http://www.kidney-international.org

© 2009 International Society of Nephrology

see commentary on page 587

Re-induction of hyponatremia after rapid overcorrection of hyponatremia reduces mortality in rats

Fabrice Gankam Kengne[1,2], Alain Soupart[1], Roland Pochet[3], Jean-Pierre Brion[3] and Guy Decaux[1]

[1]*Research Unit for the Study of Hydromineral Metabolism, Faculty of Medicine, Department of General Internal Medicine, Erasme Hospital, Université Libre de Bruxelles, Bruxelles, Belgium;* [2]*Department of Internal Medicine, University of Texas Southwestern Medical center, Dallas, TX, USA and* [3]*Laboratory of Histology, Histopathology and Neuroanatomy, Faculty of Medicine, Université Libre de Bruxelles, Bruxelles, Belgium*

Osmotic demyelination syndrome is a devastating neurologic disorder often seen after the rapid correction of chronic hyponatremia. The permeability of the blood–brain barrier is increased in experimental osmotic demyelination, and some have suggested that corticosteroids protect against this disorder by keeping the permeability of the blood–brain barrier low. We previously reported that re-lowering of the serum sodium after rapid correction of chronic hyponatremia was beneficial if performed early in the course (12 to 24 h). Here we compared mortality, blood–brain barrier permeability, and microglial activation in rats after the rapid correction of chronic hyponatremia. We studied three groups of rats after correction of chronic hyponatremia: and treated them with sodium chloride, with or without dexamethasone; or with sodium chloride followed by re-induction of hyponatremia. We found that treatment with dexamethasone or re-induction of hyponatremia effectively prevented the opening of the blood–brain barrier, reduced neurological manifestations, and decreased microglial activation; however, only re-induction of hyponatremia resulted in a significant decrease in mortality 5 days after the correction of chronic hyponatremia. Restoring the permeability of the blood–brain barrier to normal levels did not decrease mortality. Our results suggest that after inadvertent rapid correction of hyponatremia, treatment options should favor re-lowering serum sodium. The increased permeability of blood–brain barrier seen in osmotic demyelination syndrome may not be a primary pathophysiologic insult of this syndrome.

Kidney International (2009) **76,** 614–621; doi:10.1038/ki.2009.254; published online 15 July 2009

KEYWORDS: clinical nephrology; electrolytes; hyponatremia

Correspondence: *Fabrice Gankam Kengne, Service de Medecine Interne Génerale, Hopital Erasme, 808 Route de Lennik, 1070 Bruxelles, Belgium. E-mail: fgankamk@ulb.ac.be*

Received 19 January 2009; revised 5 May 2009; accepted 19 May 2009; published online 15 July 2009

Osmotic demyelination syndrome (ODS) occurs after excessive correction of chronic hyponatremia.[1,2] Histological lesions consist of symmetrical foci of demyelination, with activated microglia infiltration in different regions of the brain.[1,2] The physiopathology of this condition is poorly understood, and as a result, very few interventions have been reported to be beneficial in ODS.[3,4] The development of ODS lesions probably involves complex interactions among blood–brain barrier (BBB), brain organic osmolyte fluxes, microglia, and inflammatory cytokines.[4] The permeability of BBB in experimental ODS has been previously investigated and some have postulated that the opening of the BBB, provoked by a rapid correction of chronic hyponatremia, could result in the exposure of the brain to myelinolytic substances and subsequently induce ODS lesions.[5–7] It has recently been suggested that the administration of corticosteroids, such as dexamethasone (DXM), in an animal model of ODS could be beneficial by preventing the opening of BBB.[8] However, the facts that there is an opening of BBB in experimental ODS, that most animals with an open BBB after a rapid correction of hyponatremia will develop ODS, and finally that DEX could be efficacious in ODS because it protects against the opening of BBB, do not necessarily imply that an increase in the permeability of BBB is the initial and priming pathogenic phenomenon in this disorder. In fact, the exact role of this increase in the permeability of BBB is still debatable, as it could either represent the initial and crucial insult or simply a surrogate, secondary marker in the course of the ODS; studies addressing the relationship between BBB permeability and ODS have not fully clarified this issue. Furthermore, the net extent of protection conferred by DXM is controversial.[8–11]

Owing to the complex and mostly unknown nature of the phenomenon that induces demyelinative lesions after rapid correction of hyponatremia, few treatment options are available, and there is no consensus on the appropriate treatment strategy in ODS. Our group previously reported a benefit in mortality and neurological manifestations due to ODS, after a re-induction of hyponatremia up to 24 h after

the initial correction in asymptomatic and pauci-symptomatic rats;[12,13] the mechanisms underlying the beneficial effect of serum sodium (SNa) re-lowering are poorly understood and the action of this rescue treatment on BBB and microglial activation has never been investigated.

In this work, we aimed at comparing DXM administration and hyponatremia re-induction as preventive treatment options in ODS. We further investigated BBB permeability and microglial activation under both treatments in an attempt to clarify the role of these two components in the pathophysiology of ODS.

RESULTS

SNa values before and after correction of hyponatremia

We used a previously described animal model of ODS (See Figure 1 for experimental protocol).[8,14,15] Along with others, we previously reported that a correction of hyponatremia with a 24-h SNa gradient >25 mEq/l was associated with a high incidence of neurological damage.[15] Therefore, to avoid potential bias in survival because of inadequate correction gradient, only rats that had severe hyponatremia (SNa <120 mEq/l) and that achieved an SNa gradient $\geqslant 25$ mEq/l were included in the analysis.

