Comparative Medicine
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Vol 68, No 3
June 2018
Pages 227–232

Original Research

Hydrocephalus after Intrathecal Administration of Dextran to Rhesus Macaques (*Macaca mulatta*)

Jason P Dufour,[1,*] Kasi E Russell-Lodrigue,[1] Lara Doyle-Meyers,[1] Kathrine P Falkenstein,[1] Robert V Blair,[2] Elizabeth S Didier,[3] Nadia Slisarenko,[4] Kenneth C Williams,[5] and Marcelo J Kuroda[4]

Dextrans have been used extensively as medical therapies and labeling agents in biomedical research to investigate the blood-brain barrier and CSF flow and absorption. Adverse effects from dextrans include anaphylactic reaction and dilation of the cerebral ventricles due to administration into the subarachnoid space. This retrospective study describes 51 rhesus macaques (*Macaca mulatta*) that received dextran intrathecally. The purpose of intrathecal administration was to enable detection of long-lived, dextran-labeled macrophages and to study monocyte–macrophage turnover in the CNS of SIV- or SHIV- infected and uninfected animals by using immunofluorescence. Of the 51 dextran-treated macaques, 8 that received dextran diluted in saline developed hydrocephalus; 6 of these 8 animals exhibited neurologic signs. In contrast, none of the macaques that received intrathecal dextran diluted in PBS developed hydrocephalus. These data suggest the use of saline diluent and the duration of dextran exposure as potential factors contributing to hydrocephalus after intrathecal dextran in rhesus macaques.

Abbreviation: SPION, superparamagnetic iron oxide nanoparticles

DOI: 10.30802/AALAS-CM-17-000096

Dextran has been used extensively in the treatment of clinical conditions and as a labeling substance in research settings. Dextrans are complex polysaccharides composed of glucose molecules and were first administered in the clinical setting in 1947 as a plasma colloid volume expander.[27,32] Dextran 40 has been successfully administered epidurally to patients to treat persistent postdural-puncture headaches resistant to other treatments.[1]

Numerous dextran conjugates have been studied with regard to the CNS and serve as valuable markers for evaluating the integrity of the blood–brain barrier.[32] For example, studies examining the blood–brain barrier in adult short-tailed opossums (*Monodephis domestica*) demonstrated that intraperitoneally administered biotin–dextran conjugate was restricted from movement across tight junctions.[6] Fluorescently conjugated dextrans have been injected directly into the subarachnoid space in adult rats to examine communication of spinal subarachnoid space with dorsal root ganglia.[11] In addition, fluorescent dextran has been used in a cat model to demonstrate that the deep lymphatic system plays an integral role in clearing large-molecular-weight compounds and fluid from the subarachnoid space.[2] Radiolabeled dextran has been administered into the subdural space in rhesus macaques to examine infusate distribution and fluid movement in convection-enhanced drug distribution.[10] Furthermore, previous studies at our facility in which conjugated dextran amines were administered into the cerebellomedullary cistern of NHP have enabled the evaluation of macrophage recruitment into the CNS during AIDS pathogenesis.[25]

The administration of dextrans is not risk-free, and several complications have been noted. Reports of anaphylactic reactions to clinical intravenous dextran therapy have been recognized since the 1960s, and compounds to manage the risk of severe dextran-induced anaphylactic reaction were introduced in the 1980s.[21,35] Parenteral administration of very-high-molecular-weight (458-kDa) dextrans was acutely toxic in mice and rats, producing emboli, hemorrhage, and infarction across a wide dose range. Toxicity was reduced after the administration of similar doses of compounds that were lower in molecular weight (47 and 7.5 kDa) , with the LD_{50} of the smallest compound similar to commercial heparin.[34] In rabbits, dose-dependent toxicity in the form of cachexia and eventual osteoporosis occurred after chronic intravenous administration of low molecular weight (3 kDa) dextran solutions.[8]

Several studies demonstrate a link between administration of dextrans directly into the CSF and the development of hydrocephalus.[18,19] Hydrocephalus is defined as an abnormal condition in which CSF accumulates in the ventricles of the brain.[4,7,28] This accumulation can be due to the blockage of normal outflow of fluid from the brain or the failure of fluid to be absorbed into the bloodstream quickly enough.[17] As a result, pressure increases in the brain, and neurologic symptoms including headache, loss of muscular coordination, and ophthalmologic abnormalities can develop.[3,13,23,29,30]

This retrospective report investigates negative sequelae after the administration of dextran intrathecally in rhesus macaques and evaluates possible dose- or diluent-associated effects. The animals described here were assigned to research protocols investigating the role of monocytes and macrophages in the pathogenesis of simian AIDS, the recruitment of macrophages

Received: 01 Jun 2017. Revision requested: 11 Jul 2017. Accepted: 18 Dec 2017.
Divisions of [1]Veterinary Medicine, [2]Comparative Pathology, [3]Microbiology, and [4]Immunology, Tulane National Primate Research Center, Covington, Louisiana; and [5]Department of Biology, Boston College, Chestnut Hill, Massachusetts
[*]Corresponding author. Email: jdufour@tulane.edu

into the CNS, and associations between aging and inflammation. Dextran has been used successfully as a labeling agent to evaluate the retention compared with turnover of macrophages in the CNS.[25] Here we report the observation that hydrocephalus was present only in NHP that received dextran diluted into saline for intrathecal administration, and we discuss possible mechanisms for this outcome.

