Acute Cerebral Effects of Isotonic Crystalloid and Colloid Solutions Following Cryogenic Brain Injury in the Rabbit

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Despite the numerous studies examining the relative merits of crystalloids *versus* colloids for expansion of intravascular volume, little attention has been directed to the cerebral effects of these solutions. In particular, the effect of changes in plasma oncotic pressure on brain water content are poorly understood. The authors recently examined the acute effects of changes in plasma osmolality and colloid oncotic pressure in normal animals, and found that a 65% reduction in oncotic pressure had no detectable effect on brain water content or intracranial pressure. In an effort to extend these studies to a more clinically relevant situation, the authors have now compared the acute effects of 0.9% saline, 6% hetastarch, and 5% albumin on regional cerebral water content and intracranial pressure in an animal model of brain injury produced by focal cortical freezing. Under general anesthesia and following the production of the cryogenic brain lesion, rabbits underwent a 45-min period of isovolemic hemodilution to a hematocrit of 20–25% with one of the three selected fluids. The saline group required approximately twice as much fluid (207 ± 17 ml) to maintain a stable mean arterial pressure and central venous pressure as did the hetastarch (105 ± 14 ml) or albumin (103 ± 29 ml) groups. As intended, the oncotic pressure decreased by a mean of 9.6 ± 2.4 mmHg in the saline group, while remaining stable in the hetastarch and albumin groups. There were no significant changes in osmolality in any group during the hemodilution period. Intracranial pressure increased in all groups following production of the cerebral lesion, but there were no differences between the various experimental groups upon conclusion of the hemodilution. Brain water content was significantly increased in the vicinity of the cryogenic lesion, but there were no differences between the various hemodilution groups. This experiment suggests that a decrease in plasma oncotic pressure due to the administration of an isotonic crystalloid solution does not acutely exacerbate the cerebral edema that is produced by this model of brain injury. (Key words: Brain: edema; intracranial pressure. Complications: cerebral edema. Fluid therapy: colloids; crystalloids.)

TRAUMA VICTIMS and neurosurgical patients may require the intravenous administration of large volumes of fluids to treat or prevent the onset of hypovolemia.

Despite numerous studies comparing the relative merits of crystalloid *versus* colloid solutions for resuscitation,[1–3] there is little information on the cerebral effects of these solutions. In particular, the effects of hypo-oncotic (*e.g.*, crystalloid) solutions on cerebral edema formation are unknown. This lack of knowledge is especially distressing, as postoperative and post-traumatic intracranial hypertension is a major cause of morbidity in these patients.

Although it is often recommended that crystalloid solutions be avoided in patients with head injuries to minimize edema formation, there is a paucity of experimental evidence to substantiate this claim. Massive *peripheral* edema may follow an acute decrease in plasma oncotic pressure, but it is unclear whether this same phenomena occurs in the brain. In a previous study, we demonstrated that decrements in plasma oncotic pressure have no acute effect on brain tissue water content in regions where the blood-brain barrier remains intact.[4] However, in regions where the blood-brain barrier has been disrupted, it is impossible to predict the effects of a hypo-oncotic state on water movement between the vasculature and the interstitium.

We, therefore, investigated the acute cerebral effects of three commonly used isotonic fluids in an animal model of brain injury. Following the production of a cryogenic cerebral lesion, the animals were hemodiluted by the simultaneous withdrawal of blood and infusion of 0.9% saline, 6% hetastarch, or 5% human albumin to simulate concurrent hemorrhage and volume resuscitation. The effect of the various solutions on intracranial pressure (ICP) and brain water content were then compared.

Materials and Methods

All experiments were approved by the Animal Care Committee of the University of California at San Diego. Thirty-two New Zealand white rabbits weighing 3.3 ± 0.3 kg (mean ± SD) were anesthetized with 4% halothane in oxygen in a closed Plexiglas box. A 22-gauge catheter was inserted into a marginal ear vein and the animals were paralyzed with pancuronium bromide (1 mg iv) to facilitate oral intubation with a 3.0-mm cuffed endotracheal tube. Mechanical ventilation was begun with a tidal volume of ≈15 ml/kg at a rate of 35 breaths/min using an inspired gas mixture of 0.5–0.7% halothane in 70% nitrous oxide and oxygen. CO_2 was