Table 1 shows the SNa values in the three groups before and after correction. SNa levels at day 4 were comparable in all groups. In Group 3, the SNa gradient was re-lowered to a mean value of 14 mEq/l at the twenty-fourth hour, after a mean gradient of 29 mEq/l at the twelth hour.

Neurological manifestations and mortality after correction of hyponatremia

Table 1 summarizes the outcome of the animals at day 5 (24 h after correction) and at day 10 (5 days after correction).

At 24 h after correction of SNa, all rats became severely symptomatic in Group 1; in Group 2, 50% of the animals (8/16) were symptomatic; and in Group 3, only one animal showed neurological signs. DXM administration resulted in a significant reduction in neurological manifestations ($P=0.01$). However, animals treated with a re-induction of hyponatremia showed a better clinical status. ($P=0.01$, Group 2 vs Group 3)

By day 10 of the experiment, all the rats treated with hypertonic saline alone died (two rats were killed at day 7 because they were moribund). Only 3 of 16 rats treated with DXM survived ($P=0.17$ for DXM vs NaCl alone), and 15 of 16 rats survived in the group treated with SNa re-lowering. ($P<0.001$ for SNa re-lowering vs NaCl alone and vs DXM).

Figure 2 shows the luxol fast blue staining of myelin in the animals of each group.

IgG immunostaining

The extent and localization of BBB leakiness in the different groups were analyzed on brain sections immunolabeled for rat IgG. Representative microphotographs of IgG immunostaining carried out on brain slices are shown in Figure 3. In animals treated with DXM or with a re-induction of hyponatremia, only a faint immunostaining for IgG was present around the blood vessels, whereas brain sections of animals corrected with NaCl alone (Figure 3b) showed a massive immunostaining for IgG.

BBB permeability

Blood–brain barrier permeability was quantitatively assessed by Evans blue (EB) dye extravasation (Figure 4), as previously described.[16] The SNa values of the animals used for the EB dye experiment are given in Table 2. As shown in Figure 4,

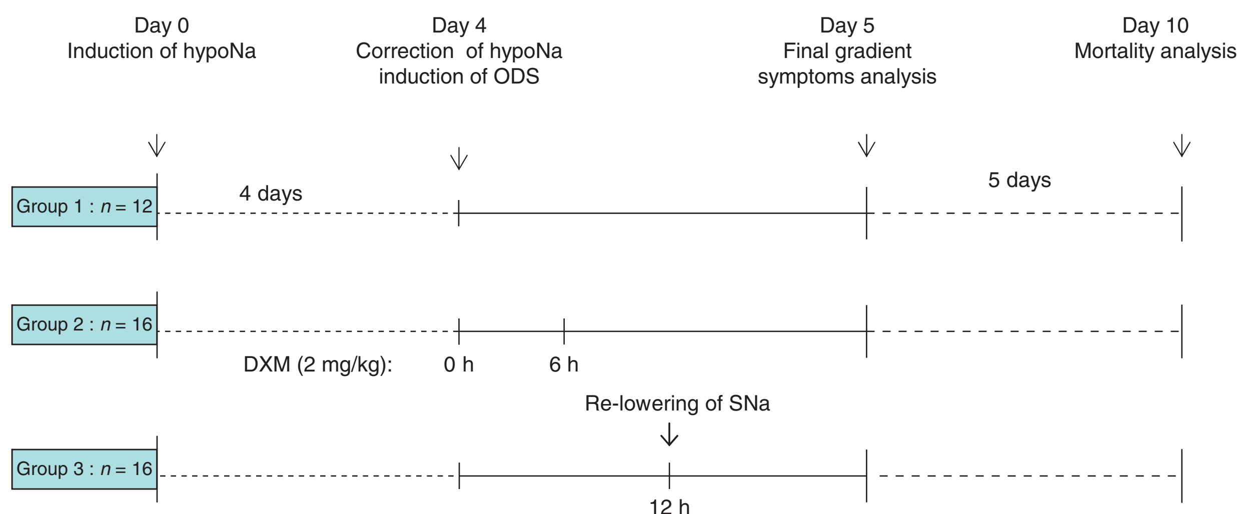

Figure 1 | Schematic representation of the experimental protocol used. Hyponatremia was induced by a combination of DDAVP infusion and liquid diet, and was maintained for 4 days. Correction was carried out using intraperitoneal hypertonic NaCl. Rats were allowed to consume food at day 5 after blood sampling and to consume water at day 6. In Group 2, dexamethasone (DXM) was administered 0 and 6 h after correction, whereas in Group 3, serum sodium was re-lowered 12 h after correction. Symptoms and mortality were recorded 1 and 5 days (day 5 and 10, respectively) after correction of hyponatremia in the three groups. HypoNa, hyponatremia; ODS, osmotic demyelination syndrome; SNa, serum sodium.

