Effects of Fluid Management on Edema Volume and Midline Shift in a Rat Model of Ischemic Stroke

Richard P. Paczynski, MD; Ramesh Venkatesan, DSc; Michael N. Diringer, MD; Yong Y. He, MD; Chung Y. Hsu, MD, PhD; Weili Lin, PhD

Background and Purpose—The purpose of this study was to investigate the effects of fluid management on brain water content (BW) and midline shift (MLS) after a focal cerebral ischemic insult.

Methods—A suture model was used to induce focal cerebral ischemia for 90 minutes (n = 44). The rats were randomly assigned to 3 groups 2.5 hours after reperfusion: dehydration (n = 24), control (n = 8), or hydration (n = 12). BW was obtained with the wet-dry weight method 24 hours after middle cerebral artery (MCA) occlusion. In addition, MRI were obtained (n = 31) 24 hours after the onset of ischemia so that the ratio of hemispheric volumes ipsilateral (IH) and contralateral (CH) to the infarct and the extent of MLS could be obtained.

Results—Across the range from moderate dehydration to intravascular volume expansion with isotonic saline, BW of the IH increased linearly as a function of change in body weight ($r^2 = 0.89$), whereas few changes in relation to body weight were observed in CH, indicating a preferential effect of fluid management on the infarcted hemisphere. Furthermore, the hemispheric volume ratio (IH/CH) and MLS also increased in relation to changes in body weight. However, paradoxical increases in BW, IH/CH, and extent of MLS were observed in comparison with controls when severe dehydration was produced with high-dose mannitol.

Conclusions—Changes in ischemic BW by fluid management correlated closely with changes in body weight except when high-dose mannitol was used. Mannitol, as a dehydrating agent, may be associated with bimodal effects, with a high dose aggravating ischemic BW. (Stroke. 2000;31:1702-1708.)

Key Words: brain edema ▪ cerebral ischemia ▪ magnetic resonance imaging ▪ rats

Cerebral edema associated with large hemispheric infarction (LHI) frequently has the character of a mass lesion that results in asymmetric shifting of the contents of the cranial container, leading to transtentorial herniation. Subtle signs of elevated tissue water content may be apparent on MRI or axial CT within hours of stroke onset, but the clinical manifestations of cerebral edema typically reach a maximum after a delay of 1 to 5 days. Massive brain edema is the leading cause of death within 1 week of ischemic strokes. The pathophysiological mechanisms underlying the development of progressive brain edema days after ischemic stroke have been extensively explored in animal stroke models. However, no effective therapies are available in the treatment of ischemic brain edema. Decompressive hemicraniectomy has been shown in preliminary studies to reduce mortality rates, but the impact of this invasive procedure on the long-term outcome and quality of life remains to be established. Modulation of osmolarity with various oncotic regimens has been attempted. Mannitol has remained as the most extensively studied agent in reducing ischemic brain edema in animal models and patients. However, potential detrimental effects of mannitol have been noted. Unfavorable effects of osmotic agents may be related to osmotic reduction in water content in the normal rather than the ischemic brain, aggravating the midline shift (MLS). Detrimental effects of mannitol have also been attributed to its retention in the damaged brain to worsen brain edema. Thus, osmotic regimens have not been widely accepted in the management of ischemic brain edema with mass effects. A question remains in clinical practice: What is the optimal osmotic condition for patients with severe ischemic brain edema with mass effect leading to inevitable transtentorial herniation? Should fluid be restricted or osmotic diuresis achieved in an attempt to reduce ischemic edema? The common practice of prophylactic fluid restriction for patients with LHI is based on the assumption that there is a direct relationship between the hydration status of the body as a whole and the potential
severity of brain edema. On the other hand, despite concerns about exacerbating brain edema, intravascular volume expansion during the acute phase of ischemic stroke has also been explored.\textsuperscript{17,21,31,32} The latter approach to fluid management is based on the assumption that viable brain tissue adjacent to core zones of severe ischemia may exist for many hours or even days after the onset of stroke and that intravascular volume expansion may improve blood flow and oxygen delivery to these marginally perfused regions. However, despite the clinical importance of these issues, few attempts at quantification of the effects of fluid management on ischemic brain edema have been made. Since there are few objective data that firmly support one mode of management over another, general decisions about the prescription of fluids, diuretics, or both are often based on local tradition and therefore tend to vary considerably from one institution to another.