added to the inspired gas mixture to maintain normocarbia as initially assessed by expired gas analysis and later by arterial blood gas measurements (Pa_{CO_2} 35–40 mmHg). Maintenance fluids consisted of 0.9% saline infused through the ear vein catheter at a rate of 4 $ml \cdot kg^{-1} \cdot h^{-1}$. Esophageal temperature was servo-controlled to 37° C through the use of heat lamps. Following infiltration with 0.25% bupivacaine, bilateral groin incisions were made for the placement of femoral arterial and central venous catheters for pressure recording and an inferior vena cava catheter for subsequent blood withdrawal. Right atrial location of the central venous catheter was confirmed by monitoring of the pressure waveform during pullback from the right ventricle. The animals were then placed in the prone "sphinx" position and the head fixed in a stereotactic frame (David Kopf Instruments; Tujunga, California) with the interaural line approximately 12 cm above the midchest. The scalp was infiltrated with 0.25% bupivacaine, incised in the midline, and reflected laterally to expose the skull, and an 18-gauge ventriculostomy needle was stereotacticly inserted into the right lateral ventricle via a burr hole located 8 mm lateral and 9 mm posterior to the bregma.

Monitored variables in all animals included mean arterial pressure (MAP), central venous pressure (CVP), ICP, arterial blood gases, hematocrit, plasma osmolality, and oncotic pressure. The ICP transducer was referenced to the interaural line, while transducers used for recording MAP and CVP were zeroed at the midchest. All pressures are reported as electrical means. Osmolality was measured by freezing point depression while oncotic pressure was determined using a Wescor 4400 colloid osmometer with an SS-030 membrane.

When the surgical preparation was complete and blood gas analysis confirmed acceptable values (i.e., Pa_{O_2} > 100 mmHg, Pa_{CO_2} 35–40 mmHg), a metal funnel with a neck diameter of 1 cm was cemented to the intact skull overlying the left hemisphere. The anterior edge of the funnel neck was situated on the coronal suture and the medial edge on the sagittal suture. Evans blue dye (1 ml, 3% solution) was administered intravenously, and MAP, CVP, ICP, hematocrit, plasma osmolality, and oncotic pressure were determined. The cryogenic lesion was then created by applying liquid nitrogen to the skull overlying the left hemispere (via the funnel) for precisely 60 s.

Immediately after the production of the cryogenic lesion, the animals were entered into one of four experimental groups. In one of these (CONTROL, n = 8), no further intervention was made and animals received only a maintenance infusion of saline (4 ml/kg/h) for the ensuing 45 min. The remaining animals underwent a 45-min period of isovolemic hemodilution. Blood was

TABLE 1. Colloid Oncotic Pressure and Osmolality of Infused Solutions

	COP (mmHg)	OSM (mOsm/kg)
Control	Not hemodiluted	
Saline	0	282
Hetastarch	23	304
Albumin	22	265

withdrawn from the inferior vena caval catheter at a rate of 2–3 ml/min using a roller pump. This was replaced with one of three selected fluids, each given via the ear vein catheter in a volume sufficient to maintain MAP and CVP at baseline (pre-dilution) values. The three fluids were: 0.9% saline (Saline, n = 8), 6% hetastarch in saline (Hespan, n = 8), or 5% albumin in saline (Albumin, n = 8). The measured osmolality and oncotic pressure of these three fluids are summarized in table 1.

Upon completion of the hemodilution period, MAP, CVP, and ICP were again recorded, a second blood sample obtained, and the animals killed with intravenous potassium chloride. Using Friedman bone rongeurs, an extensive craniotomy was performed. The brains were rapidly removed and the hemispheres individually weighed before being immersed in cold (4° C) kerosene for 15 min. Using a #11 scalpel blade and a pair of forceps, 2 mm^3 samples of the left cortex were obtained from the lesion (which was defined by intense Evans blue staining), in the perilesional area, and anterior to the lesion as well as corresponding samples from the right hemisphere (fig. 1). These were placed in a kerosene/bromobenzene density gradient for determination of their specific gravities (i.e., water content). Linearity of the gradients was verified using potassium sulfate standards following each set of determinations.

Data were analyzed using analysis of variance and multiple comparison tests when appropriate. Significance was assumed for $P < 0.05$.
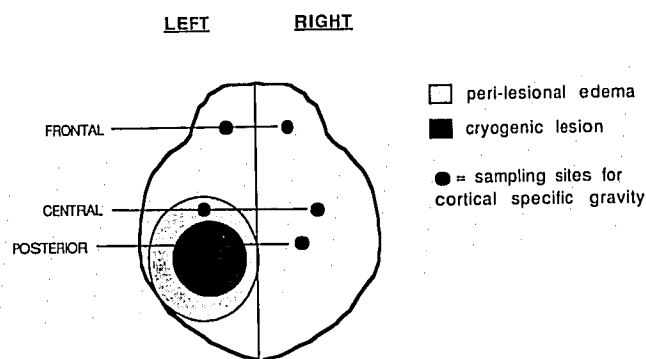

FIG. 1. Cortical sampling sites for specific gravity determinations.