Table 1 | Serum sodium values (SNas) and clinical data in the three groups of rats measured before (day 4) and after (12 or 24 h) treatment, in the experiment addressing mortality

	Group 1 NaCl alone ($n=12$)	Group 2 NaCl+DXM ($n=16$)	Group 3 NaCl+SNa re-lowering ($n=16$)
SNa at day 4 (mEq/l)	108 ± 2*	107 ± 1*	104 ± 2*
SNa 12 h after correction	—	—	133 ± 2*,†
SNa at 24 h	137 ± 2*,†	136 ± 1*,†	117 ± 2
δ-SNa at 12 h	—	—	29 ± 1*
δ-SNa at 24 h	29 ± 1*	29 ± 1*	14 ± 1
Neurological manifestations at day 5	12/12	8/16‡	1/16‡
Mortality at day 10	12/12	13/16§	1/16§

ANOVA, analysis of variance; DXM, dexamethasone; NaCl, hypertonic NaCl.
Data are presented as means ± s.e.m.
*P = NS between the three groups.
†$P < 0.01$ compared with SNa before the correction (day 4).
‡P values: Group 1 vs Group 2, <0.01; Group 3 vs Group 2, <0.01; Group 3 vs Group 1, <0.001.
§P values: Group 1 vs Group 2, NS; Group 3 vs Group 2, <0.001; Group 3 vs Group 1, <0.001.
χ^2-test and one-way ANOVA were used for statistical analysis.

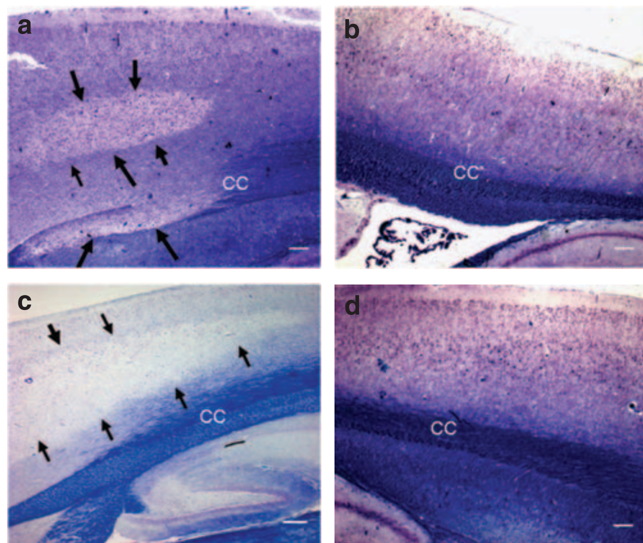

Figure 2 | Myelin staining (Luxol Fast Blue/Cresyl Violet). Representative microphotographs of brain sections stained with Luxol Fast Blue and Cresyl Violet for myelin. (**a**) Rats treated with dexamethasone (2 mg/kg body weight) at 0 and 6 h after the correction. (**b**) Rats treated with re-induction of hyponatremia. Rats treated with hypertonic saline alone (**c**) showed massive cortical demyelinative lesions (arrows), characterized as zone of pallor in the deep cortex, compared with uncorrected controls (**d**). (**a** and **d**) At 5 days after correction of chronic hyponatremia. (**c**) At 3 days after correction of chronic hyponatremia. Bar = 200 μm. CC: corpus callosum.

BBB permeability was increased in rats as early as 12 h after correction compared with that in uncorrected rats ($P<0.001$), and this increased permeability persisted for 24 h after correction. Treatment with DXM or SNa re-lowering resulted in a significant decrease in BBB permeability 12 h after correction. At 24 h after correction, treatments with DXM and re-induction of hyponatremia had the same protective effect on BBB permeability ($P<0.01$ compared with rats treated with NaCl alone, P = NS compared with uncorrected control rats).

Immunostaining for microglial activation

Representative microphotographs of activated microglia immunostaining are shown in Figure 5.

We confirmed the intense immunostaining observed in rats corrected with NaCl alone, mostly in the hippocampus, basal ganglia, and deep cortical areas (Figure 5b). In rats treated with DXM (Figure 5c) or with a re-induction of hyponatremia (Figure 5d), moderate immunostaining for activated microglia was also observed.

DISCUSSION

In these experiments, we found that, compared with rats treated with NaCl alone, rats treated with DXM presented less neurological manifestations 24 h after correction of chronic hyponatremia, but DXM failed to reduce the mortality at 5 days; on the contrary, re-lowering of SNa was efficacious in both reducing neurological manifestations and mortality. We further investigated the opening of BBB and microglial activation with the same treatments. We confirmed the previous findings with regard to the increase in BBB permeability and the protective effect of DXM on that increase (5, 7, 8, and 9). This study also suggests for the first time that a decrease in the permeability of BBB is associated with SNa re-lowering after an inadvertent correction of hyponatremia. As previously suggested,[17] we found that microglial activation occurs after correction of chronic hyponatremia with hypertonic saline alone in the rat model of ODS.

This study shows two important points: first of all, our results suggest that re-induction of hyponatremia is more efficient than DXM in the prevention of myelinolysis. This finding has an obvious clinical importance. Previous papers have reported a beneficial effect of corticosteroids in experimental osmotic demyelination[8–11] syndrome and isolated cases reported have echoed these experimental findings.[18] In our study, however, although we confirmed an early protection of DXM from neurological manifestations of ODS, we showed, with a larger series of animals, that DXM does not significantly impact mortality in this experimental

