*Acta Anaesthesiol Scand 2007; **51**: 1331–1337*
Printed in Singapore. All rights reserved

ACTA ANAESTHESIOLOGICA SCANDINAVICA
doi: 10.1111/j.1399-6576.2007.01448.x

Brain damage following severe acute normovolemic hemodilution in combination with controlled hypotension in rats

Y. L. GE[1], R. LV[2], W. ZHOU[3], X. X. MA[2], T. D. ZHONG[2] and M. L. DUAN[4]
[1]*Department of Anesthesiology, Subei People's Hospital, Xuzhou Medical College, Xuzhou, and Departments of* [2]*Anesthesiology* [3]*General Surgery, Affiliated Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, China and* [4]*Department of Anesthesiology, Jinling Hospital, Nanjing, China*

Background and aim: The reduced oxygen content and perfusion pressure during acute normovolemic hemodilution (ANH) and controlled hypotension (CH) raise concerns about hypoperfusion and ischemic injury to the brain. In this study on rats, we examined the brain damage following four different degrees of ANH combined with CH.
Methods: Forty rats were randomly assigned to receive a sham operation or CH and ANH [with a hematocrit (Hct) of 30, 25, 20 or 15%]. ANH was performed after baseline physiological parameters had been monitored for 20 min; 30 min later, CH was induced using sodium nitroprusside, and the mean arterial blood pressure was maintained at 50–60 mmHg for 1 h. Rats were killed 3.5 h after hemodilution. Ultrastructural alterations in the CA1 region of the rat hippocampus were observed, and serum concentrations of S100B and neuron-specific enolase (NSE) were measured before and after ANH.
Results: The serum S100B concentration increased significantly in the Hct 20% + CH and Hct 15% + CH groups. However, there were no significant differences in the serum levels of NSE between the groups. In the CA1 region of the rat hippocampus, marked ultrastructural alterations, such as mitochondrial denaturalization and nucleus distortion, were observed in the Hct 20% + CH and Hct 15% + CH groups.
Conclusion: Severe ANH (Hct $\leq$ 20%) combined with CH may induce cerebral damage, as confirmed by marked ultrastructural alterations in the CA1 region of the rat hippocampus and significantly increased serum levels of S100B, and should be avoided.

Accepted for publication 21 June 2007

Key words: Brain damage; controlled hypotension; hemodilution; hippocampus; neuron-specific enolase; S100B protein.

TRANSFUSION-ASSOCIATED complications have resulted in the need to reduce the exposure of patients to homologous blood (1). Acute normovolemic hemodilution (ANH) and controlled hypotension (CH) are independently effective in decreasing operative blood loss and the transfusion of allogeneic blood (2, 3). Their combination may further reduce the need for allogeneic blood transfusion (4, 5). However, the combined use of ANH and CH is still underutilized, because of concerns that reduced oxygen content and perfusion pressure may lead to ischemic and hypoperfusion injury to vital organs (6). The organ most susceptible to hypoxia is the brain. Although an effect of ANH combined with CH on cerebral oxygenation has been reported (1), their effect on direct cerebral damage, such as ultrastructural changes, has not been investigated in detail.

In recent years, protein S100B and neuron-specific enolase (NSE), as specific biochemical markers for the evaluation of cerebral damage, have been studied in various clinical settings (7–9). Many studies have demonstrated peri-operative cerebral complications, such as delayed awakening after anesthesia, cognitive dysfunction and stroke, associated with increased serum levels of these markers (10–12). S100B is an astroglial-specific protein and NSE a neuronal-originating protein. With cerebral damage and increased permeability of the blood–brain barrier, both markers may be released into the blood and have a higher concentration in serum. However, to date, no data are available on the serum levels of S100B and NSE when ANH is combined with CH.

We have conducted a study in rats to investigate the cerebral morphological changes and serum

concentrations of brain-originating proteins after treatment with four degrees of ANH combined with CH. The results of this study may help to determine the safe limit of the blood-sparing technique.