Using a rat model of ischemic cortical infarction, we recently demonstrated that multiple-dose mannitol infusions consistently increased plasma osmolality, produced total body dehydration, and reduced the water content of brain tissue. In experiments producing mild to moderate dehydration, brain tissue water content was decreased to a greater extent in the hemisphere ipsilateral to the infarct (IH) than in the contralateral hemisphere (CH), suggesting a potentially favorable effect of mannitol on cerebral tissue shifts.\textsuperscript{24} We postulated that the reduction of the water content of the infarcted hemisphere was caused by the general diuretic effect of mannitol (through reduction of interstitial fluid volume).\textsuperscript{29} Although alternative explanations relating the rheological and free radical–scavenging effects of mannitol to the severity of the primary ischemic insult are also plausible,\textsuperscript{33,34} the goal of the present study was to assess the influence of fluid management reflected by changes in body weight on the extent of ischemic brain edema after a uniform cerebral insult produced by focal cerebral ischemia–reperfusion. In addition, we also assess the degree of MLS as an adjunct endpoint of fluid management. For these purposes, we used a rat model of ischemic infarction that simulates many of the clinically relevant features of LHI\textsuperscript{35} and an MRI protocol that was sensitive to subtle changes in MLS. Although there are numerous experimental approaches to osmotic manipulation of potential interest in the management of ischemic stroke,\textsuperscript{16–24,31,32} we chose to compare the natural history of edema formation in this model with the effects of volume expansion with crystalloid (isotonic saline) and dehydration with the diuretics furosemide and mannitol because of the widespread use of these agents clinically.

**Materials and Methods**

**LHI Model in the Rat**

Adult, male Long-Evans rats weighing 275 to 340 g were used in these studies (n = 44). All procedures were approved by the Washington University Animal Care Committee and were consistent with the National Institutes of Health guidelines for small-animal experimentation. A modification of the method of Zea Longa et al\textsuperscript{15,36} was used to produce cerebral ischemia for 90 minutes by intraluminal suture occlusion of the right MCA. Both blood pressure and arterial blood gasses were maintained within the normal range during surgery, and temperature stability (37 ± 0.5°C) was ensured with the use of a thermo-controlled heating pad.

**Fluid Management Protocols**

After recovery from general anesthesia (return of righting reflexes), animals were randomly assigned to either dehydration (n = 24), control (ad lib; n = 8), or hydration protocols (n = 12). All infusions (saline, mannitol, and furosemide) were administered via indwelling intravenous catheters at a constant rate of 0.5 to 0.75 mL/min through a Sage microinfusion pump. Rats randomized to the dehydration group were divided into 1 of 3 subgroups: intravenous infusions of a 25% mannitol solution every 5 hours (250 mg/mL, 1372 mOsm/L, Abbott Laboratories) at either a high dose (1.5 g/kg; mannitol HD; n = 8), a low dose (0.5 g/kg; mannitol LD; n = 9) or intravenous infusions of furosemide at a single dose every 5 hours (0.5 mg/kg; n = 7). In each case, the first dose of diuretic agent was given 2.5 hours after reperfusion of the right MCA; a 0.5-mL bolus of isotonic saline was injected after each intravenous infusion to maintain the patency of the catheters. Mannitol-treated and furosemide-treated rats had free access to food but were restricted from drinking water or other fluids during the 24-hour experimental period. Preliminary experiments (data not shown) established the typical changes in total body weight expected from sustained administration of mannitol and furosemide at these doses and indicated that these agents were generally well tolerated and did not produce significant changes in arterial blood pressure. Control group rats (n = 8) received 0.5-mL boluses of isotonic saline via indwelling intravenous catheters every 5 hours, also beginning 2.5 hours after MCA reperfusion, but were otherwise under ad lib conditions. The rats in the hydration group were divided into 2 subgroups, the first group receiving enough isotonic saline every 5 hours to maintain constant body weight ±1% with respect to baseline (preschisma; saline A; n = 7) and the second group receiving enough isotonic saline every 5 hours to increase body weight ~5% above the baseline value (saline B; n = 5). Hydration group rats had free access to food but were restricted from drinking water.

