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Prevention of Demyelination Induced by Rapid correction of Hyponatremia in Mice

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Abstract: Central pontine myelinolysis is a serious human demyelinative disorder commonly associated with rapid correction of hyponatremia. Although its precise etiology remains unclear, the blood-brain barrier (BBB) disruption following rapid increase in serum Na^+ considered having an important role in the process. In the present study, therefore, we investigated the preventive effects of various drugs that have been reported to affect the BBB permeability, on osmotic demyelination induced by rapid correction of hyponatremia in mice. Hyponatremia was induced by liquid diet feeding and subcutaneous infusion of 1-deamino-8-D-arginine vasopressin, a vasopressin V2 receptor agonist. After 3 days, serum Na^+ was rapidly corrected with hypertonic saline injection. The following drugs were given subcutaneously just before and 5 hours after the correction. Mice in the control group showed serious neurological impairments and most of them died after the correction. Brain sections revealed marked demyelinative lesions in various areas of the brain. However, in the mice treated with aminoguanidine or 7-nitroindazole, a nitric oxide synthase inhibitor, neurological impairments were improved and the survival rates for 24 hours after the correction were markedly increased. Indomethacin, cimetidine and dexamethasone also increased the survival rates after rapid correction. Results from the present study suggest that these drugs may be useful in the prevention of osmotic demyelination after rapid correction of hyponatremia.

Key words: hyponatremia, demyelination, blood-brain barrier

Central pontine myelinolysis (CPM) is a serious and often fatal human demyelinative disorder commonly associated with rapid correction of hyponatremia, especially if the hyponatremia is chronic.[1)] It is generally accepted that rapid correction of hyponatremia increases the risk for developing CPM. However, it has not been concluded about the appropriate rate of correction to avoid the onset of CPM, and it may not be easy to increase the serum Na^+ as slowly as planned. Therefore, it is important to establish a measure that can effectively prevent the onset of CPM even if the correction was made rapidly. Although the precise etiology of CPM remains unclear, there are accumulating evidences that the blood-brain barrier (BBB) disruption following rapid correction of hyponatremia plays a critical role in the process. Rapid increase in serum Na^+ causes intracellular to extracellular water shifts in brain vascular endothelial cells resulting in the cell shrinkage and the BBB disruption.[2,3)] Under these circumstances, circulating neurotoxic factors such as complement, lymphocytes, cytokines and vasoactive amines could gain access to the central nervous system and contribute to the subsequent demyelination.[4)] It has been reported that nitric oxide (NO), prostaglandins (PGs) or histamine is involved in the regulation of BBB permeability, and that a NO synthase (NOS) or a PGs synthetase inhibitor, a histamine H_2 receptor antagonist or glucocorticoid prevents the BBB disruption induced in various conditions such as heat stress, brain ischemia or infection.[5-12)] These findings lead us to the assumption that these drugs may also be useful in the prevention of the BBB disruption following rapid increase in serum Na^+ and inhibit the onset of demyelinative lesions. In the present study, therefore, in order to establish a useful preventive measure of CPM, we evaluated the effect of aminoguanidine; an iNOS selective inhibitor, 7-nitroindazol (7-NI); a selective nNOS inhibitor, indomethacin; a PGs synthetase inhibitor, cimetidine; a histamine H_2 receptor blocker or dexamethasone on the osmotic demyelination induced by rapid correction of hyponatremia in mice.

Materials and Methods

Male ICR mice (Chubu Science Materials, Nagoya, Japan) weighing 30–35 g were maintained under the controlled conditions (24°C, lights on 0600–1800 h). Hyponatremia was induced in mice by feeding liquid diet (10% glucose, 0.2%

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NaCl and 0.15% KCl) and subcutaneous (sc) injection of 1-deamino-8-D-arginine vasopressin (dDAVP), a vasopressin V2 receptor agonist (2 μg per mouse, twice daily; Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan). On day 4 of hyponatremia, serum Na^+ levels were rapidly corrected by an intraperitoneal injection of 1.0 M NaCl (1.5 ml/100 g of body weight). The following drugs were injected sc in a volume of 0.3 ml/mice twice (just before and 5 hours after the hypertonic saline injection). Doses of the drugs were determined by the preliminary studies. 1) Aminoguanidine (200 mg/kg of body weight; Sigma, St. Louis, MO) or 7-NI (40 mg/kg of body weight, Sigma), 2) indomethacin (7 mg/kg of body weight; Sigma), 3) cimetidine (10 mg/kg of body weight; Sigma), 4) dexamethasone (2 mg/kg of body weight; Sigma). Aminoguanidine and cimetidine were dissolved in saline. Indomethacin and dexamethasone were dissolved in DMSO and then diluted with saline. 7-NI was suspended in corn oil with sonication. Vehicle solutions were used as control for each experiment. Blood samples for serum Na^+ determination were obtained via tail transection under light diethyl ether anesthesia. Serum Na^+ was measured using an auto analyzer (Hitachi Ltd., Tokyo, Japan). Mice were sacrificed by transcardial perfusion with 4% paraformaldehyde 3 days after the correction of hyponatremia if they survived till then. Brains were sectioned and stained with Luxol fast blue and cresyl violet to evaluate the demyelinative lesions. All procedures were performed in accordance with institutional guidelines for animal care at Nagoya University, which conform to the NIH animal care guideline.