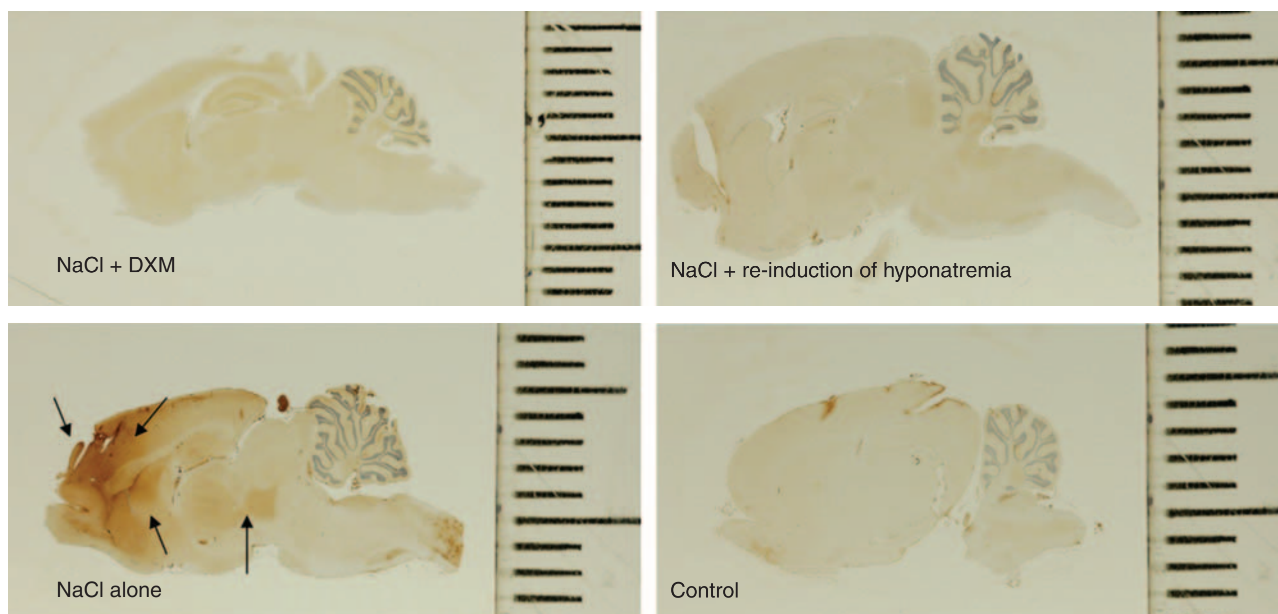

Figure 3 | Immunoreactivity for IgG in brain of rats of the four different groups. There is a significant increase in IgG reactivity in the cortical and subcortical areas, and in the basal ganglia of rats treated with NaCl alone (arrows) compared with that in all other groups (24 h after correction). DXM, dexamethasone.

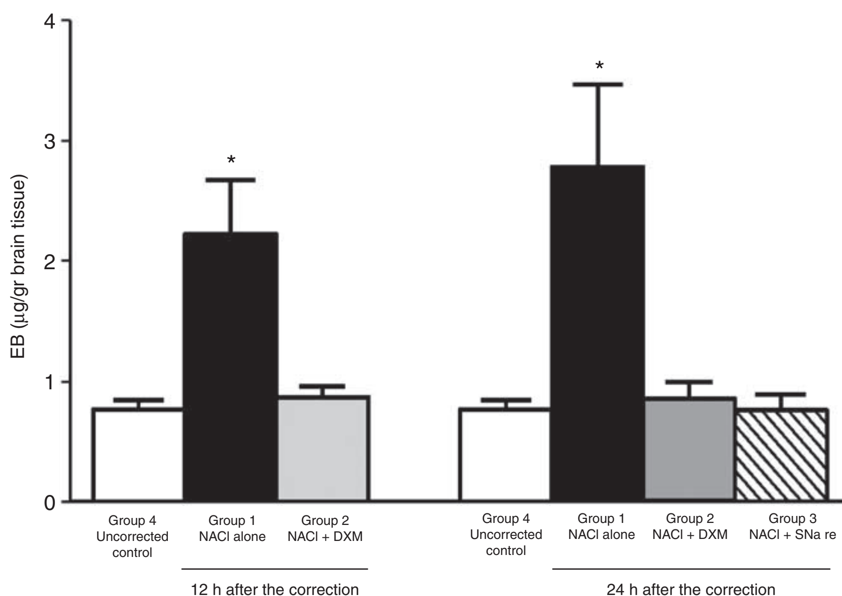

Figure 4 | Effect of the different treatments on blood–brain barrier (BBB) permeability after rapid correction of hyponatremia. $^*P<0.01$ in Group 1 vs Group 4 (uncorrected controls), and $P<0.01$ in Group 1 vs Group 2 (rats treated with NaCl alone and NaCl/DXM, respectively) and Group 3 (treated with NaCl and re-induction of hyponatremia), both 12 and 24 h after correction of serum sodium (SNa). $P=\mathrm{NS}$ between groups 2, 3, and 4, 12 and 24 h after correction. One-way analysis of variance, followed by Bonferroni LSD, was used for statistical analysis. BBB permeability was quantified using the Evans blue dye method 24 h after correction. Values are mean and s.e.m. ($n=5$–6 in each group). There is a striking increase in BBB permeability after rapid correction of hyponatremia. Treatment with DXM or SNa re-lowering resulted in a decrease in the permeability of BBB. DXM, dexamethasone; SNa re ↓, serum sodium re-lowering.

model. In contrast, SNa re-lowering was effective in protecting from both neurological manifestations and death in the animal model of ODS. These later findings are in accordance with our previous experiments,[12,13] and support recent clinical evidence of the protective role of re-induction of hyponatremia in the prevention of ODS in humans.[19–21] Our experiments suggest that the efficacy of DXM in terms of mortality should be considered with caution and that early re-induction of hyponatremia is to be preferred if the patient has been submitted to extensive correction of hyponatremia.

Table 2 | Serum sodium values (SNa) in the groups of rats used for EB analysis (data are presented as mean ± s.e.m.)

