Effect of mannitol and furosemide on blood-brain osmotic gradient and intracranial pressure

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The effect of mannitol (1.0 gm/kg) and furosemide (0.7 mg/kg), alone and in combination, on the blood-brain extracellular fluid and cerebrospinal fluid (CSF) osmotic gradient, elevated intracranial pressure (ICP), CSF and serum osmolality, and urine output was studied in 26 mongrel dogs. Mannitol and furosemide, when used together, produced a greater (62.4% versus 56.6%) and more sustained (5 hours versus 2 hours) fall in ICP than mannitol alone. This correlated with a prolongation of the reversal of the blood-brain osmotic gradient (-3.4 to +38.5 mOsm/kg) and a rate of urine formation 15 times control values. There was a transient but not significant fall in serum Na+ with the combined treatment, but the arterial pressure did not vary from pretreatment levels. The results from this present study suggest that the distal loop diuretics in a dose of less than 1.0 mg/kg act synergistically with mannitol by causing preferential excretion of water over solute in the renal distal tubule, and thereby sustaining the osmotic gradient initially established by the mannitol infusion. It is possible, but unlikely in the doses used, that the additive effect of furosemide on reducing ICP in the presence of mannitol is due to interference with CSF formation or Na+ and H2O movement across the blood-brain barrier.

KEY WORDS • blood-brain barrier • osmotic gradient • diuretic effect • intracranial pressure • osmotic and loop diuretics

The osmotic and distal loop diuretics have been shown to be effective in both the experimental and clinical setting in reducing intracranial pressure (ICP) by altering the volume of the intracranial compartments.4,11,12,21,22,23,27,29 Furosemide has been reported to lower ICP when used alone,13,14,16,20,22,24 although there is conflicting experimental evidence for this in the normal and edematous brain.9,12 The enhancement of the mannitol effect on ICP by the distal loop diuretics is well established, although the mechanism for this synergy is incompletely understood at this time.26

This present study relates the changes in the blood-brain extracellular fluid and cerebrospinal fluid (CSF) osmotic gradient upon ICP after infusion of mannitol and furosemide, alone and in combination. The aim of this investigation was to clarify the mechanism underlying the synergy between these two diuretic agents.

Materials and Methods

Protocol

Twenty-six adult mongrel dogs of both sexes, each 12 to 20 kg in weight, were used in this study. All animals received Innovar-Vet (fentanyl, 0.4 mg/ml, and droperidol, 20 mg/ml) and atropine (0.04 mg/ml) in a dose of 0.1 ml/kg, approximately 15 minutes prior to induction of anesthesia. The animals were anesthetized with pentobarbital (50 mg/ml) administered intravenously in a dose of 0.25 ml/kg. The animals were then paralyzed with 1.0 ml of pancuronium bromide (1.0 mg/ml), following which they were intubated and maintained on halothane using a mechanical ventilator. The rate of ventilation was set to achieve normal blood-acid base values. The femoral artery and vein were cannulated with polyethylene tubes, allowing continuous monitoring of arterial blood pressure via a transducer and periodic collection of blood samples, and providing a route for administration of medication. A urinary catheter was placed into the bladder and connected in line with a drop counter leading to a collection system.

Trefhine openings were made in the parietal bone bilaterally after gaining access to the skull through a midline skin incision. A solid-state Gaeltec* ICP trans-
ducer was inserted into the subdural space via one of the cranial openings, and the opposite opening was used for placement of an epidural balloon catheter. Through a midline cervical incision, the atlanto-occipital membrane was exposed and a modified No. 22 spinal needle was inserted into the cisterna magna. The arterial blood pressure, ICP, electrocardiogram, and urinary output were continuously recorded on a multichannel recorder using appropriate electromechanical interfacing devices.

