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Laboratory Investigation

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Accumulated Mannitol and Aggravated Cerebral Edema in a Rat Model of Middle Cerebral Artery Infarction

Objective : Repeated administration of mannitol in the setting of large hemispheric infarction is a controversial and poorly defined therapeutic intervention. This study was performed to examine the effects of multiple-dose mannitol on a brain edema after large hemispheric infarction.

Methods : A middle cerebral artery was occluded with the rat suture model for 6 hours and reperfused in 22 rats. The rats were randomly assigned to either control (n=10) or the mannitol-treated group (n=12) in which intravenous mannitol infusions (0.8 g/kg) were performed six times every four hours. After staining a brain slice with 2,3,5-triphenyltetrazolium chloride, the weight of hemispheres, infarcted (IH) and contralateral (CH), and the IH/CH weight ratio were examined, and then hemispheric accumulation of mannitol was photometrically evaluated based on formation of NADH catalyzed by mannitol dehydrogenase.

Results : Mannitol administration produced changes in body weight of −7.6±1.1%, increased plasma osmolality to 312±8 mOsm/L. It remarkably increased weight of IH (0.77±0.06 gm versus 0.68±0.03 gm : $p<0.01$) and the IH/CH weight ratio (1.23±0.07 versus 1.12±0.05 : $p<0.01$). The photometric absorption at 340 nm of the cerebral tissue in the mannitol-treated group was increased to 0.375±0.071 and 0.239±0.051 in the IH and CH, respectively from 0.167±0.082 and 0.162±0.091 in the IH and CH of the control group ($p<0.01$).

Conclusion : Multiple-dose mannitol is likely to aggravate cerebral edema due to parenchymal accumulation of mannitol in the infarcted brain tissue.

KEY WORDS : Brain edema · Cerebral infarction · Mannitol · Mannitol dehydrogenase · Rat · Middle cerebral artery.

INTRODUCTION

Osmotic therapy with mannitol is effective in reducing ICP by its rheological and osmotic effects[5,7,12,17]. Theoretically, its osmotic effect is produced in the brain with an intact blood-brain barrier (BBB) and extravasation of mannitol through a disrupted BBB may exacerbate vasogenic brain edema. Despite its known pharmacokinetics, the effects of multiple-dose mannitol infusions on brain edema in the setting of cerebral infarction still remain contradictory[6,8,9,13,14].

The present study was performed to evaluate the effect of multiple-dose mannitol on a brain edema in a rat model of middle cerebral artery (MCA) infarction by measuring hemispheric weight and accumulated mannitol in the hemisphere using mannitol dehydrogenase.

MATERIALS AND METHODS

Right MCA occlusion was performed with the rat suture model. Twenty-two adult male Sprague-Dawley rats weighing 290 to 320 g were used in this study. Enflurane, mixed with oxygen and nitrogen, was delivered through a nose cone with the use of a flow regulator. An uncoated 30-mm-long segment of 3-0 nylon monofilament suture with the tip rounded by flame was inserted into the stump of the common carotid artery and advanced into the internal carotid artery ~19-20 mm from the bifurcation to occlude the ostium of the MCA[18]. The rats were allowed to recover from anesthesia after skin suture. At the end of the 6 hours of MCA occlusion, the animals were re-anesthetized and the suture occluding the MCA was removed, and the animals were allowed to recover from anesthesia again. During surgery, rectal temperature was maintained at 37°C throughout the procedure with a heating lamp and heating pad connected to a rectal thermistor. The animals were randomly assigned to either control (n=10) or the mannitol-treated group (n=12).

