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DEEP CERVICAL LYMPH FLOW FOLLOWING THE INFUSION OF MANNITOL IN RABBITS

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Summary

Jugular lymph flow of anesthetized rabbits in response to infusion of mannitol solutions differing in osmolarity were measured. Either an isotonic (310 mosmol), hypotonic (100 mosmol), or hypertonic (605 mosmol) mannitol solution was infused into either the internal carotid artery (ICA) or the right lateral ventricle (RLV). Lymph was collected continuously and measured over a 60 min preinfusion period, as well as during mannitol infusion and intermittent recovery periods. The mean peak flow rates of hypertonic infusion for the first 30 min via ICA and RLV were 2.2 ± 0.4 (12% decrease) and $5.0\pm1.0\ \mu$l/min (72% ncrease), over those of isotonic infusates which were $2.5\pm0.3\ \mu$l/min (via ICA) and $2.9\pm0.5\ \mu$l/min (via RLV), respectively. In contrast, lymph flow rates of hypotonic infusate for the first 30 min via ICA and RLV were $3.9\pm0.8\ \mu$l/min and $2.3\pm0.4\ \mu$l/min, respectively. A decrease both in intracranial pressure and in lymph flow following hypertonic mannitol infusion via ICA were observed. However, intracranial pressure and lymph formation were increased following hypertonic infusion via RLV. The results indicate that the changes in jugular lymph flow could be affected by the changing in osmolarity of mannitol infusate.

Key Words: cervical lymph flow, osmolarity, mannitol infusion

Although it is generally considered that the central nervous system (CNS) contains no lymphatics, cerebrospinal fluid (CSF) or cerebrointerstitial fluid (CIF) may be associated with and fulfill some lymph functions for the brain and the spinal cord. The existence of a relationship between CSF or CIF and the lymphatic system is further strengthened by studies measuring the amount of CSF or CIF draining into the deep cervical lymphatic system in some species (1,2,3,4). Moreover, many substances after intracerebral (1), magna cisternal (4), or intraventricular (5) injection, were subsequently found in varying concentrations in the cervical lymph trunk.

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A possible route connecting the lateral ventricle or cerebral nucleus and cervical lymph has been suggested as running along the perineural spaces of the olfactory nerve, and around the cribriform plate to the nasal submucosa (1,3,4).

Further evidence from studies showing that cervical lymphatics contribute to the clearance of both CSF (4,5) and CIF (1) from the CNS suggests that the lymphatics may act as a major drainage pathway for CIF. In earlier study (6), we noted that the amount of CIF could be influenced by the changes of saline osmolarities either in the internal carotid artery (ICA) or in the right lateral ventricle (RLV), and consequently resulted in a change in cervical lymph flow. It was suggested that alteration of cell permeability of the cerebral vessel wall and the choroid epithelia following saline infusion changed the deep cervical lymph flow (6). However, according to the model for brain edema proposed by Rapoport (7,8), CIF formation depends on capillary osmotic and hydrostatic pressures, tissue compliance, and tissue hydraulic conductivities. Mannitol infusate can pass through the blood-brain barrier or blood-CSF barrier under acute hypertension with adrenergic agents and hence it may influence CIF formation and drainage (9,10). Many papers concerning lymph flow in tissues other than those of the brain have been reported (11,12,13,14,15,16,17,18). Few have carried out investigations on the effect of anisotonic solutions on cervical lymph flow (6). At present, there is little literature regarding the effect of nonelectrolyte mannitol on cervical lymph flow. This paper explores quantitative changes in deep cervical lymph flow during infusion of different osmolarities of mannitol solution via ICA and RLV in anesthetized rabbits.

Methods

Internal carotid artery infusion and intraventricular microinfusion of mannitol.
The details of the procedures of animal preparation, lymph cannulation and collection are essentially the same as described in great detail in the previous publication (6). Therefore, only mannitol infusion is described. The external carotid artery was centripetally cannulated with the catheter opening placed 1 cm distal to the carotid bifurcation. The blood flow in the internal carotid was not interrupted during the operation procedure. Each of the various mannitol solutions was infused into internal carotid artery over a 30 min period at a constant flow rate of 0.22ml/min/Kg, while the common carotid artery was temporarily clamped to prevent reverse flow into the aorta. After lymphatic cannulation, the anesthetized animal was turned over to the prone position, and a cannula inserted into each of the two lateral ventricles (right side for infusion and left side for recording pressure) through a drill-hole in the skull located (with reference to a stereotaxic atlas) at a point 7mm behind the coronal suture and 6.5mm from the midline (19). The extra-cranial body of the cannula was cemented to the skull surface with glue. At insertion, the cannulae were full of artificial-CSF equilibrated at 95% O_2 and 5% CO_2. Each mannitol solution (0.75ml/Kg) of different osmolarity in artificial-CSF was infused into the RLV over a 30 min period. The left brain cannula was connected to a pressure transducer in order to record intracranial pressure (ICP).