Results

Mice were made severely hyponatremic by a combination of liquid diet feeding and dDAVP injection. Hypertonic saline injection rapidly increased serum Na^+ levels by around 20 mEq/l within 24 hours (table 1). After hypertonic saline injection, mice in the control groups showed serious neurological impairments and many of them died within 24 hours. Fig. 1 shows typical demyelinative lesions in the brain taken from the mouse that survived for 3 days after the correction although manifesting serious neurological impairments. Demyelinative lesions were observed in various areas of the brain and most marked in the subcortical white matter as shown in Fig. 1. On the other hand, in the mice treated with aminoguanidine or 7-NI, neurological symptoms were markedly improved and most mice survived for 24 hours after rapid correction (Fig. 2A). Indomethacin also markedly improved the neurological impairments and most of the indomethacin-treated mice could survive for 24 hours after the correction (Fig. 2B). In the next experiment, all of the control mice died within 24 hours after the correction, while a half of mice treated with cimetidine survived for that period (Fig. 2C). The neuroprotective effect of dexamethasone was so prominent that all mice survived while more than a half of control mice died within 24 hours after the correction (Fig. 2D).

Table 1 Serum Na^+ levels before and after rapid correction of hyponatremia

	Serum Na^+ (mEq/l)
Normal	140 ± 2
Hyponatremic	116 ± 7*
24 hours after correction	135 ± 2*†

Values express mean ± S.E. Mice were made hyponatremic by liquid diet feeding and dDAVP infusion for 3 days, then serum Na^+ was rapidly corrected with hypertonic saline injection. *$p<0.05$ vs. normal group, †$p<0.05$ vs. hyponatremic group by Student's t-tests.

Discussion

CPM is a serious human demyelinative disorder commonly associated with rapid correction of hyponatremia. Similar demyelinative lesions have been experimentally produced by rapid correction of hyponatremia in rats and used as an animal model of CPM.[13] In the present study, we successfully produced osmotically induced demyelination in mice. Serum Na^+ increased by around 20 mEq/l within 24 hours after rapid correction of hyponatremia. This serum Na^+ increase seems to be rapid enough to cause osmotic demyelination considering the report that demyelination was observed in rats whose magnitude of correction at 24 hours exceeded 16 mEq/l.[14] In humans,

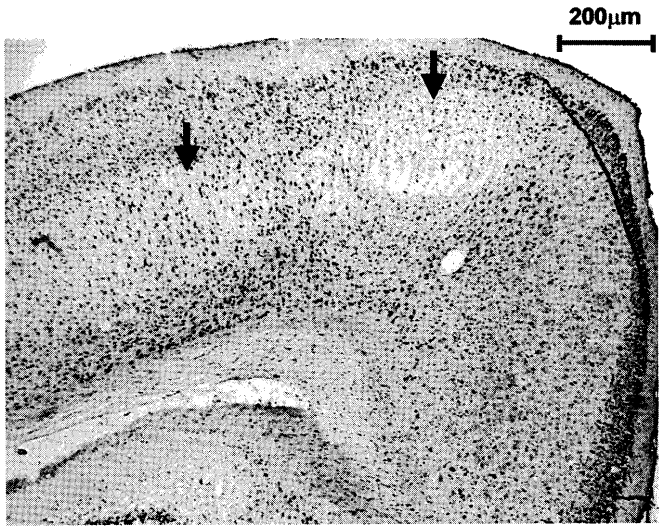

Fig. 1 Demyelinative lesions after rapid correction of hyponatremia. Brains were removed from mice 3 days after the rapid correction of hyponatremia, and processed for Luxol fast blue and cresyl violet staining. The arrows indicate demyelinative lesions in the subcortical white matter.