**MRI Protocol**

Twenty-four hours after induction of the ischemic insult, 31 of the 44 rats were reanesthetized (pentobarbital, 65 mg/kg IP) for MR brain imaging. Their heads were placed within a cylindrical MR-compatible frame that maintained a constant orientation between the sagittal plane of the cerebrum and the long axis of a custom-made receive-only radiofrequency coil. In this way, the anatomic regions corresponding to the third ventricle from which measurements of MLS were made could be positioned optimally within the coil in each case. A 3D gradient echo FLASH (fast low angle shot) sequence with radiofrequency spoiling was used to acquire T\textsubscript{1}-weighted images of the brain with a 1.5-T Siemens Vision MRI system (Siemens Medical Systems). Imaging parameters were as follows: repetition time = 25 ms; echo time = 8 ms; flip angle = 40°; matrix size = 64×64 interpolated to 128×128; field of view 55 mm; and 32 partitions with slice thickness of 1 mm.

**Quantification of Plasma Osmolality and Cerebral Edema**

All animals were killed immediately after the imaging session to maintain a consistent relationship between the end point of the fluid management protocol, MRI, and the ex vivo measurement of brain tissue water. To obtain blood samples and discern possible hemodynamic changes caused by the hydration or dehydration, a femoral arterial catheter was placed with the rat under pentobarbital anesthesia (65 mg/kg IP) immediately before the end of the experiment. Mean arterial blood pressure (MABP) was measured for 5 minutes with the use of a Micro-Med transducing system (Micro-Med, Inc.), and the average value was recorded. A sample of blood was processed for determination of hematocrit and plasma osmolality (mOsm/L) with a microcentrifuge and an automated freezing point depression micro-osmometer (Advanced Instruments, Inc.) respec-
Brain water was quantitated by the wet-dry weight method as described previously. Briefly, animals were killed by decapitation under deep pentobarbital anesthesia, the brain was removed, and the cerebral convexities were exposed in a humidified chamber. The olfactory projections and frontal poles of the brain were resected and discarded; the remainder of the forebrain was isolated from the brain stem at the level of the superior colliculus and then separated into halves by severing the corpus callosum. Each hemisphere was gently blotted with tissue paper to remove small quantities of adsorbent cerebrospinal fluid. Tissue samples were rapidly weighed with a basic precision scale (Salterius 2462, Salterius Werke) to within 0.1 mg and dried to constant weight in a vacuum oven (Precision Scientific) at 80°C and low vacuum. The percent H2O of each tissue sample was then calculated according to the following equation:

\[
\% H_2O = \frac{(\text{Wet Weight} - \text{Dry Weight})}{\text{Wet Weight}} \times 100
\]

**Data Analysis**

MLS apparent on MR images was quantitated according to adaptations of the general criteria used in the imaging studies of Ropper et al involving humans with supratentorial mass lesions. Coronal slice profiles could potentially interfere with the identification of the third ventricle, a readily identified anatomic landmark. In all rats, centroparietal slices in which the third ventricle could be identified as a distinct structure were used for measurement of MLS according to the following procedure. First, with automated image processing software (DIP Station, Hayden Image Processing Group), a horizontal score line was drawn by connecting tangents to the inferior aspects of each temporal lobe. Perpendicular lines from this horizontal base that were tangent to the right and left parietal convexities were then inscribed to form a rectangular framework unique to each coronal slice. Perpendicular projections from the parietal convexities to the center of the third ventricle were then inscribed on each image. MLS of the third ventricle was defined as the ratio of the distances from the parietal convexity to the center of the third ventricle for the IH and CH, respectively.