Experimental protocol
After preinfusion, a volume of mannitol (warmed to body temperature) was infused into either ICA or RLV over a 30 min period. Note that Evans blue dye (2%; 1.0ml) was injected into the femoral vein 5 min prior to mannitol infusion in some animals. Lymph was continuously collected and arterial blood pressure, intracranial pressure, and respiratory rate were monitored for an additional 60 min. The sequence of 30-min infusion followed by a 60-min recovery period

was repeated for each mannitol osmolarity. Finally, brains were removed 20 min after the last infusion and examined under a microscope. The osmolarities of isotonic, hypotonic, and hypertonic mannitol, as measured by freezing point depression with an osmometer (Advanced Instrument), were 310, 100, and 605 mosmols/Kg mannitol solution, respectively. Results were expressed as mean±S.E.M. and the statistical significance was determined by Dunnett's t-test.

Results

Lymph flow rate after hypertonic mannitol infusion via RLV was much greater than that of isotonic or hypotonic infusion in which lymph flow rate was the least. Lymph flow rate increased gradually at first during hypertonic infusion, and then increased sharply. On the other hand, after either isotonic or hypotonic infusion, lymph flow rate was seen to steadily increase. The changes in lymph flow rate during the preinfusion period (30-90 min) and the subsequent infusion of isotonic (91-180 min), hypotonic (181-270 min), and hypertonic (271-360 min) mannitol via ICA or RLV are listed in Table 1. The lymph flow rate at the end of the 30-min infusion of isotonic, hypertonic, and hypotonic mannitol via RLV increased to a mean peak of 2.9±0.5, 5.0±1.0, and 2,3±0.4 μl/min, respectively, and then gradually returned to the preinfusion level of 1.8±0.5 μl/min. Only the hypertonic mean peak value was shown to be significantly different from that of isotonic mannitol. The changes in lymph flow rate for each of the osmolarities infused via ICA are opposite to those infused via RLV. Thus, the lymph flow rate for hypotonic infusion via ICA was much greater than that for either isotonic or hypertonic infusion in which lymph flow rate was the least. The mean average lymph flow rate for hypotonic mannitol infusion via ICA was 3.9±0.8 μl/min, and was significantly higher than that of isotonic infusion (2.5±0.3 μl/min).

TABLE I

Changes in lymph flow rate, arterial pressure, intracranial pressure, and respiratory rate during various mannitol infusions[a]

Infusion route (via)	Infusate mannitol	Lymph flow rate (μl/min)	Arterial[b] pressure (mmHg)	Intracranial[b] pressure (mmHg)	Respiratory rate (cycles/min)
Internal carotid artery (n=10)	Preinfusion	1.7±0.4	96±5	5±3	29±3
	Isotonic	2.5±0.3	92±6	6±3	42±4
	Hypotonic	3.9±0.8*	102±6	8±3	49±6
	Hypertonic	2.2±0.4	85±5	3±1*	45±5
Right lateral ventricle (n=10)	Preinfusion	1.8±0.5	92±5	5±2	40±3
	Isotonic	2.9±0.5	98±6	8±4	45±5
	Hypotonic	2.3±0.4	103±4	7±3	47±4
	Hypertonic	5.0±1.0*	82±5	12±4*	58±6*

a:Data are mean values±S.E.M at the end of the 30-min infusion period.
b:Indicates the mean pressure between systolic and diastolic pressures.
*P<0.05 significantly compared to isotonic infusion. Intracranial pressure(ICP) was measured in the left lateral ventricle of the brain. Osmolarity of isotonic, hypotonic, and hypertonic mannitol solutions were 310, 100, and 605 mosmols, respectively.

Note that only the right side brain cortex was stained by Evans blue dye after either in hypertonic or isotonic infusion via RLV. The appearance time of staining was shorter and the concentration of dye was higher in the collected lymph during hypertonic infusion as compared to those of isotonic infusion. Neither brain staining nor lymph contamination was found during infusion via ICA. The changes in arterial blood pressure, intracranial pressure, and respiratory rate during mannitol infusion are also summarized in Table 1. Both intracranial pressure and respiratory rate rose gradually following hypertonic mannitol infusion via RLV, achieving levels significantly higher than those resulting from infusion via ICA. In fact, by the end of infusion via RLV, there were no significant differences in the changes in intracranial pressure, respiratory rate or arterial pressure between the hypotonic and isotonic groups. Moreover, venous blood pressure remained unchanged during all infusions. On the other hand, both intracranial pressure and arterial blood pressure were gradually increased during hypotonic infusion via ICA. In the meantime the lymph flow increased sharply. However, it was a significant decrease in intracranial pressure during intraarterial hypertonic mannitol infusion.