Blood samples were taken to measure pH, blood gases, osmolality, hematocrit, Na⁺, and K⁺. Urine samples were obtained for measurement of pH, osmolality, Na⁺, and K⁺. Cerebrospinal fluid (CSF) samples were obtained for osmolality determinations. Measurements were made at 15-minute intervals for the 1st hour except for the immediate posttreatment period, when the serum and CSF osmolality was measured at 1-minute intervals for the first 10 minutes, and then every 15 minutes thereafter. After the first 60 minutes posttreatment, samples were obtained at 30-minute intervals.

Experimental Groups

The animals were divided into four experimental groups based upon the treatment given after the ICP was elevated with the epidural balloon to about 25 to 35 mm Hg. In all groups, the dogs were allowed to stabilize for about 40 minutes prior to treatment. Group 1 animals received no treatment and served to indicate the changes that would be expected to occur in the parameters measured due to the effect of long-term anesthesia, dehydration, and untreated intracranial hypertension. Six animals were placed in this untreated group. Group 2 animals were given an intravenous bolus of a 25% solution of mannitol at a dose of 1.0 gm/kg body weight. This group consisted of eight animals. Group 3 consisted of six animals that received intravenous furosemide (0.7 mg/kg body weight). Group 4 animals were treated with mannitol (1.0 gm/kg) as were those in Group 2, except that they were also given furosemide (0.7 mg/kg) 15 minutes after the mannitol infusion. This group of six animals were observed for up to 300 minutes, whereas the other experimental groups were sacrificed at 180 minutes.

These experimental groups are summarized in Table 1. The values are presented as the mean ± the standard error of the mean. Each animal served as its own control and the percentage change after treatment was based on values obtained during the experimental treatment period as compared to the stabilized values in the 40-minute pretreatment period. Analysis of the level of significance was based on a paired Student's t-test, where the changes in ICP and osmolality before and after treatment in Groups 2, 3, and 4 were compared to those in the untreated control animals in Group 1 (p ≤ 0.05 was considered significant).

Results

The 26 dogs that comprised the four experimental groups had pretreatment values for hematocrit, sodium, and potassium concentration in serum of 33.6% ± 2.9%, 132.2 ± 1.8 mM/liter, and 3.8 ± 0.9 mM/liter, respectively. The values for sodium and potassium did not significantly differ from measurements made during the first 180 or 300 minutes after treatment, although there usually was a transient fall in Na⁺ concentration centered around the period of greatest diuresis. Hematocrit measurements in animals receiving treatment steadily increased throughout the posttreatment period. The mean pretreatment blood pressure of 125/70 mm Hg was generally lower, but not significantly so, during the experimental period. The arterial pH, pCO₂, and pO₂ levels were allowed to stabilize in the 40-minute pretreatment period at mean values of 7.32 ± 0.01, 33.2 ± 1.6 mm Hg, and 115 ± 2.5 mm Hg, respectively, and remained reasonably constant following treatment. In a few instances, slight adjustments of the respirator were required. The initial serum osmolality was 297.0 ± 1.2 mOsm/kg, while that of CSF was found to be 301.5 ± 3.2 mOsm/kg. The CSF osmolality was taken

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>No. of Dogs</th>
<th>Intracranial Pressure (mm Hg)</th>
<th>Blood-Brain Osmotic Gradient (mOsm/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pretreatment</td>
<td>Min. Posttreatment</td>
</tr>
<tr>
<td>Group 1 (untreated controls)</td>
<td>6</td>
<td>33.2 ± 3.7</td>
<td>29.2 ± 2.6</td>
</tr>
<tr>
<td>Group 2 (mannitol, 1.0 gm/kg)</td>
<td>8</td>
<td>24.8 ± 3.9</td>
<td>11.0 ± 2.8 (p = 0.005)</td>
</tr>
<tr>
<td>Group 3 (furosemide, 0.7 mg/kg)</td>
<td>6</td>
<td>30.5 ± 3.9</td>
<td>22.3 ± 4.9 (p = 0.32)</td>
</tr>
<tr>
<td>Group 4 (mannitol + furosemide)</td>
<td>6</td>
<td>33.8 ± 1.6</td>
<td>12.7 ± 2.4 (p = 0.001)</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means. Figures in parentheses show significance of difference compared to Group 1 (untreated control group) by Student's paired t-test (p > 0.05 is not significant). Min. = minimum; Max. = maximum.
† Blood osmolality-cerebrospinal fluid osmolality.
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to reflect the osmolality of the cerebral extracellular fluid, since these compartments are in continuity and have an identical composition, although the changes are reflected more rapidly in the extracellular fluid than in the CSF compartment.