Nonideal slice profile could potentially interfere with the identification of the contours of brain hemispheres on MR images obtained from the anterior and posterior extremes of the imaging coil. Therefore, the data set used for both MLS and volume determinations was limited to the central 10 slices. The areas of the IH and CH within each of these coronal slices were assessed by a single blinded participant who had no information as to which groups that the rats were assigned to. For the medial-most segments of the cerebral hemispheres, borders were defined by the aforementioned score lines between the third ventricle and midline structures at the base of the skull. In coronal slices anterior and posterior to the region of the third ventricle, the medial borders of each hemisphere were defined by connecting the superior and inferior longitudinal fissures. The areas within these contours were automatically determined with the DIP Station software, and the volumes of the IH and CH were then determined by summation of the elements from 10 contiguous coronal slices.

**Statistical Analysis**

Statistical analysis was performed between control, dehydrated, and hydrated groups and their respective subgroups. Differences between measured parameters (% change total body weight, measured plasma osmolality, MABP, and %H2O of the IH and CH) were tested for significance with ANOVA. The significance level between groups was assessed with post hoc Tukey tests. MRI-derived parameters (calculated hemispheric volumes and MLS) were also assessed for significance with ANOVA and the post hoc Tukey test. A value of \( P < 0.05 \) was considered significant. In addition, correlation coefficients (\( r \)) were derived from linear regression analyses of the relationships between percentage change in total body weight and %H2O of both CH and IH. In the case of the IH, this analysis was performed separately for percentage change in body weight above and below the cutoff value of 92.5%, which is the inflection point of a V-shaped regression curve plotting %H2O versus body weight (see Figure 1).

**Results**

**Impact of Fluid Management on Physiological Parameters**

The Table demonstrates the changes in total body weight produced by the different fluid management protocols in relation to other physiological variables. Dehydration with low-dose and high-dose mannitol infusions produced changes in body weight of \(-9.1 \pm 1.1%\) and \(-15.3 \pm 1.4%\), respectively. Rats receiving furosemide infusions had weight loss comparable to rats receiving low-dose mannitol (\(-9.3 \pm 1.2\%\)). In the control group (ad lib conditions) there was an average weight change of \(-5.1 \pm 0.5%\) over the course of 24 hours. The hydration group rats by definition had either

<table>
<thead>
<tr>
<th>Physiological Parameters and Percent Water of Hemispheres</th>
<th>% Change</th>
<th>Body Wt</th>
<th>pOSM</th>
<th>Hct</th>
<th>MABP</th>
<th>%H2O IH</th>
<th>%H2O CH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannitol HD (n=8)</td>
<td>-15.3 ± 1.4†</td>
<td>332 ± 12†</td>
<td>51.3 ± 0.04†</td>
<td>106 ± 14</td>
<td>83.31 ± 0.85*</td>
<td>77.8 ± 0.6*</td>
<td></td>
</tr>
<tr>
<td>Mannitol LD (n=9)</td>
<td>-9.1 ± 1.1*</td>
<td>321 ± 6*</td>
<td>46.5 ± 0.03</td>
<td>118 ± 12</td>
<td>80.53 ± 0.96*</td>
<td>78.11 ± 0.47</td>
<td></td>
</tr>
<tr>
<td>Furosemide (n=7)</td>
<td>-9.3 ± 1.2*</td>
<td>309 ± 8*</td>
<td>46.9 ± 0.03*</td>
<td>98 ± 14</td>
<td>81.57 ± 0.83</td>
<td>78.6 ± 0.52</td>
<td></td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>-5.1 ± 0.5</td>
<td>306 ± 4</td>
<td>45 ± 0.02</td>
<td>112 ± 7</td>
<td>82.28 ± 0.85</td>
<td>78.59 ± 0.36</td>
<td></td>
</tr>
<tr>
<td>Saline A (n=7)</td>
<td>-0.3 ± 0.9</td>
<td>304 ± 6</td>
<td>39 ± 0.02*</td>
<td>115 ± 12</td>
<td>84.25 ± 1.1*</td>
<td>79.29 ± 0.38*</td>
<td></td>
</tr>
<tr>
<td>Saline B (n=5)</td>
<td>4.5 ± 1.1*</td>
<td>303 ± 7</td>
<td>36 ± 0.03*</td>
<td>117 ± 12</td>
<td>84.66 ± 0.86†</td>
<td>79.28 ± 0.33*</td>
<td></td>
</tr>
</tbody>
</table>

\( p \text{OSM} \) indicates plasma osmolality; Hct, hematocrit; MABP, mean arterial blood pressure; %H2O IH, percent water content of hemisphere ipsilateral to infarct; and %H2O CH, percent water content of hemisphere contralateral to infarct.