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A 25% solution of mannitol was delivered manually via an indwelling catheter (24G, 0.75-in Angiocath) in a tail vein at a rate of 0.2 mL/min. Mannitol (0.8 g/kg of body weight) infusions were started by intravenous bolus injection after completion of 6-hour MCA occlusion and repeated every 4 hours thereafter, performing a total of 6 bolus injections (4.8 g/kg of body weight). The animals in the mannitol-treated group were sacrificed 4 hours after the last mannitol injection, 24 hours after completion of the MCA occlusion. Control animals were also sacrificed 24 hours after completion of the MCA occlusion. The rat was weighed for comparison with initial body weight, and a sample of blood was processed for determination of plasma osmolality immediately before sacrifice. Rats of both groups were allowed to have food and water freely during the experiment to reduce dehydration.

% Hemispheric infarct ratio and hemispheric weight

The rats were perfused with saline, and then brains were harvested. A 2 mm-thick coronal slice of each brain was sectioned at 3 mm distal from the frontal pole with a brain slicer (Activational System Co, Inc, USA) and was immersed in 2% 2,3,5-triphenyltetrazolium chloride (TTC) solution for 30 minutes at 37°C[2]. With TTC staining, viable brain tissue stained red whereas infarcted tissue remained pale. In order to assess extent of hemispheric infarction before edematous change, we used the % hemispheric infarct ratio that was calculated from the formula :

$$\% \text{ hemispheric infarct ratio} = (a-b) \div a \times 100$$

Where b is the stained, viable area of a hemisphere ipsilateral to infarction (IH), and a is the area of a contralateral hemisphere (CH) (Fig. 1).

After the brain tissue was divided into two through the corpus callosum including the coronal slice stained with TTC, each hemispheric tissue was weighed, and then the ratio of the weight of the IH to that of the CH was calculated as an indicator of midline brain shift and cerebral herniation. The hemispheric brain tissue was kept frozen with liquid nitrogen at -70°C for later mannitol measurement.

Photometric measurement of mannitol in the cerebral hemispheres

The frozen brain tissue was triturated and deproteinized with trichloroacetic acid. The supernatant was mixed with NAD^+ and mannitol dehydrogenase (Sigma Chemical Co., USA), and then incubated at pH 10 and at 37°C for 60 minutes.

The photometric analysis of mannitol was performed based on its oxidation to fructose and the concomitant formation of NADH catalyzed by NAD^+-dependent mannitol dehydrogenase (MDH)[4,19] :

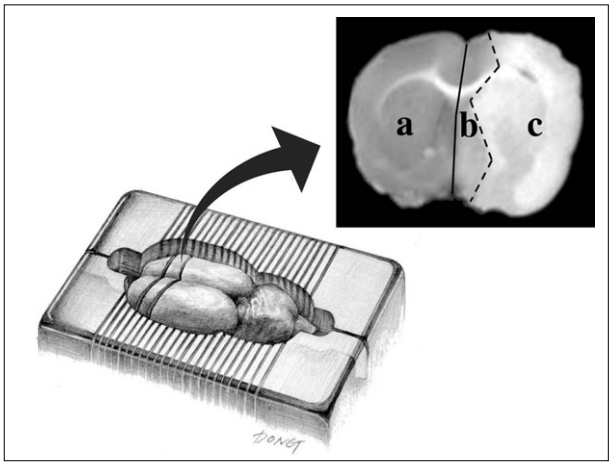

Fig. 1. Photograph showing a coronal slice sectioned at 3 mm distal from the frontal pole and stained with 2,3,5-triphenyltetrazolium chloride. % hemispheric infarct ratio=(a−b)÷a×100 where b is the stained, viable area of an ipsilateral ischemic hemisphere, and a is the area of a contralateral hemisphere.