TABLE 2. Post-hemodilution Values

	Control	Saline	Hetastarch	Albumin
MAP (mmHg)	85 ± 9	83 ± 12	88 ± 7	80 ± 6
CVP (mmHg)	4 ± 1	4 ± 1	4 ± 1	3 ± 2
ICP (mmHg)	13 ± 6	16 ± 16	19 ± 10	16 ± 10
pH	7.36 ± .04	7.34 ± .04	7.42 ± .08	7.38 ± .08
Pa_{CO_2} (mmHg)	36 ± 4	38 ± 2	37 ± 4	36 ± 5
Pa_{O_2} (mmHg)	178 ± 18	172 ± 15	170 ± 14	170 ± 10
HCT (%)	38 ± 3*	24 ± 2	20 ± 1	23 ± 2
Fluid in (ml)	0	207 ± 17*	105 ± 14	103 ± 29
Blood out (ml)	0	89 ± 19	89 ± 13	75 ± 16
Δ OSM (mOsm/kg)	0.8 ± 5.4	1.2 ± 4.4	7.1 ± 7.2	2.5 ± 8.5
Δ COP (mmHg)	−0.6 ± 1.0	−9.6 ± 2.4*	0.6 ± 0.7	−0.2 ± 1.8

All values are mean ± SD upon completion of 45 min of hemodilution.   * $P < 0.05$.

Results

Prior to hemodilution, there were no differences in any physiologic variables between the four groups. The values recorded upon completion of the study period are shown in table 2. No intergroup differences existed for MAP, CVP, ICP, or arterial blood gases. As expected, the hematocrit was significantly lower in the three hemodiluted groups as compared with Controls (20–24% vs. 38%). In spite of the similar volumes of blood withdrawn, animals in the Saline group required significantly more fluid (207 ± 17 ml) than the Hespan (105 ± 14 ml), or Albumin (103 ± 29 ml) animals during hemodilution to maintain a stable MAP and CVP (table 2). As intended, oncotic pressure decreased in the saline group (Δ oncotic pressure = −9.6 ± 2.4 mmHg, 53% reduction from baseline, $P < 0.001$). There were no significant changes in osmolality in any group during hemodilution.

Visual inspection of the cortical surfaces of all of the brains revealed a well-circumscribed area (≈ 1 cm diameter) of dark Evans blue staining corresponding to the site of application of the liquid nitrogen. This was surrounded by a pale blue rim of edematous tissue (fig. 1).

Left hemispheric cortical samples showed decreasing specific gravity (*i.e.,* increasing water content) as they approached the lesion (fig. 2). There were, however, no differences in cortical specific gravity between the various hemodilution groups at any one site. Corresponding samples from the right hemisphere showed no differences in specific gravity between any of the three sampling sites or for any of the hemodilution groups (fig. 3).

The wet weight of the lesioned hemisphere was significantly greater than that of the non-lesioned hemisphere (3.84 ± 0.32 grams vs. 3.53 ± 0.34 grams, $P < 0.001$).

Discussion

There are little experimental data to guide the fluid management of patients with brain injury and decreased intracranial compliance. Although it is well known that the administration of hypo-osmolar solutions will increase brain water content and worsen intracranial hypertension,[5] the effect of a hypo-oncotic state (as may be produced through the administration of isotonic crystalloid solutions) is unknown. We, therefore, examined the acute cerebral effects (regional brain water content and ICP) of decreasing plasma oncotic pressure in an animal model of brain injury.

These experiments were designed to complement and extend previous studies performed in normal rabbits. We chose to use a cryogenic lesion in these studies to model the pathologic changes seen following brain injury. Although "artificial," this lesion closely mimics
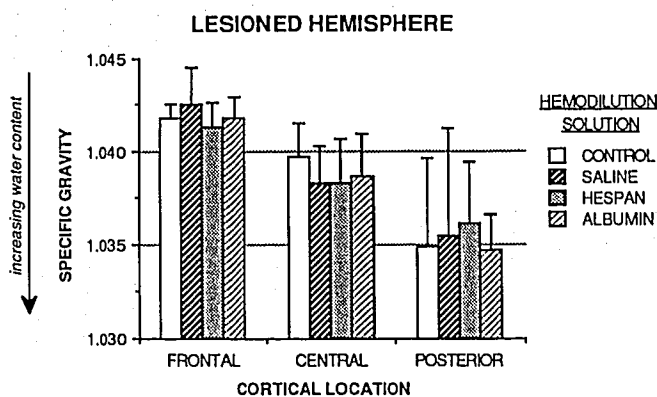
LESIONED HEMISPHERE

