Intravcerebroentricularly Injected Dextran Is Rapidly Cleared Into Serum Through Blood Brain Barrier In Normal Rats

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Introduction

Hydrocephalus is a central nervous system (CNS) disorder that manifests as an abnormal accumulation of cerebrospinal fluid (CSF) in the cerebral ventricles. Hydrocephalus can be experimentally induced by producing a sustained increase in CSF osmolarity[1,2,3]. This implies that macromolecular content in the CSF is critical in determining the water content. We have previously shown that intraventricularly injected dextran is rapidly concentrated in the perivascular space surrounding microvessels throughout the brain. To explore how the brain clears the macromolecules in CSF for maintaining its osmotic gradient, we investigated the kinetics of distribution and clearance of fluorescently-labeled 10KD dextran (FITC-D) from CSF in the normal rat brain.

Method

Sprague-Dawley rats were used in this study. All procedures and housing were carried out in accordance with the experimental protocol was approved by IACUC. Rats were anesthetized with Ketamine/Xylazine. Head was mounted in an animal stereotactic instrument. Using aseptic techniques, all rats received one time CSF injection at 1µl/s of total 15µl through cisterna magna (CM) with either FITC-D solution (1ug/ul) in group I (n=7) or sterile saline (control) in group II (n=7). Blood and urine samples were collected prior to injection, and every 30 minutes for 4 hours after injection. Both serum and urine samples were examined by spectrophotometric analysis for tracing the FITC-D particles.

Statistical analysis: Statistical analysis was done by using nonparametric tests (Wilcoxon two sample tests and signed rank tests). P-values between 0.01 and 0.05 were interpreted as significant whereas p-values less than 0.01 were considered highly significant.

Result

The p-value for the comparisons of FITC-D vs control is 0.046 for urine and serum at both 30 and 60 minutes. For comparisons within the FITC-D injecting group, the p-value for pre vs 30 and 60 minutes was 0.015 for both urine and serum. For urine, 30 vs 60minutes the p-value was 0.031. For serum,30 vs 60minutes the p-value was 0.234. FITC-D concentrations in blood serum reached a peak between 30 and 60 minutes postinjection, whereas peak concentration in urine occurred at 60 minutes postinjection. By 90 minutes postinjection, FITC-D concentrations had declined from their peak values but remained significantly elevated in both blood serum and urine for at least four hours post-injection (F-1). CM injected FITC-D is present in perivascular space throughout the brain including the neocortex in a series of coronal sections (F-2). Distribution of FITC-D in coronal sections of the rat brain at 1- 2- and 4-hours after CM injection show FITC-D distributed within perivascular space (F-3). Vesicular uptake of FITC-D by perivascular glia cells (F-4).

Conclusions

Intraventricular dextran is cleared rapidly through blood brain barrier in normal rat to maintain its normal osmotic gradient. Dysregulation of CSF osmolarity resulting from a sustained influx of macromolecules in to CSF and/or compromise of clearance mechanisms may be the underlying cause of hydrocephalus. These processes may offer novel therapeutic targets for the treatment of clinical hydrocephalus.

Reference

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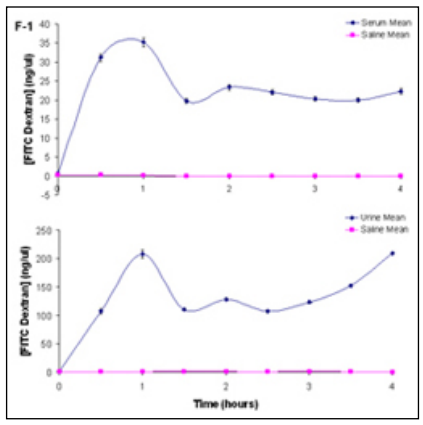
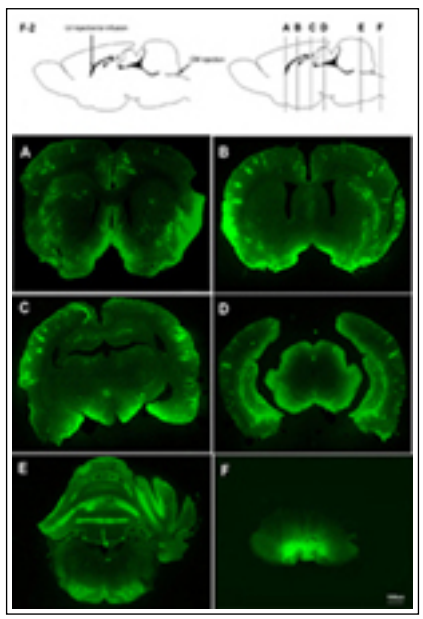
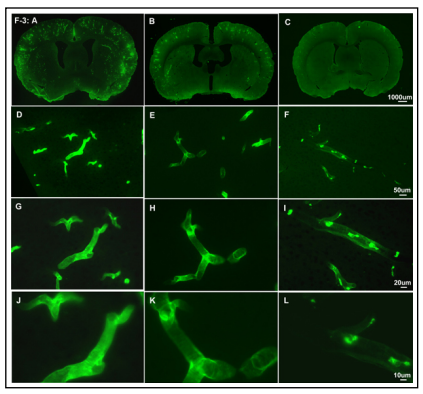


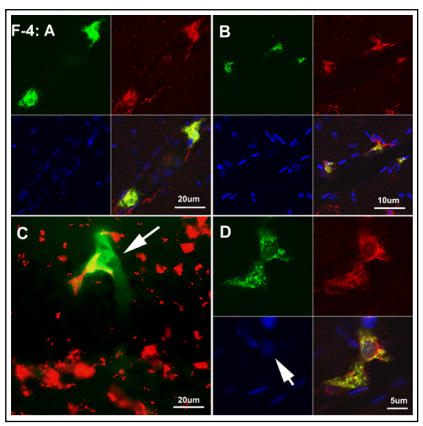
F-1: Concentration of FITC-D in serum (A) and urine (B). (**, P < 0.05)



F2: A series of coronal sections from A to F for Group I.



F3: Distribution of FITC-D in coronal sections of the rat brain at 1h (1st column)- 2h (2nd column)- and 4h(3rd column) after CM injection. FITC-D is distributed throughout the brain and is concentrated within perivascular space. FITC-D is noticeably reduced throughout the brain as time passed.



F4: FITC-D is green particles and DAPI is blue for staining nuclei. FITC-D is observed within perivascular astrocytes labeled with GFAP-Cy3 (A: red) and microglia with Iba-1-Cy3 (B: red). Dextran is observed within perivascular space surrounding a capillary, perhaps reflecting local exocytosis (arrow) of FITC-D from the adjacent perivascular microglia (C). FITC-D is observed undergoing vesicular sequestration/transport within perivascular microglial cell (D).