Materials and Methods

Animals. Studies involved in this retrospective analysis were conducted at the Tulane National Primate Research Center (Covington, LA), which is fully AAALAC-accredited. All procedures were IACUC-approved and were performed in accordance with the *Guide for the Care and Use of Laboratory Animals*.[9] The animal housing rooms were maintained on a 12:12-h light:dark cycle, with a relative humidity of 30% to 70% and a temperature of 64 to 84 °F (18 to 29 °C). All animals were fed a standard, commercially formulated NHP diet with fruit offered at least 3 times weekly, as part of the enrichment program. The facility's records database was examined for all animals that received dextran intrathecally. All 51 macaques identified were included in this retrospective review.

Dextran administration. Animals were anesthetized with tiletamine hydrochloride and zolazepan hydrochloride (5 to 8 mg/kg IM; Tiletamine–zolazepam, Zoetis, Kalamazoo, MI) and placed in either ventral recumbency, with the rear limbs positioned in a fully flexed, 'frog leg' position, or in lateral recumbency. The head was stabilized and ventroflexed, and a 22-gauge butterfly catheter was used for the administration of dextran. The catheter needle was inserted into the cisterna magna until a flow of CSF was initiated. An equal volume (1 mL) of CSF was removed prior to the administration of dextran. Several formulations of 10,000MW dextran were administered (D1820, Fluorescein; D1860, Amino; D22910, Alexa Fluor 488; D22914, Alexa Fluor 647; D1817, fluoro-ruby; D1956, biotin; Molecular Probes, Eugene, OR), after which the macaques were monitored for complications. To provide analgesia, buprenorphine hydrochloride (0.03 mg/kg IM twice daily; Buprenex, Reckitt and Colman, Slough, United Kingdom) was administered for 3 d or sustained-release buprenorphine hydrochloride (0.2 mg/kg SC; Buprenorphine SR, ZooPharm, Laramie, WY) was administered once prior to the procedure.

Pathology. Hydrocephalus was evaluated by making serial 4-mm coronal sections of the cerebrum to the level of the pons. Hydrocephalus was defined and diagnosed in any animal that exhibited dilation of the lateral ventricles on coronal sections, as determined by a board-certified veterinary pathologist.

Statistics. For descriptive statistics, data were analyzed by using Prism (version 5, GraphPad Software, La Jolla, CA). When possible, odds ratios and 95% confidence intervals were calculated to identify potentially significant risk factors. Fisher exact tests were performed to determine statistical significance, with a P value of less than 0.05 used to define significance.

Results

Demographics of study cohort. We examined the clinical records of the 51 rhesus macaques that received dextran intrathecally between 2004 and 2016. Overall, the 51 subjects ranged from 1.9 to 23.4 y of age and weighed between 2.9 and 14.5 kg at the time of dextran administration. Specifically, the 33 males had a median age of 5.3 y (range, 1.9 to 10.1 y) and weighed 9.2 kg (2.9 to 14.5 kg); the 18 females had a median age of 8.2 y (5.0 to 23.4 y) and weighed 6.5 kg (4.9 to 9.7 kg).

Dextran administration. For the entire study population, the total dose of dextran administered ranged from 2.25 to 50 mg (0.23 to 17.54 mg/kg). Dextran was prepared for injection by dilution with either 0.9% normal saline (n = 24) or PBS (n = 27). The time between dextran administration and necropsy ranged from 0 to 672 d, with a median of 28 d.

Additional research procedures and considerations. The NHP were enrolled in a variety of research studies at the time of dextran administration. Of the 51 animals in this retrospective review, the majority (n = 43, 84.3%) had been experimentally exposed to SIV or SHIV, and 36 of these 43 animals (83.7%) received intrathecal dextran after virus inoculation. The macaques that received dextran before inoculation, the median time between dextran exposure and virus inoculation was 91 d (310 to 23 d before inoculation). For those that were exposed to dextran after SIV or SHIV inoculation, the median time between dextran administration and virus inoculation was 66.5 d (12 to 609 d after inoculation). In addition, 16 of the 43 (37.2%) SIV- or SHIV-inoculated animals had undergone depletion of $CD8^+$ T cells prior to necropsy, and some (13.7%) received an additional macrophage-labeling agent, superparamagnetic iron oxide nanoparticles (SPION) intrathecally.