Discussion

It has been demonstrated by some investigators that substances, particles or ions can pass from the cerebral ventricle through brain interstitial fluid into lymph ducts in the cervical region (4,5). Investigators consider the cervical lymphatics as a major drainage pathway (30-35% in the rabbit) for cerebrointerstitial fluid (1) or CSF (5) and believe that a route must exist from the interstitial fluid to the lymph (1). A possible drainage route of cerebrointerstitial fluid into deep cervical lymph is along the olfactory nerve, around the cribriform plate, and through the perineural spaces of nasal submucosa (3,4,5). It has been stated that hypertonic saline (5M) induced the opening of blood-brain barrier(20). A previous study (6) demonstrated that administration of hypertonic saline could increase cerebrovascular permeability, resulting in an enhanced cervical lymph flow. However, when an osmotic diuretic such as mannitol was used as the infusate as in the present study, the lymph flow rate was shown decrease during infusion of hypertonic manniotl via ICA, while it was significantly increased during infusion via RLV. Contrarily, lymph flow rate increased significantly when hypotonic mannitol was infused via ICA and decreased slightly when it was infused via RLV, as cpmpared to those of isotonic infusates.

Cervical lymph flow, as stated in previous study (6), can be affected by fluid formation in either the brain extracellular space or around the perineural space in nasal submucosa, not to mention the force that drives the fluid into lymphatic vessels. The formation of cerebrointerstitial fluid, as proposed by Rapoport (7,8), is determined by capillary osmotic and hydrostatic pressures, capillary and tissue hydraulic conductivities, as well as tissue compliance. The interrelation between these factors may be influenced in a different way by any one of the three (isotonic, hypotonic, and hypertonic) mannitol solutions examined in this study. As stated by Murphy and Johanson (9), there are two ways for nonelectrolyte mannitol to cross either the blood-brain or CSF-brain barrier, diffusion and perhaps pinocytosis. Indeed, two of the most important processes for increasing fluid formation in brain spaces (that is, increasing intracranial pressure) are diffusion and osmosis. Intracerebroventricular infusion of hypertonic mannitol solution may increase ventricular hydrostatic pressure which subsequently leads to flow of infusate into the brain parenchyma, resulting in minimal water flow into brain interstitial space (9). Moreover, a hypertonic infusate in the ventricles can pass through the CSF-brain barrier and causes removal of water from the blood, increasing CSF, and thus cerebrointerstitial fluid volume and intracranial pressure. This was evidenced by staining brain cortex and contaminating lymph fluid with Evan's blue dye in the animal infused with hypertonic mannitol via RLV. Since lymph

formation depends primarily on cerebrointerstiital fluid volume (as indicated by intracranial pressure), which is the largest in hypertonic mannitol infusion via RLV. Therefore, lymph flow rate is the greatest during intraventricular infusion of hypertonic mannitol. On the other hand, ICA infusion of hypertonic mannitol solution, which under normotension conditions cannot permeate cerebral capillary walls (9), will cause an increase in capillary osmotic pressure. This will result in a marked deprival of brain water content, and thus a reduction in intracranial pressure and lymph flow. Basically, infusion of hypertonic mannitol via ICA tends to cause water to leave the brain and enter the blood via capillary, as evidenced by the fact that it decreases renal reabsorption and reduces blood pressure. It is reasonable to assume that the ICA infusion of hypertonic mannitol could cause more water to leave the brain by osmosis than enter it by hydrostatic pressure. However, this situation is inverted during infusion of hypotonic mannital via ICA. The origin of the additional fluid (lymph) during intraarterial infusion of hypotonic mannitol may result from the more water entering the brain by hydrostatic pressure than it leaving by osmosis at cerebral capillary walls.

In summary, the present study has demonstrated that an increase in brain ventricle osmotic pressure during hypertonic mannitol infusion drives CSF into brain interstitial space, induces the formation of a large volume of cerebrointerstitial fluid. From here, the brain fluid may pass along such perivascular spaces as the subarachnoid space, and olfactory nerve or perineural spaces, to the cervical lymphatics. Thus, lymph flow rate was the greatest after hypertonic mannitol infusion via RLV. It is concluded that lymph flow in the deep cervical lymph trunk can be affected by changes in brain interstitial fluid volume resulting from changes in osmotic pressure, induced by infusion of different osmolarities of mannitol solution.

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