	12 h After the correction of SNa		24 h After the correction of SNa			Controls
	Group 1: NaCl alone ($n=6$)	Group 2: NaCl+DXM ($n=6$)	Group 1: NaCl alone ($n=5$)	Group 2: NaCl+DXM ($n=5$)	Group 3: NaCl+SNa re-lowering ($n=5$)	Group 4: uncorrected controls ($n=6$)
SNa at day 0 (mEq/l)	106 ± 1	104 ± 1	102 ± 2	104 ± 2	102 ± 2	104 ± 1
SNa 12 h after correction	135 ± 4	135 ± 2	—	—	131 ± 1	—
SNa at 24 h			133 ± 2	134 ± 1	106 ± 1	—
δ-SNa at 12 h	30 ± 2	31 ± 2	—	—	29 ± 2	—
δ-SNa at 24 h	—	—	31 ± 2	30 ± 2	4 ± 2	—

DXM, dexamethasone; EB, Evans blue stain.
P = NS for SNa at day 4 between all groups; P = NS for SNa and δSNa at 12 h between all the compared groups; P = NS for SNa and δSNa at 24 h between Group 1 and Group 2; P < 0.001 for SNa at 24 h between Group 3 and Groups 1 and 2.

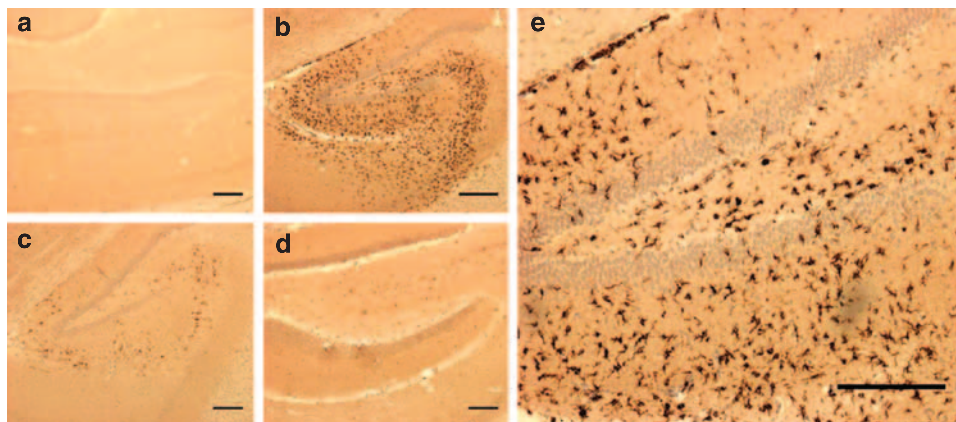

Figure 5 | Immunostaining for microglial activation. Representative microphotograph of activated microglia in the hippocampus of rats of the four different groups. (**a**) Uncorrected controls. Massive immunoreactivity was observed in rats treated with NaCl alone (**b**), in rats treated with dexamethasone (DXM) (**c**), and in those treated with re-induction of hyponatremia (**d**). On the right, a higher magnification of the hippocampus of a rat treated with NaCl alone (**e**). Bar = 200 μm.

Some differences in experimental design and methodology may account for the discordance between our results and those of previously published experiments. In these reports, particularly worrisome have been the end points studied, the SNa gradient of animals included in the analysis, and the sample size. The other experiments[9–11] mostly addressed the number or severity of lesions in the DXM-treated group compared with that in the control group: some reported[10,11] a decrease in the number of demyelinative lesions in rats treated with corticosteroids or in those that are repeatedly tail transected compared with that in rats treated with hypertonic saline alone, with no significant difference in the number of affected animals (7 of 10 in tail transected and 8 of 10 in steroid treated vs 10 of 10 in control group). Compared with previous papers, we used a larger number of rats ($n = 12$–16) and we included in the analysis only rats with a large increase of SNa (δ-SNa ≥ 25 mEq/l/day). By choosing this set point of SNa gradient, we were more likely to disclose any beneficial effect on mortality after treatment with either DXM or hyponatremia re-induction, as such a gradient is invariably associated with a high incidence of neurological manifestations and death.[15] In the previous studies addressing this issue, animals had an SNa gradient of < 25 mEq/l/day, which could explain the lower mortality rate usually found and a possible overestimation of the protective role of DXM.

The second key conclusion from our results is regarding the role of BBB in ODS pathophysiology.

Some have hypothesized that the opening of BBB could allow the passage of large blood-borne molecules (IgG and complement) that could damage oligodendrocytes and induce demyelination.[5,6] In other animal models, as well as in *in vitro* studies, osmotic opening of the BBB has been shown to be involved in the transient modifications of cellular cytoskeletal architecture, leading to a shrinkage of tight junctions of endothelial cells.[22–25] These changes have been related to both the intensity and duration of the osmotic stimulus and involve some proteins of the tight junction complex, such as ZO 1, occluding, and claudin.[22–24] In parallel, various inflammatory stimuli (cytokines and nitric oxide) are known to regulate the permeability of BBB.[26] Some of these inflammatory cytokines are shown to be present in ODS.[17] Taking into account the results of previous experiments, we can hypothesize that the increase in the permeability of BBB in ODS might involve two distinct but overlapping components: one early, reversible osmotic

component mediated by rapid changes in plasma tonicity with the resultant mechanical shrinking of tight junctions of the BBB and a late inflammatory component mediated by the secretion of proinflammatory cytokines after propagation of the osmotic insult.