Clinical and pathologic outcomes. Antemortem neurologic abnormalities were reported in 7 (13.7%) macaques after intrathecal dextran administration. These abnormalities included but were not limited to ataxia, head tilt, intention tremors, seizures, proprioceptive defects, paresis, visual impairment, negative pupillary light response, and dilated pupils. Clinical signs developed between 20 and 275 d (median, 86 d) after dextran administration. The time between the development of clinical signs and necropsy ranged from 0 to 235 d (median, 17 d). Hydrocephalus was found in 8 (15.7%) of the 51 subjects at the time of necropsy, of which 6 had exhibited clinical abnormalities previously (Figure 1).

Animals with hydrocephalus. All 8 macaques exhibiting hydrocephalus received dextran dissolved in 0.9% normal saline, whereas none of the animals given dextran in PBS developed hydrocephalus; diluent was identified as a significant risk factor (odds ratio, 28.33; 95% CI, 1.53 to 524.00; Fisher exact test, P = 0.0012; Table 1). Of the 8 hydrocephalic animals, the total dose of dextran administered was 50 mg (4.72 to 17.54 mg/kg) in 6, 23.75 mg (3.13 mg/kg) in 1, and 15.75 mg (4.20 mg/kg) in the remaining macaque. In addition, the nonhydrocephalic animal that received the highest saline-diluted dose (15.63 mg/kg) had no history of virus administration, but excessive CSF was present on gross necropsy at 28 d after dextran exposure. These data suggest that dose may be an important factor independent of diluent; however, when we stratified data to examine the potential association between the development of hydrocephalus in macaques receiving less than 5 mg/kg compared with 5 mg/kg or greater, no significant association was revealed (odds ratio, 0.2897; 95% CI, 0.0604 to 1.39; Fisher exact test, P = 0.1309). All 8 animals with hydrocephalus were male, and 7 were younger than 5 y at the time of dextran administration. We do not have sufficient data in the current retrospective review to determine whether sex is an independent risk factor regardless of age or whether there is an interaction between sex and age. Age (< 5 y, ≥ 5 y) was significantly associated with hydrocephalus (Fisher exact test, $P < 0.0001$); however, normal differences in the brains of juvenile compared with adult macaques might influence this statistical association.[16,31] Furthermore, 5 of the 8 hydrocephalic macaques had also been inoculated with SIV or SHIV. None of the 8 animals with hydrocephalus received SPIONs, but 2 had a history of $CD8^+$ lymphocyte depletion. Hydrocephalus was

