjusted accordingly.

Introduction

Pharmacokinetic and research studies in rats and mice are most effectively and humanely performed using a chronically implanted catheter/cannula that allows repeated sampling and dosing. Direct dosing of compounds to the brain is achieved the by placing a cannula into the intracerebroventricle or a specific brain target.

Charles River provides intracerebroventricle cannulation (IVC) for scientists for research studies. The longevity of IVC patency directly affects study outcomes. However, the long term patency of IVC has not been studied. In this study, we investigated the patency longevity of the IVC model in rats and mice.

Materials and Methods

Animals

22 adult male 225-275 g CD® IGS rats [Crl:CD(SD)] and 22 adult male 25-28 g CD-1® IGS mice [Crl:CD1(ICR)] produced by Charles River Laboratories were used. They were maintained in polycarbonate cages in a dedicated rodent surgical complex that was kept at 21 ± 2 ºC with a relative humidity of 60 ± 5% and a 12/12 hour light/dark cycle. Commercially produced, sterilized feed, bedding and water were provided *ad libitum*. All conditions of animal preparation and use were in accordance with recommendations set forth in the Guide for the Care and Use of Laboratory Animals and approved by the institutional IACUC. The animals were of a VAF/Plus® health status.

Surgical Procedures

The animals were anesthetized with Ketamine (75 mg/kg for rats, 71.5 mg/kg for mice) and Xylazine (6 mg/kg for rats, 14.5 mg/kg for mice) administered intraperitoneally. Fur was removed from the dorsal cranium and the skin prepared using chlorhexidine and alcohol. Animals were placed in a stereotaxic apparatus. Bregma and lambda were identified and guide holes were drilled, through which anchoring screws (4 for rats, 3 for mice) were mounted onto the skull. A guide cannula was loaded onto the holder of the stereotaxic apparatus, the tip of the cannula was pointed directly over the bregma and the zero coordinates recorded. Based on the coordinates (see below), a hole was drilled through the skull and the guide cannula was inserted via the hole to reach the left intralateral ventricle of the brain. A layer of cranioplastic powder was applied to affix the cannula and cover the exposed portion of the skull, then a small amount of cranioplastic liquid was applied to the powder. Finally, a dummy cannula was inserted into the guide cannula.

Patency Test Procedure

IVC patency was tested weekly in GROUP1 and GROUP3 animals and every other week in GROUP2 and GROUP4 animals for 13 weeks using brain cannulation instructions (Ref). To check patency, the dummy cannula was removed, the internal injector cannula was completely inserted into the guide cannula and artificial CSF (5-6 μL for rats, 2-3 μL for mice) was slowly injected. The IVC was considered patent if artificial CSF could be injected into the intracerebroventricle without any resistance.

Stereotaxic Coordinates for IVC

	Rats	Mice
Incisor Bar - IB (mm)	-3.3	Individually adjusted
Anterior Posterior - AP (mm)	-0.8	-0.22
Medial Lateral - ML (mm)	-1.5	-1.0
Dorsal Ventral - DV (mm)	-4.8	-2.3

Cannula for IVC

guide cannula *dummy cannula* *internal cannula*

Experimental Design

The 22 rats were randomly allocated into 2 groups consisting of 11 rats each for GROUP1, GROUP2.
The 22 mice were randomly allocated into 2 groups consisting of 11 mice each for GROUP3, GROUP4.

Table 1: Experimental Design (P indicates patency test)

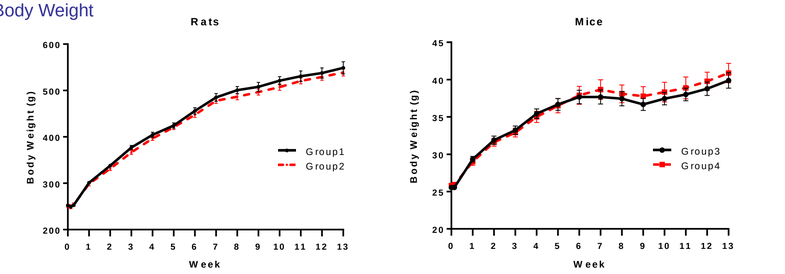
Species	Time Frame (Week)	1	2	3	4	5	6	7	8	9	10	11	12	13
Rats	GROUP1 (weekly)	P	P	P	P	P	P	P	P	P	P	P	P	P
	GROUP2 (biweekly)	P		P		P		P		P		P		P
Mice	GROUP3 (weekly)	P	P	P	P	P	P	P	P	P	P	P	P	P
	GROUP4 (biweekly)	P		P		P		P		P		P		P

Results

General Observations

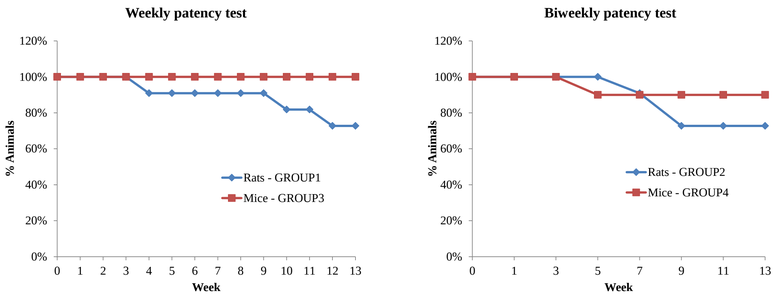
- 2 mice in GROUP3 and 1 mouse in GROUP4 failed to recover from anesthesia.
- All animals remained clinically healthy throughout the study.
- All of the animals showed similar weight gains after surgery (i.e., no difference between treatment groups, p > 0.05).
- During the study, animals were removed from the study due to dislodgment of the IVC.

Body Weight



IVC Patency

The number of animals that entered the study in GROUP1, GROUP2, GROUP3 and GROUP4 was 11, 11, 9 and 10, respectively.



- In GROUP1, 10 of 11 rats (91%) remained at week 4 and decreased to 8 of 11 (73%) at week 13.
- In GROUP2, 10 of 11 rats (91%) remained at week 7 and decreased to 8 of 11 (73%) at week 13.
- In GROUP3, 9 of 9 mice (100%) remained at week 13.
- In GROUP4, 9 of 10 mice (90%) remained at week 5 and continued until study end.

All of the animals that completed the study were patent through study end at 13 weeks.

Conclusion

Greater than 70% rats and 90% mice completed the 13-week study and maintained IVC patency throughout the study. The cause of IVC dislodgement was not determined, but this observation highlights that failures unrelated to catheter patency may occur during longer-term studies and animal numbers should be adjusted accordingly when planning such studies.

Ref : http://www.criver.com/files/pdfs/surgery/ss_r_brain_cannulation_handling.aspx