The effect of DXM on BBB has been well characterized in both *in vivo* and *in vitro* studies, and DXM has been shown to modulate the changes in tight junctional proteins and other components involved in the regulation of BBB physiology with, as net effect, an increase in the stability of BBB.[27,28] Regarding the re-lowering of SNa, we showed that the permeability of BBB returned to near normal values after the re-induction of hyponatremia; it is likely that the withdrawal of the osmotic insult (by re-induction of hyponatremia) acts on BBB by limiting the consequences of the inflammatory processes triggered by a sustained osmotic stress.

Dexamethasone, to a similar extent as SNa re-lowering, prevented the opening of the BBB observed after rapid correction of SNa, but did not significantly affect mortality. This suggests that the opening of the BBB component in ODS is distinct from the initial demyelinative injury.

The hypothesis of the marginal contribution of the opening of the BBB as the initial insult causing ODS is further supported by other observations: first, the osmotic opening of BBB has been extensively studied in the clinical setting and has been shown to be transient,[24,25,29] whereas all papers studying this topic with respect to ODS reported a long-lasting opening of the BBB, suggesting that the increased permeability of BBB after 24 h is not related to a mechanical osmotic phenomenon. Second, although lovastatin has been shown to decrease the permeability of BBB in various experimental settings, it has failed to show a significant efficacy in ODS.[17] Third, an increase in the permeability of BBB has been well characterized in other models of neurological injury; yet, such a specific pattern of demyelination does not occur. These facts, when associated with the results of our experiments, strongly imply that the opening of BBB could count as a surrogate marker in ODS resulting from the inflammatory milieu, which is distinct from the priming insult.

We showed that rats could still develop ODS and die of it, despite a closed BBB. However, neither our study nor the previous one could fully decipher the exact role of BBB in this disorder, and other experimental approaches are needed to understand how the increased permeability of BBB observed in ODS could be implicated in the complex pathophysiology of this disorder.

In summary, although corticosteroids could decrease the permeability of BBB and the neurological symptoms after rapid correction of hyponatremia, this treatment does not result in an increase in the overall survival rate in an experimental model, suggesting that BBB permeability is rather a surrogate marker of secondary phenomenon than initial insult after rapid correction of chronic hyponatremia.

Our results are not readily transposable to human practice, and it is important to stress on the fact that the two treatment strategies compared in this study are clinically different, because re-induction of SNa is a rescue maneuver, whereas administration of DXM is strictly preventive. Therefore, both the treatments can be combined for maximal efficacy. Nevertheless, these experiments stress on the fact that re-induction of hyponatremia is an efficacious treatment option after an inadvertent correction of chronic hyponatremia.

Our experiments further show how complex the pathophysiology of ODS is. Besides SNa re-lowering, the only interventions that resulted in such a striking reduction in mortality after rapid correction of chronic hyponatremia were myoinositol[30] administration and exogenous or endogenous uremia.[31,32] All these treatments affected the organic osmolyte content of the brain. Indeed, important variations in the organic osmolyte content in the brain have been shown in chronic hyponatremia.[33,34] These imbalances in the 'buffering potential' of the cell could further lead to a poor response to a rapid increase in the tonicity of extracellular space, which could in turn trigger a cellular lesion in oligodendrocytes with secondary inflammation, finally leading to demyelination.

MATERIAL AND METHODS

Animals

Male Wistar rats (250–300 g) were used for all the experiments. The animals were housed in individual cages under conditions of constant temperature (23 °C) and a 12-h/12-h light/dark cycle. They were allowed to adapt for 3–4 days and were fed with standard pelleted chow with *ad libitum* access to water before the beginning of the experiments. All procedures were performed in accordance with guidelines for animal care at Université Libre de Bruxelles.

Induction of hyponatremia

In all animals, hyponatremia was induced by previously described methods.[8,14] In brief, an osmotic minipump (Model 2001 Alzet, Palo alto, CA, USA) was filled with desmopressin acetate (0.4 μg/ml) (Ferring, Sweden) and inserted into the back of the animal, which was administered light halothane anesthesia at the beginning of the experiment (Day 0). On the day of the insertion, rats were switched to a liquid diet with a low sodium content (AIN 76, Technilab BMI, Someren, Netherlands). The liquid diet was maintained for a total of 4 days.

Correction of hyponatremia

After 4 days of liquid feeding (day 4 of the experiment), hyponatremia was rapidly corrected by administration of NaCl 1 M (2 ml/100 g body weight) intraperitoneally in a single dose. Desmopressin infusion was maintained all through the experiment.

Pelleted chow was given to the animals on day 5 of the experiment, after blood sampling, for the determination of final 24-h SNa and to avoid incidental re-lowering occurring at day 5; water was given only on day 6.

Blood measurements

Blood samples (0.3 ml) were collected by tail transection at days 0 and 1 after administering light anesthesia, for SNa analysis. Electrolyte measurements were determined using MODULAR p800 (Roche Diagnostics, Vilvoorde, Belgium).

Evaluation of neurological manifestations and mortality

In a first set of experiments (Figure 1), we addressed the effect of DXM and SNa re-lowering in rats after rapid correction of chronic hyponatremia in terms of neurological manifestations and mortality. In these experiments, a total of 44 rats were randomly assigned to three groups depending on the treatment regimen. All groups were studied concurrently.

Group 1 ($n = 12$) consisted of hyponatremic rats, the SNa of which was rapidly corrected with hypertonic saline without further treatment.