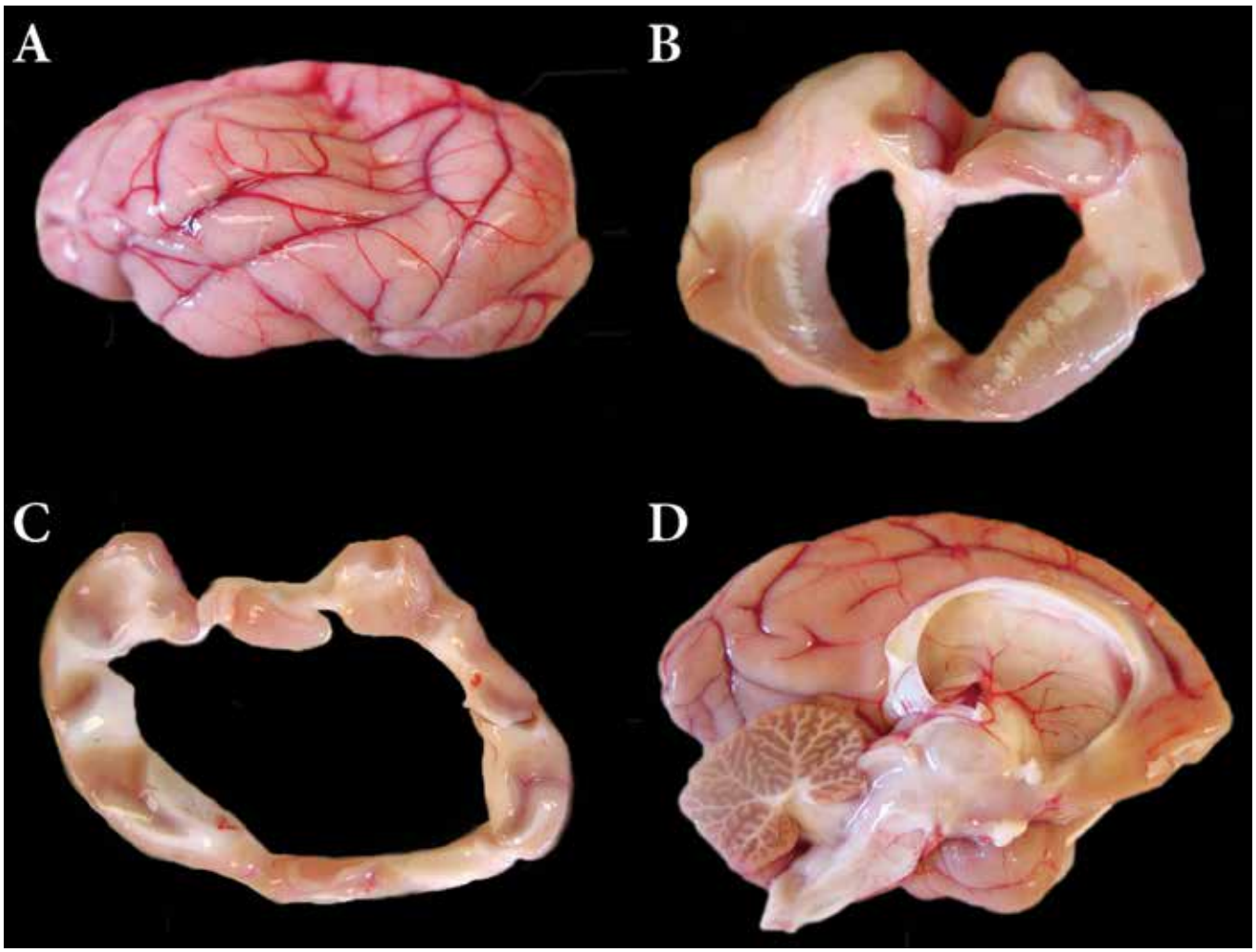

Figure 1. Hydrocephalus discovered at necropsy. (A) Depression of the cerebral cortex, (B) Dilation of the lateral ventricles, approximately 25 mm cranial to interaural, (C) Loss of brain tissue due to compression by excess CSF, approximately at interaural, (D) Enlarged lateral ventricle.

not significantly associated with SIV exposure (Fisher exact test, $P = 0.0993$), $CD8^+$ depletion (Fisher exact test, $P = 0.6936$), or the administration of SPION (Fisher exact test, $P = 0.5787$).

Discussion

Dextran administration reportedly produces hydrocephalus due to a hyperosmotic state in the cerebral ventricles, which exerts osmotic force on blood and possibly the interstitial fluid of the brain, or due to an increase in water influx generated from CSF secretion from the choroid plexus in an attempt to equilibrate any osmotic gradients.[19] In addition, dextran can increase ventricular volumes when administered at isoosmotic concentrations.[18] Therefore, the purpose of this retrospective review was to relate potential factors associated with hydrocephalus observed during studies on SIV–SHIV and macrophage biology in rhesus macaques in which dextran was injected into the subarachnoid space. One of our key findings is that only macaques that received dextran that had been diluted in saline—and not PBS—developed hydrocephalus. On comparing the 2 diluents, we found that the osmolarity of PBS and saline are similar (280 to 315 mOsmol/L and 310 mOsmol/L, respectively), whereas the pH of saline is 5.6, but that of PBS is more physiologic (pH 7.4).

Compared with nonhydrocephalic macaques, those that had hydrocephalus received higher doses of dextran in saline. The median dose in saline was 2.83 times higher than the median dose in PBS (7.38 mg/kg [range, 3.13 to 17.54 mg/kg] compared with 2.61 mg/kg [0.23 to 4.76 mg/kg], respectively); however, these doses did not differ significantly. In addition, we found that the follow-up time was longer for animals that developed hydrocephalus than those that did not. Specifically, the median time from dextran administration to necropsy for animals that exhibited hydrocephalus with saline as the diluent was 111 d compared with a median time from dextran administration in animals that didn't display hydrocephalus of 33 d for the saline diluent group and 8 d for the PBS diluent group. Whether more animals would have developed hydrocephalus had the experimental endpoints occurred longer after dextran administration is unknown. Dextran doses for 3 of the animals in the saline diluent group that demonstrated hydrocephalus (3.125, 4.2, and 4.72 mg/kg) were within the dose range of the animals in the PBS diluent group, in which none of the animals demonstrated hydrocephalus (Figure 2).

An acknowledged theory of CSF production and absorption is that these are continuous processes and that CSF is reabsorbed back into circulation through the arachnoid villi at the superior sagittal sinus.[15,19,22,24] This continuous flow produces the 'sink effect,' which is designed to reduce the steady-state concentration of molecules penetrating the CSF.[22,33] The brain controls its internal environment containing CSF by using the blood–brain