Methods

Animals and treatment

Adult male Sprague-Dawley rats (body weight, 350–400 g) were obtained from Shanghai Animal Center, Shanghai, China, and kept in accordance with the Institutional Animal Care Committee guidelines. The study protocols were approved by the Institutional Animal Care Committee of Sir Run Run Shaw Hospital. The animals were housed in a room with an ambient temperature of 22 °C and a 12-h light/dark cycle. Animals were allowed at least 7 days to acclimatize before the experiments. They were anesthetized by the intraperitoneal administration of 1250 mg of urethane (Shanghai Chemical Reagent Co., Shanghai, China) per kilogram of body weight, and then intubated and mechanically ventilated with a volume-control mode [tidal volume, 8 ml/kg; frequency, 60 breaths/min; fraction of inspired oxygen (F_iO_2), 1.0]. From blood gas analysis (Radiometer ALB 500, London Scientific, London, ON, Canada), ventilation parameters were adjusted to maintain the arterial oxygen saturation (S_aO_2) at greater than 95% and the pressure of arterial carbon dioxide (P_aCO_2) within the normal physiological range.

The tail vein was cannulated with a polyethylene catheter for the intravenous administration of solutions. The macrohemodynamic parameters, including the mean arterial blood pressure (MAP), heart rate (HR) and central venous pressure (CVP), were measured through the left femoral artery and vein catheterized with a microtip transducer (Abbot Laboratories Ltd., Chicago, IL).

Experimental protocol

The rats were randomly divided into five treatment groups (eight rats per group): sham operation and four degrees of ANH [hematocrit (Hct) of 30, 25, 20 and 15%] plus CH. After monitoring of the baseline parameters for 20 min, the rats underwent ANH, where arterial blood from the femoral artery was simultaneously exchanged with an equivalent volume of hydroxyethyl starch (HES 130/0.4, 6%; medium molecular weight, low degree of substitution; Fresenius Kabi, Bad Homburg, Germany) infused via the tail vein. To reach different Hct targets, the volume (V) of blood to be removed was calculated as follows (13):

$$V = \mathrm{EBV} \times (H_i - H_f)/H_{av}$$

where EBV is the estimated blood volume (70 ml/kg), H_i is the initial Hct, H_f is the final Hct after ANH and H_{av} is the mean Hct (mean of H_i and H_f). Thirty minutes after ANH, CH was induced with the infusion of 0.01% sodium nitroprusside (SNP) at 0.15–15 μg/kg/min to maintain MAP at 50–60 mmHg for 1 h. After ANH and sham operation for 3.5 h, the animals were killed by an overdose of urethane.

During the experiment, a heating pad and heating lamp were used to maintain the temperature of rats at about 37 °C.

Parameter measurements

MAP, HR, CVP and the body temperature (measured via the anus) were continuously monitored throughout the experiment. MAP, HR, CVP and arterial blood gas were recorded immediately before hemodilution (T_0), just before CH (T_1), in the middle of CH (T_2), at the end of CH (T_3) and 30 min after recovery from CH (T_4).

Protein levels of S100B and NSE in serum

In each group, 1 ml of arterial blood was collected for S100B and NSE measurement at baseline and at the end of the experiment. After centrifugation (12,000 ***g***, 4 °C) for 5 min, the serum samples were frozen at –70 °C for further analysis. To avoid the impact of hemodilution on serum protein levels, we also collected exchanged blood. Practical serum protein concentrations were calculated as follows:

$$C_p = C_e \times V_e/\mathrm{EBV} + C_f$$

where C_P is the practical serum protein concentration, C_e is the concentration of proteins in exchanged blood, V_e is the volume of exchanged blood, EBV is the estimated blood volume (70 ml/kg) and C_f is the final serum protein concentration. Serum S100B protein and NSE concentrations were analyzed by enzyme-linked immunosorbent assay (ELISA). Values are expressed as micrograms per litre of serum.

Histological and ultrastructural studies

After the systemic circulation had been perfused with 0.9% NaCl, the skulls of the rats were opened carefully, the hippocampus was immediately removed and the CA1 region was cut into standardized sections. These were fixed for 48 h in Somogyi solution containing paraformaldehyde, glutaraldehyde 25% and picric acid, pH 7.4, for electron microscopy (14). The tissue was then dehydrated and embedded in epoxy resin.

Only the ultrastructural changes in mitochondria were evaluated semi-quantitatively (15, 16). The changes

in other cell organelles and components are simply described. Five electron micrographs were taken of each biopsy, and, in each micrograph, 20 mitochondria were selected at random. Each mitochondrion was graded on a scale of 0–3 (Fig. 1): 0, normal structure; 1, slight change but clear crests; 2, edematous change; 3, accumulation of amorphous material.