Group 2 ($n = 16$) consisted of the DXM-treated group. DXM (Aacidexam, Organon, Brussels, Belgium) was administered subcutaneously at a dose of 2 mg/kg body weight at the time of correction of hyponatremia and 6 h later. This administration scheme has been previously reported to be the most protective in this setting.[8]

Group 3 ($n = 16$) consisted of hyponatremic rats corrected with hypertonic saline, followed by re-induction of hyponatremia 12 h later in asymptomatic rat. Blood samples were taken for serum analysis, and only rats that achieved an SNa gradient of correction $\geqslant 25$ mEq/l were treated with re-induction of hyponatremia. Hyponatremia was re-induced by an intraperitoneal administration of water (10% of body weight) to achieve a final SNa gradient $\leqslant 20$ mEq/l over 24 h.[12,13]

Groups 1 and 2 included only rats with a 24-h SNa gradient $\geqslant 25$ mEq/l. This value of SNa gradient has been previously shown to be inevitably associated with brain demyelinative lesions in animals corrected with NaCl alone.[15]

Animals were observed for neurological manifestations of ODS 1 day and 5 days after correction of hyponatremia. Rats were considered symptomatic if they showed one of the following: slow or awkward gait, limb weakness and/or paralysis, seizures, severe motor deficits, or a complete inability to move.

Immunohistochemistry

Animals were killed if they were found to be moribund in their cages; otherwise, all surviving animals at day 10 of the experiment (5 days after correction of chronic hyponatremia) were killed by decapitation. Brain was divided into two hemispheres and was fixed overnight in formalin (10%) before rinsing with phosphate-buffered saline. Specimens were then embedded in paraffin and cut into sagittal sections of 7 μm thickness using a microtome.

Immunohistochemical detection of IgG was performed on rat brain sections, as previously described.[5] After deparaffination and rehydration of tissue sections, endogenous peroxidases were blocked (0.3% H_2O_2 in methanol) and non-specific binding of the antibody was inhibited using appropriate species' normal serum at 1/10 ratio. Slides were then incubated with a biotinylated goat anti-rat IgG antibody (1:100; Vector, Burlingame, CA, USA) for 3 h, followed by avidin–biotin–peroxidase (Vector Labs, Labconsult, Brussels, Belgium) and diaminobenzidine (Dako, Heverlee, Belgium). Immunohistochemistry for activated microglia was performed using a mouse anti-ED1 antibody[16] (1/200, Serotec, Dusseldorf, Germany), followed by a biotinylated horse anti-mouse antibody (1:100; Vector).

Evaluation of BBB by EB dye extravasation

In a second set of experiments, EB was used to determine BBB permeability at 12 and 24 h after correction of hyponatremia in the different groups studied, as well as in one group of uncorrected hyponatremic rats serving as controls (Group 4).

In each animal, EB solution (Sigma Chemical, St Louis, MO, USA) (2% in saline; 3 ml/kg) was injected intravenously 24 h after rapid correction of chronic hyponatremia and was allowed to circulate for 40 min. The chest was subsequently opened and the brain was transcardially perfused with 100 ml of saline through the left ventricle at 100-mm Hg pressure until an almost colorless perfusion fluid was obtained from the right atrium. Brains were then weighed and placed in 50% trichloroacetic acid solution. After homogenization and centrifugation,[16] the absorbance of the supernatant was analyzed spectrophotometrically at 610 nm and the content of EB, expressed per gram of brain tissue, was calculated using a linear standard curve derived from known amounts of dye.

Statistical analysis

Results were expressed as mean ± s.e.m. The Mann–Whitney nonparametric test, Fischer's exact test on proportions, and one-way analysis of variance followed by Bonferroni's LSD were used when appropriate. P values <0.05 were considered to be significant.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

We thank Charles Nicaise and Michelle Authelet for their helpful technical assistance. This study was supported by a grant from the Fonds National de la Recherche Scientifique, conventions 3.4509.03 and 3.4656.09, by a grant from foundation Erasme and by a grant from the Belgian PAI/IAP P6/43. Part of this work was presented at the ASN annual meeting November 2008 and at the annual meeting of the Belgian Society of Internal Medicine December 2008.

REFERENCES

1. Kleinschmidt-DeMasters BK, Norenberg MD. Rapid correction of hyponatremia causes demyelination: relation to central pontine myelinolysis. *Science* 1981; **211**: 1068–1070.
2. Kleinschmidt-DeMasters BK, Norenberg MD. Neuropathologic observations in electrolyte-induced myelinolysis in the rat. *J Neuropathol Exp Neurol* 1982; **41**: 67–80.
3. Kleinschmidt-Demasters BK, Rojiani AM, Filley CM. Central and extrapontine myelinolysis: then and now. *J Neuropathol Exp Neurol* 2006; **65**: 1–11.
4. Murase T, Sugimura Y, Takefuji S *et al.* Mechanisms and therapy of osmotic demyelination. *Am J Med* 2006; **119**(7 Suppl 1): S69–S73.
5. Baker EA, Tian Y, Adler S *et al.* Blood-brain barrier disruption and complement activation in the brain following rapid correction of chronic hyponatremia. *Exp Neurol* 2000; **165**: 221–230.