Table 1. Summary data stratified by diluent and presence of hydrocephalus

		Hydrocephalus	No hydrocephalus
	Diluent	*n* = 8	*n* = 43
Dose (mg/kg)	Saline	8.4 (3.1–17.5)	7.3 (3.5–15.6)
	PBS	—	2.6 (0.3–4.8)
Age (y)	Saline	3.6 (1.9–6.3)	6.6 (2.0–23.4)
	PBS	—	6.1 (4.6–12.8)
Time (d) between dextran and necropsy	Saline	111 (23–528)	33 (0–672)
	PBS	—	8 (0–121)
Clinical signs			
Yes	Saline	6 (75%)	1 (2.3%)
	PBS	—	—
No	Saline	2 (25%)	15 (35%)
	PBS	—	27 (63%)
Sex			
Male	Saline	8 (100%)	4 (9%)
	PBS	—	21 (49%)
Female	Saline	—	12 (28%)
	PBS	—	6 (14%)
Dextran to SIV exposure			
No SIV Exposure	Saline	3 (38%)	1 (2%)
	PBS	—	4 (9%)
Dextran prior to SIV exposure	Saline	4 (50%)	3 (7%)
	PBS	—	—
Dextran after SIV exposure	Saline	1 (13%)	12 (28%)
	PBS	—	23 (53%)
CD8 depletion?			
Yes	Saline	2 (25%)	—
	PBS	—	17 (40%)
No	Saline	6 (75%)	16 (37%)
	PBS	—	10 (23%)
SPION			
Yes	Saline	—	—
	PBS	—	7 (16%)
No	Saline	8 (100%)	16 (37%)
	PBS	—	20 (47%)

Data are presented as the median and the range of observed values or percentage of subjects in a particular group with a given attribute.

barrier. The blood–brain barrier is composed of continuous tight junctions of the endothelial cells lining the cerebral blood vessels. These tight junctions seal off intercellular spaces to all but very small molecules.[33]

An intriguing fact was the time of onset of neurologic deficits after dextran administration. Intraventricular injections of FITC–dextran (10 KDa) in rats produced significant increases in ventricular volumes at the 30-min time point,[18] proving that increases in CSF osmolarity can lead to a hydrocephalic state. Particularly interesting in the rat study is that the ventricular volumes returned to preinjection volumes at the 24-h time point, thus suggesting that 10-kDa dextran is sufficiently cleared within 24 h.[18] Thereafter the animals included in that study appeared bright, alert and responsive, and maintained weight and hydration.[18] In another study, rats that had received kaolin (aluminum silicate) injections into the subarachnoid space demonstrated rapid asymptomatic progressive enlargement of the ventricles during the initial 2 wk after injection and then a more delayed symptomatic slow ventricular enlargement until 2 mo afterward.[12] In addition, kaolin administration into the subdural space in rats led to only minimal cognitive deficits, even in animals with ventricular enlargement, until late in the experiments, when animals were necropsied at 9 mo after administration.[5] The macaques we report here exhibited clinical signs after a median of 86 d after dextran administration. Although this value includes a single outlier that didn't develop neurologic signs until 275 d after dextran administration, the earliest that neurologic deficits were apparent was 20 d afterward. The results of the 2 kaolin studies described earlier regarding hydrocephalus-associated neurologic signs better align with the onset of clinical signs in the macaques we report here.

The rat study that reported the resolution of increased ventricular volumes at 24 h after intraventricular injection in rats prompts the question of why this resolution does not occur in

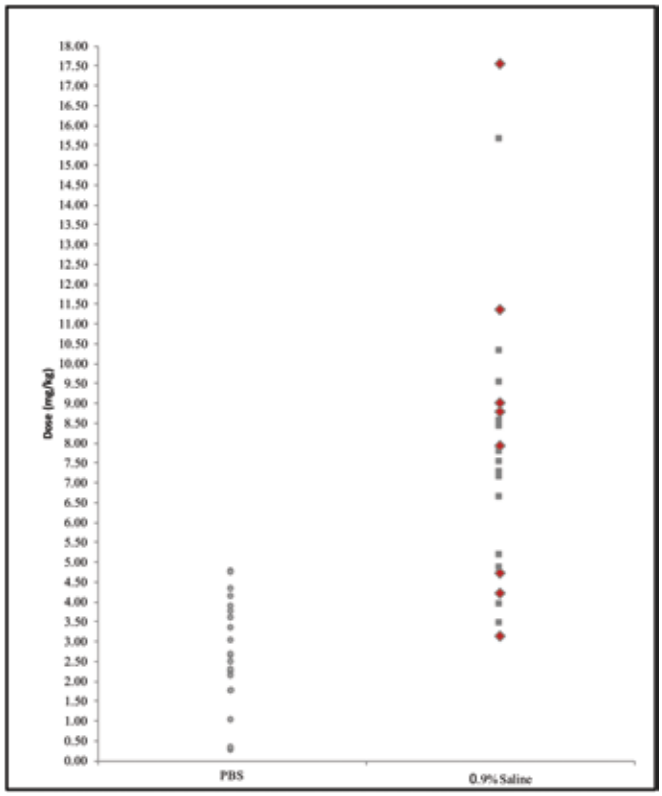

Figure 2. Relationship between dose and diluent of dextran that was administered intrathecally and the development of hydrocephalus. Red diamonds indicate animals with hydrocephalus.