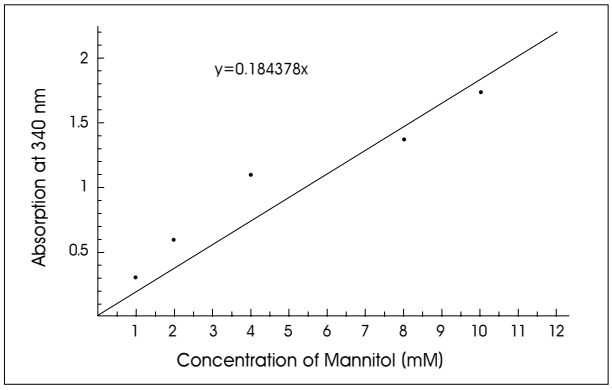

Fig. 2. Mannitol standard curve. Sample mannitol concentrations and the corresponding net absorption measured at 340 nm are given.

$$\text{D-Mannitol} + NAD^+ \xrightarrow{\text{MDH}} \text{D-Fructose} + \text{NADH} + H^+$$

As such, spectrophotometric absorption at 340 nm is determined by the NADH formed by oxidation of mannitol in addition to the natural NADH in the brain tissue. Thus, increase in the absorption at 340 nm from the infarcted and contralateral hemispheres of the control group determined parenchymal accumulation of mannitol. A mannitol standard curve was made by measuring absorption at 340 nm of test samples containing 1 mM, 2 mM, 4 mM, 8 mM, and 10 mM mannitol and linear (Fig. 2).

Statistical analysis

The data in this report are presented as mean ± standard deviation. T-test was used to compare such values as % change in total body weight, plasma osmolality, % hemispheric infarct ratio, hemispheric weight, IH/CH weight ratio, photometric absorption at 340 nm between control and mannitol-treated groups and between ipsilateral infarcted and contralateral

Table 1. Comparison of mannitol-treated and control rats after 6-hour occlusion of right middle cerebral artery*

Characteristic	Control (n=10)		Mannitol-treated (n=12)	
	CH	IH	CH	IH
% change in body weight	−3.3±0.7†		−7.6±1.1	
Plasma osmolality	301±4†		312±8	
% hemispheric infarct ratio†	67.6±13.0		69.6±16.2	
Hemispheric weight (g)	0.59±0.03§	0.68±0.03**	0.62±0.05§	0.77±0.06
IH/CH weight ratio	1.12±0.05†		1.23±0.07	
Absorption at 340 nm	0.162±0.091‖	0.167±0.082**	0.239±0.051§	0.375±0.071

*Values are expressed as means±SDs. †% Hemispheric infarct ratio=(a−b) / a, where b is the stained, viable area of an infarcted hemisphere, and a is the area of a contralateral hemisphere. ‡Significant difference ($p<0.01$) in values between control and mannitol-treated groups. §Significant difference ($p<0.01$) between CH value and IH value. ‖Significant difference ($p<0.05$) in CH values or IH values between control and mannitol-treated groups. **Significant difference ($p<0.01$) in CH values or IH values between control and mannitol-treated groups. Abbreviations : CH=hemisphere contralateral to infarction; IH=hemisphere ipsilateral to infarction

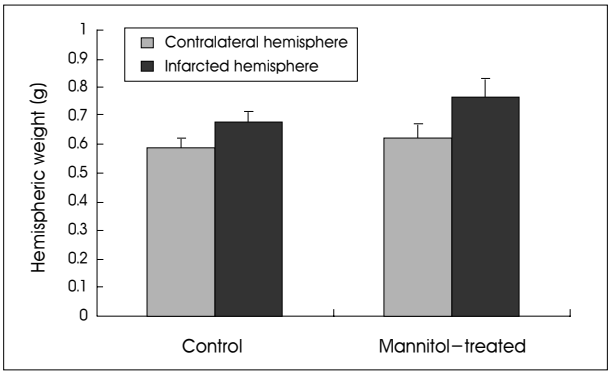

Fig. 3. Graph showing weight of hemispheres ipsilateral (IH) and contralateral (CH) to middle cerebral artery infarction. Multiple mannitol infusions remarkably increased weight of IH and the IH/CH weight ratio.