6. Adler S, Martinez J, Williams DS *et al.* Positive association between blood brain barrier disruption and osmotically-induced demyelination. *Mult Scler* 2000; **6**: 24–31.
7. Adler S, Verbalis JG, Williams D. Effect of rapid correction of hyponatremia on the blood-brain barrier of rats. *Brain Res* 1995; **679**: 135–143.
8. Sugimura Y, Murase T, Takefuji S *et al.* Protective effect of dexamethasone on osmotic-induced demyelination in rats. *Exp Neurol* 2005; **192**: 178–183.
9. Ke QH, Liang TB, Yu J *et al.* A study of the pathogenesis and prevention of central pontine myelinolysis in a rat model. *J Int Med Res* 2006; **34**: 264–271.
10. Oh MS, Choi KC, Uribarri J *et al.* Prevention of myelinolysis in rats by DXM or colchicine. *Am J Nephrol* 1990; **10**: 158–161.
11. Rojiani AM, Prineas JW, Cho ES. Protective effect of steroids in electrolyte-induced demyelination. *J Neuropathol Exp Neurol* 1987; **46**: 495–504.
12. Soupart A, Penninckx R, Stenuit A *et al.* Re-induction of hyponatremia improves survival in rats with myelinolysis-related neurologic symptoms. *J Neuropathol Exp Neurol* 1996; **55**: 594–601.
13. Soupart A, Penninckx R, Crenier L *et al.* Prevention of brain demyelination in rats after excessive correction of chronic hyponatremia by serum sodium lowering. *Kidney Int* 1994; **45**: 193–200.
14. Verbalis JG, Drutarosky MD. Adaptation to chronic hypoosmolality in rats. *Kidney Int* 1988; **34**: 351–360.
15. Soupart A, Stenuit A, Perier O *et al.* Limits of brain tolerance to daily increments in serum sodium in chronically hyponatraemic rats treated with hypertonic saline or urea: advantages of urea. *Clin Sci (Lond)* 1991; **80**: 77–84.
16. Uyama O, Okamura N, Yanase M *et al.* Quantitative evaluation of vascular permeability in the gerbil brain after transient ischemia using Evans blue fluorescence. *J Cereb Blood Flow Metab* 1988; **8**: 282–284.
17. Takefuji S, Murase T, Sugimura Y *et al.* Role of microglia in the pathogenesis of osmotic-induced demyelination. *Exp Neurol* 2007; **204**: 88–94.
18. Takei Y, Akahane C, Ikeda S. Osmotic demyelination syndrome: reversible MRI findings in bilateral cortical lesions. *Intern Med* 2003; **42**: 867–870.
19. Soupart A, Ngassa M, Decaux G. Therapeutic relowering of the serum sodium in a patient after excessive correction of hyponatremia. *Clin Nephrol* 1999; **51**: 383–386.
20. Oya S, Tsutsumi K, Ueki K *et al.* Reinduction of hyponatremia to treat central pontine myelinolysis. *Neurology* 2001; **57**: 1931–1932.
21. Perianayagam A, Sterns RH, Silver SM *et al.* DDAVP is effective in preventing and reversing inadvertent overcorrection of hyponatremia. *Clin J Am Soc Nephrol* 2008; **3**: 331–336.
22. Lu TS, Chen HW, Huang MH *et al.* Heat shock treatment protects osmotic stress-induced dysfunction of the blood-brain barrier through preservation of tight junction proteins. *Cell Stress Chaperones* 2004; **9**: 369–377.
23. Dobrogowska DH, Vorbrodt AW. Immunogold localization of tight junctional proteins in normal and osmotically-affected rat blood-brain barrier. *J Mol Histol* 2004; **35**: 529–539.
24. Rapoport SI. Osmotic opening of the blood-brain barrier. *Ann Neurol* 1988; **24**: 677–684.
25. Rapoport SI, Fredericks WR, Ohno K *et al.* Quantitative aspects of reversible osmotic opening of the blood-brain barrier. *Am J Physiol* 1980; **238**: R421–R431.
26. Chavarria A, Alcocer-Varela J. Is damage in central nervous system due to inflammation? *Autoimmun Rev* 2004; **3**: 251–260.
27. Romero IA, Radewicz K, Jubin E *et al.* Changes in cytoskeletal and tight junctional proteins correlate with decreased permeability induced by dexamethasone in cultured rat brain endothelial cells. *Neurosci Lett* 2003; **344**: 112–116.
28. Frequin ST, Barkhof F, Lamers KJ *et al.* The effects of high-dose methylprednisolone on gadolinium-enhanced magnetic resonance imaging and cerebrospinal fluid measurements in multiple sclerosis. *J Neuroimmunol* 1992; **40**: 265–272.
29. Zünkeler B, Carson RE, Olson J *et al.* Hyperosmolar blood-brain barrier disruption in baboons: an in vivo study using positron emission tomography and rubidium-82. *J Neurosurg* 1996; **84**: 494–502.
30. Silver SM, Schroeder BM, Sterns RH *et al.* Myoinositol administration improves survival and reduces myelinolysis after rapid correction of chronic hyponatremia in rats. *J Neuropathol Exp Neurol* 2006; **65**: 37–44.
31. Soupart A, Schroëder B, Decaux G. Treatment of hyponatraemia by urea decreases risks of brain complications in rats. Brain osmolyte contents analysis. *Nephrol Dial Transplant* 2007; **22**: 1856–1863.
32. Soupart A, Silver S, Schroöeder B *et al.* Rapid (24-h) reaccumulation of brain organic osmolytes (particularly myo-inositol) in azotemic rats after correction of chronic hyponatremia. *J Am Soc Nephrol* 2002; **13**: 1433–1441.
33. Lien YH, Shapiro JI, Chan L. Study of brain electrolytes and organic osmolytes during correction of chronic hyponatremia. Implications for the pathogenesis of central pontine myelinolysis. *J Clin Invest* 1991; **88**: 303–309.
34. Verbalis JG, Gullans SR. Rapid correction of hyponatremia produces differential effects on brain osmolyte and electrolyte reaccumulation in rats. *Brain Res* 1993; **606**: 19–27.