all cases of hydrocephalus that are due to a hyperosmotic state.[18] This outcome might be explained by inflammation caused by large-molecular–weight substances. Kaolin, which we suspect to be much more inflammatory than dextran, induces hydrocephalus and severe inflammation in the CNS, leading to fibrosis which may obstruct or otherwise reduce CSF absorption, thereby leading to hydrocephalus. In addition, inflammation causing scarring and fibrosis of the subarachnoid space has been described as a cause of nonobstructive or communicating hydrocephalus. Nonobstructive or communicating hydrocephalus refers to a condition in which there is no visible obstruction of the flow of CSF from the ventricles to the subarachnoid space.[26] In models of communicating or nonobstructive hydrocephalus, dilation of all portions of the ventricular system is exhibited with enlargement of the lateral ventricles proportionately greater.[26] Increases in CSF outflow resistance increase lateral ventricular pressure, causing enlargement of the ventricles, thus supporting other work demonstrating that inflammation due to foreign substances delivered into the CSF could cause fibrosis of arachnoid villi, subsequently impairing CSF absorption, thereby leading to communicating or nonobstructive hydrocephalus.[20,26] Furthermore, changes in the reabsorption of CSF into the sagittal sinus at the arachnoid villi is associated with hydrocephalus.[20]

From the earlier-described reports involving the administration of a foreign, irritative substance such as kaolin and resulting inflammation and fibrosis, it is plausible that a large-molecular-weight dextran might induce inflammation that minimizes or inhibits the absorption of CSF. Histologic analyses were performed on 50 of the 51 animals examined, and 16 demonstrated histologic abnormalities associated with the meninges. In addition, 15 of these 16 macaques showed inflammation of the meninges, and one animal demonstrated edema of the meninges and dura. These NHP were assigned to infectious disease research, and 14 of the 16 macaques with histologic abnormalities were infected with either SIV or SHIV, some of which were infected with neurotropic strains of SIV. In addition, 6 of the 16 animals were treated with $CD8^+$ lymphocyte-depleting antibodies during early SIV infection that would have accelerated onset of AIDS and SIV encephalitis. Although it is impossible to associate the meningeal inflammation specifically to either the experimental infections or dextran, it is also impossible to exclude dextran as a cause of the meningeal inflammation in some of these animals. Furthermore, meningeal inflammation caused by the infectious disease might have participated in reducing CSF absorption.

The hydrocephalic state we describe here likely was multifactorial. The administration of large molecules, such as dextran, can induce hydrocephalus due to hyperosmolar solutions.[14,19] In addition, infusion of isoosmolar dextran caused increases in ventricular volume, possibly due to the saturation of clearance mechanisms that regulate CSF osmolarity.[18] Although the osmolarity of the dextran solutions administered to the current animals were not evaluated at the time of administration, all solutions had the same concentrations, for the purpose of pH and osmolarity testing, and they were essentially isotonic to normal CSF (287 to 296 mOsm/L). Although the presence of hydrocephalus and the dose administered were not significantly correlated, we determined that macaques with hydrocephalus received high doses of dextran. Therefore, the high doses of dextran might have led to the saturation of clearance mechanisms combined with inflammation from a large molecular-weight compound.

There are limitations to this study, as is the case with most retrospective reviews. The study lacked a neuroimaging component, which would have provided valuable insight regarding the onset of the hydrocephalic state and its persistence; however, dextran had previously been administered intrathecally at a total dose of 25 mg to 21 NHP at our facility with no adverse clinical signs noted. These 21 animals had been maintained on study from a few hours to 4 mo after dextran administration and exhibited no abnormalities on gross necropsy related to the use of dextran, including hydrocephalus. In addition, 9 of these 21 animals had received multiple intrathecal injections of various dextran conjugates. As stated earlier regarding the animals that displayed clinical signs and hydrocephalus, the median time from dextran administration to the onset of clinical signs was 86 d, and the median time from dextran administration to necropsy was 106 d, well within the 4-mo range of the initial study. At that point, there was no indication to include a neuroimaging component to the subsequent study protocols.

After hydrocephalus occurred in macaques intrathecally injected with 50 mg of dextran in saline, we interrupted the studies to evaluate this particular arm of the research protocol. An initial controlled pilot study examined the effect of administering dextran at 1/10 the published dose (reduced to a maximal total dose of 2.5 mg) and changing the diluent to PBS.[25] This modification proved to be safe and effective in labeling macrophages. At the time of submitting the current report, we have performed dextran administration in 8 animals at the maximum dose of 2.5 mg diluted in PBS via intrathecal routes and have observed no evidence of hydrocephalus.

Valid questions remain unanswered regarding the cause of the hydrocephalus that we have described in this retrospective case review. We determined that diluent was a significant risk factor in the development of hydrocephalus, and it appears that

dose may be an important factor independent of diluent regarding dextran administered intrathecally and the formation of hydrocephalus. Results from the most recent cohort of animals that received a maximum dose of 2.5 mg dextran diluted in PBS and administered intrathecally appeared to be safe for avoiding the development of hydrocephalus; careful evaluations will continue.