FIG. 2. Mean ± SD specific gravity of cortical samples from lesioned hemisphere. Specific gravity decreases (*i.e.,* water content increases) as the sampling site approaches the lesion. At any given site, there are no differences between the various hemodilution groups.

many of the changes seen following traumatic injury. Histologic examination of brain tissue following a cryogenic lesion demonstrates cellular disruption, vascular congestion, and interstitial edema.[6,7] Breakdown of the blood-brain barrier is demonstrated by the extravasation of intravascular markers (albumin, Evans blue dye) into the interstitium,[6,8] a finding repeated here. Finally, such an injury leads to progressive increases in ICP and evidence of increased water content (i.e., edema) in the lesioned and peri-lesional cortex.

The method by which fluids were given is also important to understanding the results of this experiment. The simple infusion of the volumes of fluid needed to alter the oncotic pressure would clearly result in marked hypervolemia, increasing CVP, and an elevated cerebral venous pressure—changes that can themselves alter ICP and increase cerebral edema. We, therefore, chose to employ a model of isovolemic hemodilution, which permits much greater control over plasma composition while maintaining stable arterial and venous pressures. By carefully adjusting the composition of the infused fluids, it was possible to selectively reduce oncotic pressure by over 50% without any significant changes in plasma osmolality. It should be noted that we did not elect to use the previously reported technique of hollow-fiber plasmapheresis (which permits plasma composition to be altered without an accompanying change in hematocrit).[1] Unfortunately, the requisite degree of heparinization needed for this method lead to an unacceptable incidence of intracranial hemorrhage in these injured animals.

Lastly, some mention needs to be made of the use of microgravimetry to detect changes in regional brain water content. The validity of this method has been repeatedly confirmed,[9-11] and its sensitivity is again demonstrated in this experiment as we were able to detect the increased water content of the lesioned and perilesional tissue. Although there may be concern that hemodilution-produced alterations in plasma protein content may invalidate this method, a recent study concluded that the main determinant of brain tissue specific gravity is tissue water content, and that alterations in serum protein content have little or no effect on this relationship.[12] Specific gravities can be reported as the more familiar percent water content; however, as we were primarily interested in this study in comparing water contents between the various groups as opposed to measuring its absolute value, we chose to report the specific gravity values that were observed.

As intended, cortical cryogenic injury resulted in a number of obvious cerebral abnormalities, including an increase in hemispheric weight, a reduction in regional specific gravity, the extravasation of Evan's blue labeled protein, and intracranial hypertension. However, more
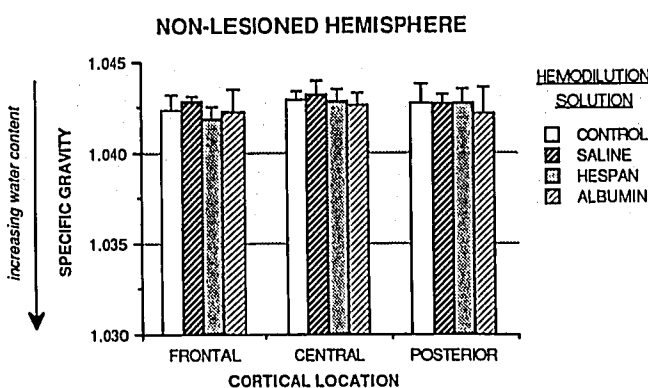

NON-LESIONED HEMISPHERE

FIG. 3. Mean ± SD of specific gravity of cortical samples from the non-lesioned hemisphere. No differences exist between the various sampling sites or between the hemodilution groups at any particular site.