Data analysis

Data are expressed as the means ± standard deviation (SD), and were analyzed by SPSS 11.0. Comparisons between groups involved analysis of variance (ANOVA) and Student's *t*-test. Significant differences within groups by time, as compared with baseline, were tested by repeated measures analysis. $P < 0.05$ was considered to be significant.

Results

During the entire study period, all animals were anesthetized. The total doses of SNP administered did not differ between the treated groups, and the need for additional doses of urethane to kill the rats was similar.

Effect of ANH with CH on macrohemodynamics and arterial blood gas parameters

MAP, HR and CVP were comparable between the groups at baseline (Table 1). MAP and HR did not differ at T_0 and T_1 between the groups. With ANH and CH treatment, HR was increased at T_2 and T_3, and MAP was lower at T_4 than at baseline. Throughout the experiment, CVP was stable. P_aO_2 and P_aCO_2 were within the normal ranges at all the measured time points (Table 2), but the pH decreased significantly at T_3 and T_4 in the Hct 15% + CH group.

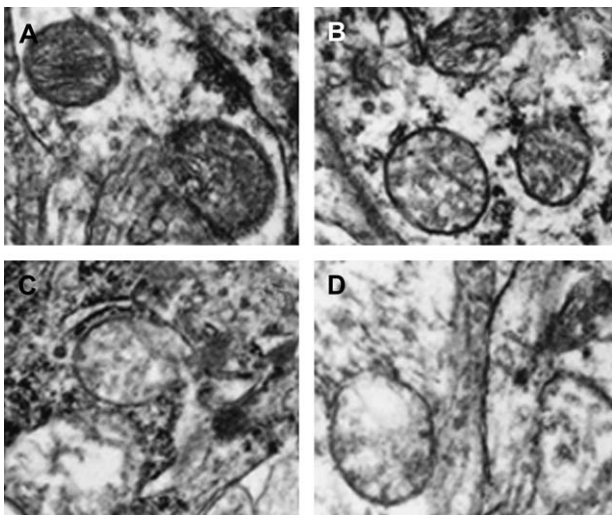

Fig. 1. Postulated stages of mitochondrial reaction in the CA1 region of rat hippocampus following acute normovolemic hemodilution (ANH) combined with controlled hypotension (CH). (A) Mitochondrial score of 0, normal structure; (B) mitochondrial score of 1, slight change but clear crests; (C) mitochondrial score of 2, edematous change; (D) mitochondrial score of 3, accumulation of amorphous material.

Effect of ANH with CH on serum concentrations of S100B and NSE

The baseline values of the serum S100B protein and NSE concentrations were comparable between the groups (Fig. 2). The serum S100B protein concentration increased significantly in the Hct 20% + CH and Hct 15% + CH groups at the end of the experiment, with no significant changes in NSE concentration in any group throughout the entire study.

Effect of ANH with CH on ultrastructural changes in the hippocampus

The Hct 20% + CH and Hct 15% + CH groups showed uniform alterations in cerebral ultrastructure induced by ANH with CH. The mean mitochondrial scores in the two groups were significantly higher than those in the sham-operated group (Fig. 3). Most nuclei appeared normal in the sham-operated, Hct 30% + CH and Hct 25% + CH groups (Fig. 4). Margination and clumping of chromatin were seen occasionally in the Hct 20% + CH group, but more frequently in the Hct 15% + CH group. The nuclear membrane was slightly irregular in the Hct 20% + CH group, and became more irregular in the Hct 15% + CH group. The phenomenon of glial activation, such as swollen glia assembling around the neurons, could be seen in the Hct 20% + CH and Hct 15% + CH groups.

Discussion

Both ANH and CH have independently shown efficacy as blood-saving techniques (2, 3). Consequently, their combination may produce even better conservation of blood. However, currently, a confirmed safe limit of ANH with CH has not been reported, and the greater blood-sparing benefits of the combination must be weighed against the risk of inadequate tissue oxygenation (4). The brain is the most susceptible organ to ischemia and hypoxia, and so care must be taken to avoid brain damage when this combined blood-sparing technique is used. ANH can increase the cerebral blood flow (CBF) to compensate for decreased arterial oxygen content (17), but, in a study on dogs, ANH with CH reduced the

Table 1

Effect of acute normovolemic hemodilution (ANH) combined with controlled hypotension (CH) on the macrohemodynamic parameters in rats.