Acknowledgments

We gratefully acknowledge funding from the NIH to TNPRC (grant no. OD011104), MJK (MH107333, AI097059), and KW (R01NS40237).

References

1. **Aldrete JA.** 1994. Persistent postdural–puncture headache treated with epidural infusion of dextran. Headache **34:**265–267. https://doi.org/10.1111/j.1526-4610.1994.hed3405265.x.
2. **Bradbury MWB, Cole DF.** 1980. The role of the lymphatic system in drainage of cerebrospinal fluid and aqueous humour. J Physiol **299:**353–365. https://doi.org/10.1113/jphysiol.1980.sp013129.
3. **Chawla JC, Woodward J.** 1972. Motor disorder in 'normal pressure' hydrocephalus. Br Med J **1:**485–486. https://doi.org/10.1136/bmj.1.5798.485.
4. **Del Bigio MR, Slobodian I, Schellenberg AE, Buist RJ, Kemp-Buors TL.** 2011. Magnetic resonance imaging indicators of blood–brain barrier and brain water changes in young rats with kaolin-induced hydrocephalus. Fluids Barriers CNS **8:**1–13. https://doi.org/10.1186/2045-8118-8-22.
5. **Del Bigio MR, Wilson MJ, Enno T.** 2003. Chronic hydrocephalus in rats and humans: white matter loss and behavior changes. Ann Neurol **53:**337–346. https://doi.org/10.1002/ana.10453.
6. **Ek CJ, Dziegielewska KM, Stolp H, Saunders NR.** 2006. Functional effectiveness of the blood–brain barrier to small water-soluble molecules in developing and adult opossum (*Monodelphis domestica*). J Comp Neurol **496:**13–26. https://doi.org/10.1002/cne.20885.
7. **Ettinger SJ, Feldman EC.** 1995. Textbook of veterinary internal medicine. Philadelphia (PA): W B Saunders Publishing.
8. **Hint HC, Richter AW.** 1958. Chronic toxicity of dextran sulphate in rabbits. Br J Pharmacol Chemother **13:**109–112. https://doi.org/10.1111/j.1476-5381.1958.tb00203.x.
9. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
10. **Iyer RR, Butman JA, Walbridge S, Gai ND, Heiss JD, Lonser RR.** 2011. Tracking accuracy of T2- and diffusion-weighted magnetic resonance imaging for infusate distribution by convection-enhanced delivery. J Neurosurg **115:**474–480. https://doi.org/10.3171/2011.5.JNS11246.
11. **Joukal M, Klusáková I, Dubový P.** 2016. Direct communication of the spinal subarachnoid space with the rat dorsal root ganglia. Ann Anat **205:**9–15. https://doi.org/10.1016/j.aanat.2016.01.004.
12. **Jusué-Torres I, Jeon LH, Sankey EW, Lu J, Vivas-Buitrago T, Crawford JA, Pletnikov MV, Xu J, Blitz A, Herzka DA, Crain B, Hulbert A, Guerrero-Cazares H, Gonzalez-Perez O, McAllister JP 2nd, Quiñones-Hinojosa A, Rigamonti D.** 2016. A novel experimental animal model of adult chronic hydrocephalus. Neurosurgery **79:**746–756. https://doi.org/10.1227/NEU.0000000000001405.
13. **Kirkpatrick M, Engleman H, Minns RA.** 1989. Symptoms and signs of progressive hydrocephalus. Arch Dis Child **64:**124–128. https://doi.org/10.1136/adc.64.1.124.
14. **Klarica M, Miše B, Vladić A, Radoš M, Orešković D.** 2013. 'Compensated hyperosmolarity' of cerebrospinal fluid and the development of hydrocephalus. Neuroscience **248:**278–289. https://doi.org/10.1016/j.neuroscience.2013.06.022.
15. **Klarica M, Orešković D.** 2014. Enigma of cerebrospinal fluid dynamics. Croat Med J **55:**287–290. https://doi.org/10.3325/cmj.2014.55.287.
16. **Knickmeyer RC, Styner M, Short SJ, Lubach GR, Kang C, Hamer R, Coe CL, Gilmore JH.** 2009. Maturational trajectories of cortical brain development through the pubertal transition: unique species and sex differences in the monkey revealed through structural magnetic resonance imaging. Cereb Cortex **20:**1053–1063. https://doi.org/10.1093/cercor/bhp166.
17. **Kondziella D, Lüdemann W, Brinker T, Sletvold O, Sonnewald U.** 2002. Alterations in brain metabolism, CNS morphology and CSF dynamics in adult rats with kaolin-induced hydrocephalus. Brain Res **927:**35–41. https://doi.org/10.1016/S0006-8993(01)03320-0.