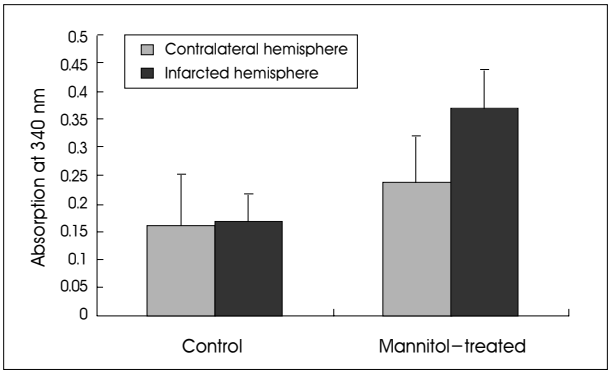

Fig. 4. Graph showing spectrophotometric absorption at 340 nm of hemispheres ipsilateral and contralateral to middle cerebral artery infarction.

hemispheres. Differences were considered significant at values of $p<0.05$.

RESULTS

Physiological parameters and % hemispheric infarct ratio

Free water intake avoided severe dehydration. MCA infarction with and without mannitol administration produced changes in body weight of −6.6±1.1% and −3.3±0.7%, respectively over the course of 24 hours. Plasma osmolality was increased minimally and moderately to 301±4 mOsm/L and 309±8 mOsm/L, respectively in the control and mannitol-treated groups.

The coronal slice stained with TTC demonstrated complete MCA infarction in all rats. The % hemispheric infarct ratio was 67.6±13.0 % in the control group and 69.6±16.2 % in the mannitol-treated group. Thus, both groups were composed of rats with similar territory of MCA infarction and comparable in evaluating the effect of mannitol on cerebral edema following infarction.

Weight of cerebral hemispheres and IH/CH weight ratio

Cerebral hemispheres ipsilateral to MCA infarction were heavier than contralateral hemispheres in the control group (0.68±0.03 gm versus 0.59±0.03 gm : $p<0.01$). The IH/CH weight ratio in the control group was 1.12±0.05. In the mannitol-treated group, weight of IH was much greater than the CH (0.77±0.06 gm versus 0.62±0.05 gm : $p<0.01$). The IH/CH weight ratio was increased to 1.23±0.07.

Multiple mannitol infusions remarkably increased weight of IH (0.77±0.06 gm versus 0.68±0.03 gm : $p<0.01$) and the IH/CH weight ratio (1.23±0.07 versus 1.12±0.05 : $p<0.01$), whereas the weight of contralateral hemispheres was not changed significantly (Table 1), (Fig. 3).

Hemispheric concentration of mannitol

Photometric absorption at 340 nm of the brain tissue in the control group is determined by natural NADH concentration before and after cerebral infarction without mannitol administration. The absorption of the CH and IH in the control group was 0.162±0.091 and 0.167±0.082, respectively. However, the difference did not reach statistical significance.

The absorption at 340 nm of the cerebral tissue in the mannitol-treated group was increased in both IH and CH. The mannitol administration increased the absorption to 0.239±0.051 in the CH, and the absorption in the IH was much increased to 0.375±0.071. The difference reached statistical significance ($p<0.01$). An increase from the mean absorption at 340 nm of the control group was 0.208±0.071 in the IH and 0.077±0.051 in the CH (Table 1), (Fig. 4).

Hemispheric concentration of mannitol was determined by the increase in the photometric absorption using the

mannitol standard curve. The concentration of mannitol was 1.13±0.39 mmol/L in the IH and 0.42±0.28 mmol/L in the CH. Multiple mannitol infusion in this study led to mannitol accumulation in both hemispheres, ipsilateral and contralateral to the MCA infarction. However, hemispheric concentration of mannitol in the IH was approximately three times greater than that in the CH.