Variable	Group	T_0	T_1	T_2	T_3	T_4
MAP (mmHg)	S	109 ± 9	110 ± 8	106 ± 8	108 ± 6	105 ± 7
	30%	115 ± 9	115 ± 6	56 ± 6	60 ± 4	95 ± 4*
	25%	109 ± 9	109 ± 9	54 ± 5	57 ± 8	93 ± 4*
	20%	105 ± 8	108 ± 7	56 ± 5	58 ± 5	89 ± 5*
	15%	112 ± 10	114 ± 6	56 ± 6	55 ± 4	86 ± 4*
HR (beats/min)	S	348 ± 18	347 ± 15	342 ± 15	339 ± 13	340 ± 17
	30%	342 ± 19	352 ± 9	369 ± 12*	365 ± 10*	344 ± 13
	25%	350 ± 17	350 ± 14	375 ± 13*	378 ± 12*	341 ± 12
	20%	352 ± 38	355 ± 14	382 ± 22*	379 ± 19*	350 ± 17
	15%	349 ± 14	347 ± 11	380 ± 20*	377 ± 16*	351 ± 16
CVP (mmHg)	S	7.13 ± 0.83	7.25 ± 0.71	7.63 ± 0.52	7.24 ± 0.48	7.13 ± 0.64
	30%	6.69 ± 1.06	7.00 ± 0.76	7.00 ± 0.76	7.13 ± 0.54	6.38 ± 0.52
	25%	6.63 ± 1.07	6.75 ± 1.28	6.78 ± 1.07	6.70 ± 0.62	6.18 ± 0.83
	20%	6.75 ± 1.13	7.00 ± 1.07	6.75 ± 1.04	6.95 ± 0.98	6.35 ± 1.25
	15%	6.50 ± 0.93	6.65 ± 0.70	6.75 ± 1.05	6.48 ± 0.89	6.28 ± 1.0

CVP, central venous pressure; Hct, hematocrit; HR, heart rate; MAP, mean arterial blood pressure; S, sham-operated group; T_0–T_4 defined in the text; 30%, Hct 30% ANH combined with CH; 25%, Hct 25% ANH combined with CH; 20%, Hct 20% ANH combined with CH; 15%, Hct 15% ANH combined with CH.
Data are means ± standard deviation (SD).
*Significant intragroup differences ($P < 0.05$) from baseline.

increased CBF (6). Cerebral autoregulation maintains CBF constant, usually to a cerebral perfusion pressure of 50 mmHg (18), whereas the combination of ANH and CH impairs the augmented CBF induced by hemodilution (19). Thus, the security of ANH seems to decrease on combination with CH, and the combination may result in cerebral tissue ischemia and hypoxia, followed by cellular damage. However, the risk increases only when ANH involves extremely low Hct values. We aimed to investigate the acceptable Hct range for the ANH–CH combination to help develop a technique for its safe use.

In our study, the combination of ANH and CH did not cause serious hemodynamic instability. HR only increased moderately during CH, which may have been associated with the compensation mechanism for decreased blood pressure. In addition, at T_4 (recovery after CH), MAP was lower than at baseline,

Table 2

Effect of acute normovolemic hemodilution (ANH) combined with controlled hypotension (CH) on arterial blood gas measurements in rats.

Variable	Group	T_0	T_1	T_2	T_3	T_4
P_{aO_2} (kPa)	S	42.8 ± 2.4	43.9 ± 2.3	44.0 ± 2.1	44.4 ± 1.9	45.5 ± 1.9
	30%	43.5 ± 2.1	43.1 ± 2.0	42.5 ± 2.0	42.8 ± 1.7	44.0 ± 1.7
	25%	42.1 ± 2.8	42.0 ± 2.0	41.3 ± 1.3	41.6 ± 2.1	43.1 ± 2.7
	20%	42.8 ± 3.3	43.7 ± 2.8	42.5 ± 2.1	42.1 ± 2.8	45.3 ± 2.8
	15%	42.5 ± 2.5	42.4 ± 2.7	41.6 ± 2.7	41.3 ± 2.1	43.7 ± 2.1
P_{aCO_2} (kPa)	S	5.6 ± 0.4	5.5 ± 0.3	5.2 ± 0.5	5.1 ± 0.4	5.3 ± 0.5
	30%	5.1 ± 0.4	5.1 ± 0.5	4.9 ± 0.4	5.2 ± 0.5	5.3 ± 0.7
	25%	5.5 ± 0.3	5.5 ± 0.4	5.3 ± 0.4	5.1 ± 0.5	5.2 ± 0.5
	20%	5.2 ± 0.3	5.2 ± 0.4	5.3 ± 0.7	5.5 ± 0.4	5.2 ± 0.4
	15%	5.6 ± 0.5	5.3 ± 0.3	5.2 ± 0.4	5.3 ± 0.5	5.5 ± 0.8
pH	S	7.38 ± 0.05	7.40 ± 0.04	7.39 ± 0.05	7.40 ± 0.08	7.39 ± 0.03
	30%	7.39 ± 0.04	7.39 ± 0.03	7.38 ± 0.06	7.40 ± 0.07	7.41 ± 0.06
	25%	7.40 ± 0.05	7.41 ± 0.02	7.37 ± 0.05	7.36 ± 0.05	7.39 ± 0.05
	20%	7.41 ± 0.06	7.42 ± 0.03	7.35 ± 0.03	7.34 ± 0.06	7.38 ± 0.06
	15%	7.42 ± 0.05	7.40 ± 0.04	7.32 ± 0.04	7.28 ± 0.07*	7.29 ± 0.05*