* \( P < 0.05 \) from ANOVA comparing ad lib with treated subgroups.
† \( P < 0.01 \) from ANOVA comparing ad lib with treated subgroups.
Impact of Fluid Management on Water Content of Cerebral Hemispheres

When \( \%H_2O \) in the IH was related to the changes in total body weight resulting from fluid management (ie, decreased by dehydration, decreased mildly in ad lib, maintained or increased by hydration), a distinctly bimodal relationship could be discerned with an inflection point corresponding to an \( \approx \)10\% reduction in body weight (Figure 1). Across the range of values to the right of this inflection point (including rats with moderate dehydration by either low-dose mannitol or furosemide, ad lib controls, and hydration group rats), \( \%H_2O \) in the IH increased linearly \( (r^2=0.89) \) as a function of increase in body weight. Across the range of values to the left of this inflection point, \( \%H_2O \) in the IH also increased in a linear fashion \( (r^2=0.83) \) in relation to severe dehydration. The percent \( H_2O \) in the CH increased only modestly in relation to increases in total body weight and did not exhibit a bimodal distribution.

**IH/CH Volume Ratios**

Hemispheric volumes were calculated from MR images, and the ratios of the volumes of the IH to those of the CH were generated as an index of cerebral volume asymmetry, as shown in Figure 2. The IH/CH ratios in the low-dose mannitol and furosemide-treated subgroups were both lower than those of controls, but the differences reached statistical significance only in the low-dose mannitol subgroup (\( P<0.05 \)). In contrast, significant increases in the IH/CH ratio in comparison with control values were observed at both extremes of fluid management (high-dose mannitol–induced dehydration, \( P<0.05 \), and hydration with isotonic saline, \( P<0.01 \)). These results are congruent with the \( \%H_2O \) data presented above.

**Impact of Hydration Status on MLS**

A comparison of MLS between different groups is shown in Figure 3. As expected, the IH/CH volume ratio and the extent of MLS were well correlated \( (r^2=0.89) \). In comparison with the control group, MLS was less in both the furosemide-treated and low-dose mannitol–treated subgroups, and this difference reached statistical significance in the latter \( (P<0.05) \). In contrast, MLS was significantly greater for rats in both hydration subgroups \( (P<0.05) \) and the high-dose mannitol subgroup \( (P<0.05) \) of the study. Again, the latter was associated with the most severe total body dehydration.

**Discussion**

Given that brain edema must ultimately be derived from the reservoir of intravascular fluid, diffusion and bulk flow of plasma or plasma ultrafiltrate from the intravascular to the interstitial spaces are basic processes that will influence tissue swelling.\(^{41}\) Particularly when reperfusion has occurred, transvascular movement of fluid into the infarct may modulate the volume of ischemic edema.\(^{42,43}\) Therefore, one obvious parameter that may influence the volume of ischemic brain edema would be the total body hydration status, which can be reflected by body weight and plasma osmolarity. These considerations drove the hypothesis tested in this study, namely that the pattern of net body fluid balance during the evolution of ischemic brain edema would influence the ultimate brain water content (BW) and mass effects.

Although indirect evidence can be found,\(^{44,45}\) to our knowledge, there are no data available that specifically relate total body hydration status to the extent of brain edema after a focal cerebral ischemic insult. Our findings indicate decreased water content in the IH in moderately dehydrated rats in comparison with controls and increased \( \%H_2O \) in the IH in rats subjected to isotonic intravascular volume expansion. The paradoxical effect of high-dose mannitol on BW and MLS is addressed below. Although the experiments presented here were not designed to specifically address the mechanisms by which edema fluid distributes within the brain, the most likely explanation of
nearly equivalent degree of total body dehydration brought about by repeated infusions of intravenous furosemide (at a dose previously shown not to produce significant acute changes in MABP) was associated with modest elevations of hematocrit and a trend toward a decrease in $\%H_2O$ in the IH in comparison with controls that did not reach statistical significance. Low-dose mannitol resulted in a somewhat greater reduction in the $\%H_2O$ of the IH than furosemide, although there were no statistically significant differences between these dehydration subgroups. The comparable effects of moderate dehydration induced with either furosemide or low-dose mannitol lend support to the notion that the reduction of water content of the IH associated with these agents may simply reflect their impact on total body water through a common diuretic action.