Group 1 animals did not receive any form of treatment for an ICP elevated to 33.2 mm Hg (Table 1). The fall in ICP of about 13% in this untreated group was not significant (Fig. 1A). The usual osmotic gradient that exists between the CSF or brain extracellular fluid (BEF) and blood (blood < CSF or BEF) was unchanged during the total experimental period.

Group 2 dogs demonstrated a rapid fall in ICP of 56.6% about 30 minutes after receiving the intravenous bolus of mannitol in a dose of 1.0 gm/kg (Fig. 1A). This fall in ICP was associated with a significant reversal of the blood-brain osmotic gradient from −3.4 to 38.5 mOsm/kg (Table 1). This reversal of some 40 mOsm at its peak (Fig. 1B) was primarily due to an increase in the serum osmolality which achieved its peak during the first 5 minutes after mannitol infusion (Fig. 1C). These changes in the osmolar gradient generally reverted back toward the normal relationship within 1 hour (Fig. 1B), while the effect on ICP was maintained for about 2 hours posttreatment (Fig. 1A). The increase in the rate of urine formation coincided with both the fall in ICP and rise in serum osmolality. The peak in urine formation of about 2.2 ml/min occurred at 15 minutes after mannitol infusion (Fig. 1D).

Group 3 animals, which were treated only with furosemide, showed no statistically significant change in ICP or blood-brain osmotic gradient in the posttreatment period (Table 1), as compared to the control animals (Group 1) or pretreatment values (Fig. 1A, B, and C). The urine output, however, was significantly increased from pretreatment levels but reverted to baseline levels within 60 minutes after administration of the diuretic agent (Fig. 1D).

Group 4 animals, which received an intravenous dose of furosemide 15 minutes after an intravenous bolus injection of mannitol (1.0 gm/kg), demonstrated a maximum fall in ICP of some 62.4% at about 30 minutes...
into the experimental period. The magnitude of the ICP decrease was greater than that observed when the animals received only mannitol (Table 1). Although the maximum decrease was greater in the combined therapy (Group 4), it was the prolongation of this fall that most characterized this group (Fig. 1). In all animals treated with both mannitol and furosemide, the reduction in ICP as compared to the pretreatment values of this group or the values in the control dogs (Group 1) is significant up to 5 hours posttreatment. It should be appreciated that the maximum decrease in ICP at any time period in Group 4 animals appears to be less than that of Group 2 animals (see Fig. 1A) only because the timing of this decrease tended not to be precisely the same in each individual animal in Group 4. It is for the same reason that the values for the maximum fall in ICP presented in Table 1 are greater than those in any time period in Fig. 1A. Table 1 presents the maximum mean values regardless of when they occurred posttreatment in the individual animal. The magnitude of the reversal in the blood-brain osmotic gradient (Table 1) and the enhanced serum osmolality in Group 4 dogs is similar to that found in the animals treated only with mannitol (Group 2), although the reversal (Fig. 1B) or enhancement (Fig. 1C) is continued for a longer period of time, which coincides with the prolongation of the decrease in ICP (Fig. 1A). The rate of urine formation in Group 4 animals is almost 15 times that of the control values and significantly greater than in dogs treated with either mannitol or furosemide alone (Fig. 1D). The urine osmolality was lower than the pretreatment values and those found in Group 2 animals.

Values for serum electrolytes were not significantly changed following the diuretic treatment. Except for a brief rise of less than 10 mm Hg in arterial blood pressure during the bolus injection, the vascular pressure remained stable throughout the experiments.