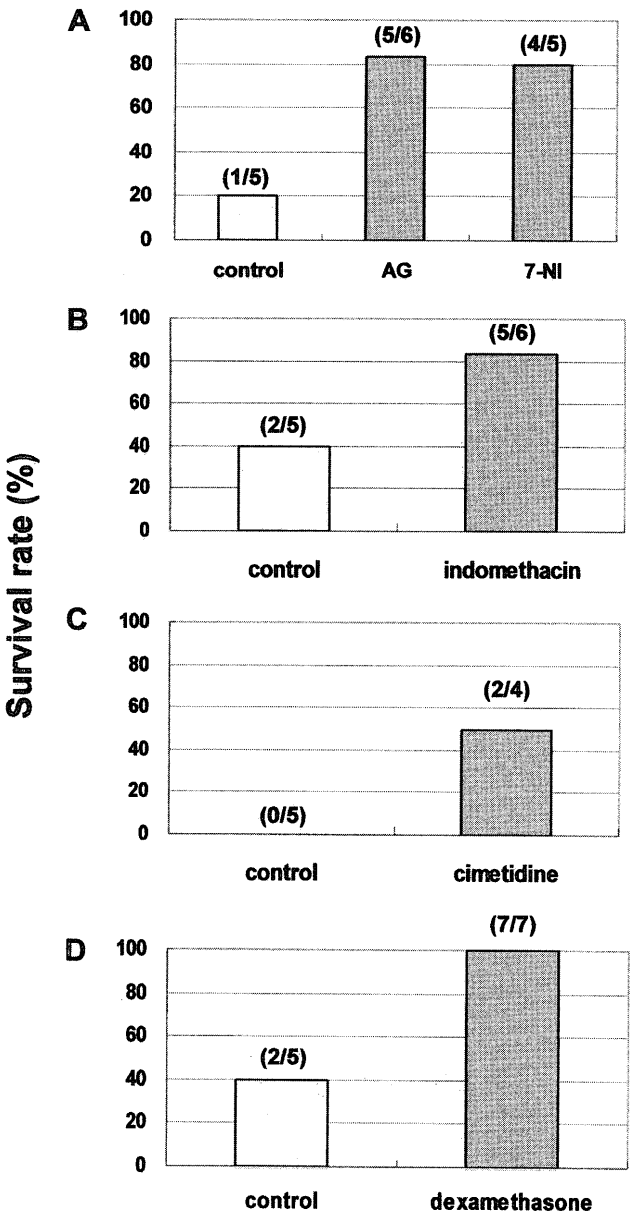

Fig. 2 The effects of drugs on survival rates for 24 hours after rapid correction of hyponatremia in mice.
Mice were made hyponatremic for 3 days, and then serum Na^+ was rapidly corrected with hypertonic saline injection. The following drugs were injected subcutaneously twice, just before and 5 hours after the correction. **(A)** aminoguanidine (AG) and 7-NI, **(B)** indomethacin, **(C)** cimetidine, **(D)** dexamethasone. Values express survival rates for 24 hours after the correction. Numbers in the parentheses indicate absolute number of mice for each group as survived/total.

CPM has been reported with 20 mEq/l increase of serum Na^+ within 3 days and with daily increase of 12 mEq/l.[1] Consequently, mice showed serious neurological impairments after the correction and many of them died within 24 hours. Brain tissues revealed marked demyelinative lesions in various areas of the brain. Therefore, it is likely that severe neurological symptoms after the correction are associated with demyelinative lesions in the brain.

Although the precise etiology of CPM remains unclear, the BBB disruption following rapid correction of hyponatremia is thought to play an important role in the process.[2-4] This led us to the speculation that it may be possible to prevent the onset of CPM if the BBB disruption is inhibited. To test this hypothesis, we evaluated the preventive effects of various drugs that have been reported to decrease the BBB permeability on osmotic demyelination in our mice model. First, we showed that aminoguanidine markedly increased the survival rate after rapid correction. Aminoguanidine is a selective iNOS inhibitor and iNOS has been reported to be important in the BBB disruption observed in meningitis.[11] We also showed that 7-NI improved clinical signs after the correction. 7-NI is a selective nNOS inhibitor and so far there is no report about the involvement of nNOS in the regulation of BBB permeability. Accordingly, the mechanism that 7-NI improved the clinical course after correction may be different from prevention of the BBB disruption. Further experiments will be required to clarify this point. Next, we showed that indomethacin, a PGs synthetase inhibitor, markedly increased the survival rate of mice after rapid correction. It has been reported that PGs are involved in the regulation of BBB permeability and indomethacin prevents the BBB disruption following heat stress or brain ischemia.[8,10] We, then, showed that cimetidin, a histamine H_2 receptor blocker, markedly increased the survival rate after the correction. Histamine has been reported to be involved in the regulation of BBB permeability and cimetidine prevents the BBB disruption induced by heat shock stress.[9] Mice sometimes had gastrointestinal bleeding after the correction. It is well known that stress ulcers often occur in patients with central nervous system diseases such as brain injury or stroke. Since cimetidine is a widely used anti-ulcer drug, it may be also useful for the prevention of stress ulcers following the correction. We also showed that dexamethasone markedly improved neurological impairments after the correction. Dexamethasone has been reported to decrease the BBB permeability induced by hyperosmolality or hypertension.[5,6] Our result is compatible with the previous report by other group that dexamethasone prevents the demyelination after rapid correction of hyponatremia, although they used rats and the protocol for making hyponatremia and rapid correction is different from the method we used in this study.[15]

In conclusion, we showed in the present study that NOS inhibitors, a PGs synthetase inhibitor, a histamine H_2 antagonist and glucocorticoid, each of which is reported to modify the BBB permeability, markedly improved the neurological signs and increased the survival rates after rapid correction of hyponatremia in mice. These results suggest that these drugs are clinically useful in the prevention of osmotic demyelination following rapid correction of hyponatremia possibly by inhibiting the BBB disruption.

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