DISCUSSION

The intravenous administration of mannitol, a hyperosmolar agent, has become a cornerstone of medical management of increased intracranial pressure (ICP), which can be caused by severe head injury, spontaneous subarachnoid hemorrhage, intracerebral hemorrhage, cerebral infarction, and other space occupying lesions. Mannitol is thought to decrease ICP by reducing blood volume due to vasoconstriction and decreasing cerebral water content and cerebrospinal fluid volume. The first effect of mannitol is rheological. An immediate expansion of intravascular volume lowers the hematocrit and blood viscosity while increasing cerebral blood flow and cerebral oxygenation which lead to vasoconstriction and decrease in ICP[5,12,17]. The second effect is an osmotic effect to shift brain tissue water into the intravascular space and reduce cerebral extracellular free water by the establishment of an osmotic gradient along the blood-brain barrier[6,7]. The third is to decrease production and reabsorption of CSF with an overall decrease in the volume of CSF[3]. In the meanwhile, intraarterially administered mannitol has been used as a permeabilizer to open the bold-brain barrier to increase the delivery of various molecules to the brain parenchyma.

Mannitol can be administered intravenously for deteriorating patients due to cerebral edema and increased ICP after large hemispheric infarction[1]. However, the effect of multiple-dose mannitol on cerebral edema is contradictory. Extravasation of mannitol through a disrupted BBB in the infarcted brain tissue has been the focus of contradictory studies against the effectiveness of mannitol[6,8,9], whereas systemic dehydration caused by osmotic diuresis reduced the brain water content in the edematous brain in some animal studies[13,14]. The brain with a disrupted BBB is primarily affected by a systemic dehydration whereas the brain with an intact BBB is affected by osmotic cerebral dehydration.

Extravasation of multiple-dose mannitol aggravating vasogenic cerebral edema was demonstrated in a cat model of a cortical cold injury by Kaufmann et al.[6] However, in the study of Paczynski et al.[14] using a rat model of MCA infarction, low dose (0.5 g/kg, four times) mannitol reduced the water content of edematous brain. Regarding to this controversy, the authors attempted to evaluate the effect of multiple-dose mannitol on the infarcted rats with allowance of minimal dehydration.

In the current study, the concentration of mannitol of the brain tissue was photometrically evaluated based on formation of NADH catalyzed by NAD^+-dependent MDH. The NADH preexisting in the brain tissue before mannitol administration was determined with photometrical absorption at 340 nm of the control group. The redox state of NAD/NADH is an assessment of mitochondrial function, and cerebral ischemia results in the increase in the level of NADH in the previous studies[11,16,20]. In the current study, photometrical absorption of the infarcted hemispheres was increased from that of contralateral noninfarcted hemispheres, reflecting increase in the NADH after cerebral infarction, although it was not statistically significant.

Brain edema following an MCA infarction results in increase in volume and weight of the ipsilateral hemispheres due to an increase in water content of the infarcted cerebral tissue. The brain edema can be assessed by several methods. Brain water can be directly quantitated by the wet-dry weight method or the gravimetric technique[6,10,13,14]. Increase in volume of infarcted hemisphere can be assessed with midline brain shift and other morphological and volumetric evaluations. The difference in weight between the infarcted and contralateral hemispheres is proportional to the volume of hemispheric infarction, and can be used to assess the extent of cerebral infarction and edema[15]. The authors used the IH/CH weight ratio as an index of cerebral weight asymmetry and of brain tissue shifts.

In patients with malignant hemispheric infarction, mannitol can be clinically used at 0.25 to 0.5 g/kg, and can be given every 6 hours. However, the present study suggests mannitol administration as a temporizing measure before patients undergo decompressive craniectomy or early decompressive craniectomy without mannitol administration.

CONCLUSION

In the setting of large hemispheric infarction in the rats, repeated administration of mannitol increased hemispheric weight of infarction and the IH/CH weight ratio aggravating brain tissue shift, in accordance with accumulation of mannitol in the infarcted brain tissue. The current study suggests that multiple-dose mannitol is likely to aggravate cerebral edema due to parenchymal accumulation of mannitol in the infarcted brain tissue.

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