Discussion

This study supports the numerous clinical and laboratory investigations that have shown the effectiveness of osmotic diuretics in lowering normal and increased ICP in the presence of an intact blood-brain barrier (cerebral capillary).\(^{4,16,20-22,29}\) We have also shown that the osmotic gradient which normally exists between brain and blood is transiently reversed following a bolus injection of mannitol, yet is effective in significantly contracting the intracranial compartments for almost 2 hours. Since the endothelial junctions of the cerebral capillary and the cerebral cellular membranes provide a more formidable barrier to the passage of hydrophilic molecules than the blood-CSF barrier (choroidal epithelium), it is more likely that the van't Hoff (osmotic) pressure is greatest at the parenchymal interfaces, and therefore more water is removed from brain tissue than from the CSF compartment.\(^{1,6,17}\) The decrease in CSF formation with osmotic therapy, however, may still be significant, as shown from the alterations in CSF formation with hypertonic solutions,\(^{19}\) and the slow penetration of radiolabeled mannitol into the CSF as well as brain.\(^{28}\) In any event, when mannitol is given as a bolus, it effectively lowers ICP without resulting in significant changes in serum electrolyte concentration. This is in general agreement with a recent study in humans in which mannitol was used as the osmotic agent in a dose of 1.4 gm/kg.\(^{21}\)

The combination of mannitol and furosemide was shown to be a very effective treatment for increased ICP in the dog (Table 1). The reduction of ICP achieved with this treatment regimen was 62.4%, which is in the range of previously reported values of 60% to 74% using furosemide or ethacrylic acid.\(^{5,16,27}\) The quantitative data from the present study (Table 1 and Fig. 1A) also support the observation by Schettini, et al.,\(^{21}\) in human subjects that the degree and time course of brain shrinkage was greater with the combined treatment than was seen with mannitol alone. In our present study, we did not observe the transient but statistically significant fall in serum sodium that Schettini, et al., found in humans during the period of enhanced diuresis that followed combined therapy. This observation has suggested caution in the use of combined therapy under certain clinical conditions without proper electrolyte replacement therapy.\(^{28}\) It is unlikely that rapid correction of this transient hyponatremia will lead to central pontine myelinosis in most clinical cases treated with combined therapy, as the clinical syndrome is usually associated with alcoholism, prolonged hyponatremia, and rapid overcorrection with salt solutions. Our inability to demonstrate the same changes in serum electrolytes in dogs may be due to the lack of fluid replacement therapy in our animals which may have led to some hemoconcentration.

Various mechanisms have been suggested to explain the synergistic action of osmotic and loop diuretics in lowering ICP.\(^{21,27}\) The first of these is the effect of the loop diuretics (furosemide and ethacrylic acid) on CSF formation and the movement of sodium and water across the blood-brain barrier. This will be discussed later. The second and most likely mechanism for the synergy between mannitol and the loop diuretics is the action of these agents on the renal tubule so that the osmotic gradient established by the infusion of mannitol is maintained well beyond the time found with mannitol alone. The importance of the renal effect of furosemide in this phenomenon is supported in our study by the prolongation of the blood-brain osmotic gradient (Fig. 1B) and elevated serum osmolality (Fig. 1C) that resulted from the enhanced diuresis (Fig. 1D) following combined therapy. This effect is secondary to the secretion of furosemide (or ethacrylic acid) into the fluid of the proximal renal tubule. On reaching the distal (ascending) tubule, the drug blocks active chloride transport (the lumen into the tubular wall), thereby blocking reabsorption of all major cations and water.\(^7\) Since the urinary excretion of mannitol and its serum...
Blood-brain osmotic gradient

level would not be expected to be significantly altered by the loop diuretics (confirmed by kinetic studies using radiolabeled mannitol: PA Roberts, et al., unpublished data, 1983), it can be concluded that the sustained elevation of serum osmolality in the combined-therapy experiments was due to the loss of water in excess of solute in the distal tubule. There is little question of the enhanced diuresis in the combined-drug experiments (Group 4), with a urine osmolality that was less than in animals treated with mannitol alone. Although we propose this as the major explanation for the increased effectiveness of combined therapy, this does not minimize the possible additive effects of furosemide and ethacrynic acid (at appropriate doses) secondary to their effect on sodium and water transport across the blood-brain and blood-CSF (choroid plexus) barriers.