18. **Krishnamurthy S, Li J, Schultz L, Jenrow KA.** 2012. Increased CSF osmolarity reversibly induces hydrocephalus in the normal rat brain. Fluids Barriers CNS **9:**13. https://doi.org/10.1186/2045-8118-9-13.
19. **Krishnamurthy S, Li J, Schultz L, McAllister JP 2nd.** 2009. Intraventricular infusion of hyperosmolar dextran induces hydrocephalus: a novel animal model of hydrocephalus. Cerebrospinal Fluid Res **6:**16. https://doi.org/10.1186/1743-8454-6-16.
20. **Linninger AA, Sweetman B, Penn R.** 2009. Normal and hydrocephalic brain dynamics: the role of reduced cerebrospinal fluid reabsorption in ventricular enlargement. Ann Biomed Eng **37:**1434–1447. https://doi.org/10.1007/s10439-009-9691-4.
21. **Ljungström KG, Renck H, Hedin H, Richter W, Wiholm BE.** 1988. Hapten inhibition and dextran anaphylaxis. Anaesthesia **43:**729–732. https://doi.org/10.1111/j.1365-2044.1988.tb05741.x.
22. **Maraković J, Oresković D, Rados M, Vukić M, Jurjević I, Chudy D, Klarica M.** 2010. Effect of osmolarity on CSF volume during ventriculo–aqueductal and ventriculo–cisternal perfusions in cats. Neurosci Lett **484:**93–97. https://doi.org/10.1016/j.neulet.2010.07.058.
23. **Maurice-Williams RS.** 1975. Mechanism of production of gait unsteadiness by tumours in the posterior fossa. J Neurol Neurosurg Psychiatry **38:**143–148. https://doi.org/10.1136/jnnp.38.2.143.
24. **McComb JG.** 1983. Recent research into the nature of cerebrospinal fluid formation and absorption. J Neurosurg **59:**369–383. https://doi.org/10.3171/jns.1983.59.3.0369.
25. **Nowlin BT, Burdo TH, Midkiff CC, Salemi M, Alvarez X, Williams KC.** 2015. SIV encephalitis lesions are composed of $CD163^{+}$ macrophages present in the central nervous system during early SIV infection and SIV-positive macrophages recruited terminally with AIDS. Am J Pathol **185:**1649–1665. https://doi.org/10.1016/j.ajpath.2015.01.033. Erratum: Am J Pathol **187**:1436.http://dx.doi.org/10.1016/j.ajpath.2017.03.002
26. **Orešković D, Klarica M.** 2011. Development of hydrocephalus and classical hypothesis of cerebrospinal fluid hydrodynamics: facts and illusions. Prog Neurobiol **94:**238–258. https://doi.org/10.1016/j.pneurobio.2011.05.005.
27. **Paull JD.** 1987. Dextrans. Dev Biol Stand **67:**133–138.
28. **Pease RW Jr, editor.** 1986. Webster's medical desk dictionary. Springfield (MA): Merriam–Webster
29. **Persson EK, Anderson S, Wiklund LM, Uvebrant P.** 2007. Hydrocephalus in children born in 1999–2002: epidemiology, outcome and ophthalmological findings. Childs Nerv Syst **23:**1111–1118. https://doi.org/10.1007/s00381-007-0324-7.
30. **Peters NJ, Mahajan JK, Bawa M, Sahu PK, Rao KL.** 2013. Factors affecting quality of life in early childhood in patients with congenital hydrocephalus. Childs Nerv Syst **30:**867–871. https://doi.org/10.1007/s00381-013-2335-x.
31. **Pierre PJ, Hopkins WD, Taglialatela JP, Lees CJ, Bennett AJ.** 2008. Age-related neuroanatomical differences from the juvenile period to adulthood in mother-reared macaques (*Macaca radiata*). Brain Res **1226:**56–60. https://doi.org/10.1016/j.brainres.2008.06.001.
32. **Saunders NR, Dziegielewska KM, Møllgård K, Habgood MD.** 2015. Markers for blood–brain barrier integrity: how appropriate is Evans blue in the 21st century and what are the alternatives? Front Neurosci **9:**385. https://doi.org/10.3389/fnins.2015.00385.
33. **Saunders NR, Habgood MD, Dziegielewska KM.** 1999. Barrier mechanisms in the brain, I. Adult brain. Clin Exp Pharmacol Physiol **26:**11–19. https://doi.org/10.1046/j.1440-1681.1999.02986.x.
34. **Walton KW.** 1954. Investigation of the toxicity of a series of dextran sulphates of varying molecular weight. Br J Pharmacol Chemother **9:**1–14. https://doi.org/10.1111/j.1476-5381.1954.tb00809.x.
35. **Zinderman CE, Landow L, Wise RP.** 2006. Anaphylactoid reactions to dextran 40 and 70: reports to the United States Food and Drug Administration, 1969 to 2004. J Vasc Surg **43:**1004–1009. https://doi.org/10.1016/j.jvs.2006.01.006.