importantly, it is clear that the marked reduction in oncotic pressure achieved in the saline-diluted animals was not associated with any additional alterations in either regional specific gravity or ICP as compared with the two colloid-diluted groups. This result is essentially identical to that observed in normal animals, and may be surprising to some. Nevertheless, a theoretical explanation can be found by carefully examining the Starling equation.[13] This equation states that the hydrostatic forces that act to drive water out of the intravascular space are counteracted by an osmotic gradient that functions to draw fluid back into the capillary. In peripheral tissues, this osmotic effect is almost entirely the product of an intravascular-extravascular difference in protein concentration (and is hence an "oncotic" gradient), since small ions (e.g., Na^+) easily move across the capillary bed and, hence, cannot contribute to the production of a gradient. If plasma oncotic pressure is reduced, there will be a increased movement of fluid into the interstitium and edema will result if there is inadequate lymphatic drainage. Such a phenomenon is commonly seen in patients resuscitated with large volumes of crystalloid. However, the brain, unlike peripheral tissues, is isolated from the vasculature by the blood-brain barrier. When intact, the blood-brain barrier is impermeable to most plasma solutes (Na^+, Cl^-, etc).[14] Under such circumstances, plasma osmolality (instead of oncotic pressure, which represents only a tiny fraction of the total osmolality) becomes the primary determinant of water movement between the vasculature and interstitum. The relatively weak gradient that can be produced by even substantial reductions in oncotic pressure is insufficient to produce any measurable net water movement into the brain interstitium.

If the blood-brain barrier is rendered permeable to plasma proteins, as it is following head trauma and after cryogenic injury (witness the extravasation of Evan's

blue-labeled albumin), neither an oncotic nor osmotic gradient can be established in the region of the injury. Under these circumstances, edema formation becomes entirely dependent on hydrostatic forces. Plasma oncotic pressure is no longer a determinant of water flux, and decreasing oncotic pressure by the infusion of an isotonic crystalloid cannot exacerbate edema formation. Since altering oncotic pressure is also without effect in non-injured tissue, it can be concluded the plasma oncotic pressure is probably unimportant in determining water movement between the vasculature and the interstitium in the brain.

Do these observations have any implications for the clinical management of neurosurgical patients or trauma victims with concomittant head injuries? As noted above, we recognize that the lesion used in these studies is "artificial" (although, in many ways, it resembles those changes seen following traumatic injury). The experiment was also brief, and it may be difficult to predict whether there might be any "chronic" effects of changes in oncotic pressure. Nevertheless, these observations do not support the belief that isotonic crystalloids should be withheld from patients with decreased intracranial compliance, particularly when the administration of such fluids is deemed necessary for the achievement of hemodynamic stability.

References

1. Gammage G: Crystalloid versus colloid: Is colloid worth the cost? Int Anesthesiol Clin 25:37–60, 1987
2. Kaufman BS, Rackow EC, Falk JL: Fluid resuscitation in circulatory shock. Curr Stud Hematol Blood Transfus 53:186–198, 1986
3. Tranbaugh RF, Lewis FR: Crystalloid versus colloid for fluid resuscitation of hypovolemic patients. Adv Shock Res 9:203–216, 1983
4. Zornow MH, Todd MM, Moore SS: The acute cerebral effects of changes in plasma osmolality and oncotic pressure. ANESTHESIOLOGY 67:936–941, 1987
5. Dodge PR, Crawford JD, Probst HJ: Studies in experimental water intoxication. Arch Neurol 3:513–529, 1960
6. Clasen RA, Cooke PM, Pandolfi S, Boyd D: Experimental cerebral edema produced by focal freezing. 1. An anatomic study using vital dye techniques. J Neuropath Exp Neurol 21:579–596, 1962
7. Clasen RA, Brown DV, Leavitt S, Hass GM: The production by liquid nitrogen of acute closed cerebral injuries. Surg Gynecol Obstet 96:605–616, 1953
8. Gazendam J, Go KG, Zanten AK: Composition of isolated edema fluid in cold-induced brain edema. J Neurosurg 51:70–79, 1979
9. Nelson SR, Mantz ML, Maxwell JA: Use of specific gravity in the measurement of cerebral edema. J Appl Physiol 30:268–271, 1971
10. Marmarou A, Tanaka K, Shulman K: An improved gravimetric measure of cerebral edema. J Neurosurg 56:246–253, 1982
11. Shigeno T, Brock M, Shigen S, Fritschka E, Cervos-Navarro J: The determination of brain water content: Microgravimetry versus drying-weighing method. J Neurosurg 57:99–107, 1982
12. Bothe HW, Bodsch W, Hossmann KA: Relationship between specific gravity, water content, and serum protein extravasation in various types of vasogenic brain edema. Acta Neuropathol (Berl) 64:37–42, 1984
13. Pappenheimer JR, Soto-Rivera A: Effective osmotic pressure of the plasma proteins and other quantities associated with the capillary circulation in the hindlimbs of cats and dogs. Am J Physiol 152:471–491, 1948
14. Fenstermacher JD: Volume regulation of the central nervous system, Edema. Edited by Staub NC, Taylor AE. New York, Raven Press, 1984, pp 383–404