In contrast, when a much larger dose of mannitol (1.5 g/kg) was infused at the same frequency, weight loss was severe, mean plasma osmolality increased by $\approx 30$ mOsm/L above control levels, and $\%H_2O$, IH/CH volume ratio, and MLS were significantly increased in comparison with control rats. The paradoxical results from the high-dose mannitol subgroup suggest that a potentially beneficial effect of osmotic diuresis on the volume of ischemic edema may be obtained only when mild to moderate degrees of dehydration are produced. Beyond that level, countervailing processes associated with mannitol infusions may result in an actual increase in brain edema. Although these experiments were not designed to identify the mechanism(s) by which mannitol-induced dehydration could increase brain edema, our observations are consistent with the explanation postulated by other investigators that exogenous osmoles may accumulate within damaged tissue and thereby produce an increase in BW through the establishment of a “reverse” osmotic gradient.22 This process would seem more likely to occur under conditions of severe dehydration in which renal clearance of mannitol would be impaired, resulting in its accumulation within plasma and therefore in brain. Another possibility is that the adverse effects of severe dehydration on blood rheology or arterial blood pressure may have compromised cerebral blood flow to marginally perfused tissues, resulting in a larger infarction. However, even in association with high-dose mannitol infusions there were no significant reductions in MABP noted in this series or in preliminary work. Finally, as pointed out by Fishman and colleagues,50,51 the induction of “idiogenic osmoles” within the brain parenchyma in association with high-dose mannitol infusions could cause cell swelling and thereby an increase in brain water. To further investigate the paradoxical effects of high-dose mannitol, an assessment of cerebral blood flow may be useful in our understanding of the biphasic effects of mannitol on brain edema.

The results of this study ultimately must be placed in the context of the clinical pathophysiology of ischemic brain edema. However, the limited spatial resolution of the MR images of the rat brain available for this study precluded analysis of axial displacements of brain structures in relation to the tentorium; our study was therefore limited to an analysis of the influence of simulated clinical fluid management on the position of the third ventricle relative to its normal midline position. It is recognized that the unique craniocebral anatomy of the rat limits the scope of the conclusions that can be drawn from this study regarding changes in MLS observed in clinical situations. Furthermore, obvious differences in the water metabolism of humans and rodents preclude an extrapolation of our data on body weight change to what may be considered clinically relevant in the fluid management of clinical
stroke. Nevertheless, these data support the basic concept that fluid balance during the evolution of a cerebral infarct may influence the volume of edema and the associated distortions of intracranial geometry. Mild to moderate dehydration with low doses of mannitol or furosemide produced “optimal” results, neither of which, however, were strikingly different from ad lib conditions. Increases in edema volume and frank worsening of MLS were observed at both extremes of the hydration-dehydration spectrum.

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References
Brain edema is known to be the major cause associated with mortality and morbidity in stroke patients. The pathophysiological mechanisms underlying ischemic brain edema are multifactorial and are partly associated with oxidative stress. However, effective therapies are still lacking. Hyperosmolar agents, including mannitol or glycerol, are commonly used to reduce brain edema in stroke patients. In this carefully done study, Paczynski and colleagues have employed both hyperosmolar and diuretic agents, which are used clinically, to correlate the infarction volume with brain swelling in rats after ischemic cortical infarction. They have found that the hyperosmolar mannitol, despite being a dehydrating agent, is associated with high brain water content (swelling). These data confirm and extend previous findings that in addition to the dehydration effect, idiogenic osmoles and blood-brain barrier opening are also known to be produced when brain parenchyma respond to hyperosmolar agents. These data also provide evidence that strategies and agents other than the hyperosmolar mannitol are needed to reduce brain swelling and blood-brain barrier opening in a clinical stroke setting. It is suggested that alternative efforts such as molecular strategies may be considered to study the pathophysiology of ischemic edema.

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