We were unable to confirm the reported usefulness of furosemide alone in lowering an elevated ICP in the doses used in this present study. Many clinical and experimental studies appear to indicate that the distal loop diuretics reduce ICP in the normal and edematous brain, but reports to the contrary are also available. In most studies in which these drugs have been effective, the doses were in the range of 5 to 50 mg/kg, although in a few instances a dose equal to that used in this present study was successful in reducing ICP. It has been suggested that furosemide decreases ICP by inhibiting the formation of CSF. Sahar and Tsipstein have demonstrated an inhibition of this secretory process in the range of 25% with low-dose therapy (0.7 mg/kg) and up to 94% at a dose of 20 mg/kg. McCarthy and Reed have reported a maximum inhibition of 50% with a furosemide dose of 50 mg/kg. Other studies, however, were unable to demonstrate any effect of this drug at low or high doses. At the dose used in our study, and the rate of inhibition reported by Sahar and Tsipstein, the volume of CSF produced would be expected to fall from a normal of 0.06 ml/min to 0.048 ml/min. It is unlikely that a decrease in CSF flow of 0.012 ml/min would lead to a significant reduction in ICP. This lack of effect on ICP was observed in our Group 3 animals. On the other hand, if the rate of inhibition (94%) found with high doses is used to compute the reduction of CSF formation that might occur with furosemide therapy, the resulting loss of CSF volume could possibly lead to a decrease in elevated ICP.

It has also been proposed that the loop diuretics lower ICP in normal and edematous brain by reducing water and ion penetration across the intact and altered blood-brain barrier. In some studies using high-dose therapy (50 mg/kg), penetration of both Na and water into an injured area is decreased as compared to findings in untreated animals, although there are similar studies with contrary results. Most investigators have assumed that furosemide reduces ICP by decreasing brain sodium levels and water transport as well as lowering CSF volume by adversely affecting cation transport into the ventricular cavity. More recently, it has been suggested that the elevated ICP associated with cerebral edema can be reduced by the removal of edema fluid via the ventricular system. This process presumably can be enhanced in response to inhibition of choroid plexus sodium transport. The proposed mechanism for this process is that the decrease in CSF secretion and volume that follows the inhibition of choroid plexus activity enhances the transepidermidal movement of edema fluid from brain into the ventricular cavity down a pressure gradient (brain greater than CSF). The edema fluid would then be subsequently absorbed into the blood via the arachnoid villi with a decrease in brain bulk. Although this is a reasonable notion as to the mechanism for the removal of edema fluid, there is some evidence that the ventricular system may not serve as a sink for cerebral edema fluid, and therefore this proposed mechanism by which furosemide lowers ICP requires further study before it can be accepted without doubt.

Another explanation for the effectiveness of furosemide in improving the development of cerebral edema is the effect of this drug on arterial pressure. Sklar has shown in cats that furosemide therapy (1.0 mg/kg) can lead to a fall in mean arterial pressure of about 10%, and therefore would decrease the edema front by reducing the cerebral perfusion pressure and the tissue pressure gradients. Millison, et al., also found a significant drop in arterial pressure (14%) with furosemide therapy. In this present study and the clinical study of Samson and Beyer, no permanent or significant changes in vascular pressure were noted. In spite of the conflicting evidence, most clinical studies support the usefulness of furosemide in decreasing brain bulk in a dose range usually 30 to 50 times the diuretic dose. Further experimental studies relating dose, kinetics of Na penetration into the brain and CSF, cerebral blood flow and volume, and ICP will be required before we can reconcile the conflicting laboratory evidence with the apparently favorable effect, in a clinical environment, of the loop diuretics on increased ICP.

References

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