P_{aCO_2}, pressure of arterial carbon dioxide; P_{aO_2}, pressure of arterial oxygen; S, sham-operated group; T_0–T_4 defined in the text; 30%, Hct 30% ANH combined with CH; 25%, Hct 25% ANH combined with CH; 20%, Hct 20% ANH combined with CH; 15%, Hct 15% ANH combined with CH.
Data are means ± standard deviation (SD).
*Significant intragroup differences ($P < 0.05$) from baseline.

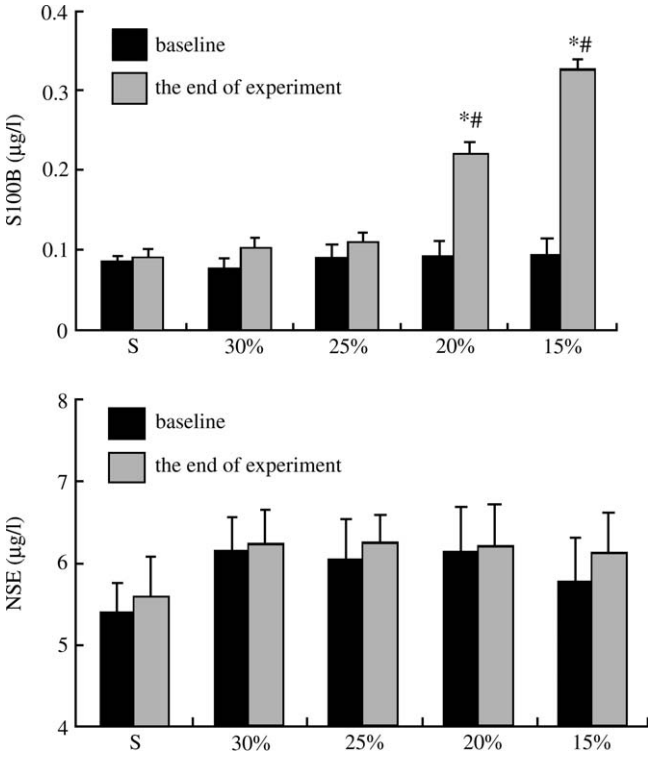

*Fig. 2. Serum concentrations of S100B protein and neuron-specific enolase (NSE). S, sham-operated group; 30%, Hct 30% + CH group; 25%, Hct 25% + CH group; 20%, Hct 20% + CH group; 15%, Hct 15% + CH group. Values are means ± standard deviation (SD). *P < 0.05 vs. baseline values. #P < 0.05 vs. sham-operated group at the end of the experiment. CH, controlled hypotension; Hct, hematocrit.*

possibly because of the decreasing effect of HES 130/0.4 on blood volume expansion at that time, which was 2 h after the infusion of HES. As CVP was not significantly different between the groups, it was not a sensitive parameter, unlike MAP and HR. P_{aO_2} and P_{aCO_2} were both within the normal ranges at the measured time points. P_{aCO_2} may have fallen within the normal range because we always adjusted the ventilation parameters according to the results of arterial blood gas analysis. P_{aO_2} did not differ from baseline levels during ANH and CH, but showed a decreasing trend, especially in the Hct 15% + CH group, which may have resulted in the decreased pH. In addition, tissue hypoperfusion induced by CH may be another explanation for the low pH.

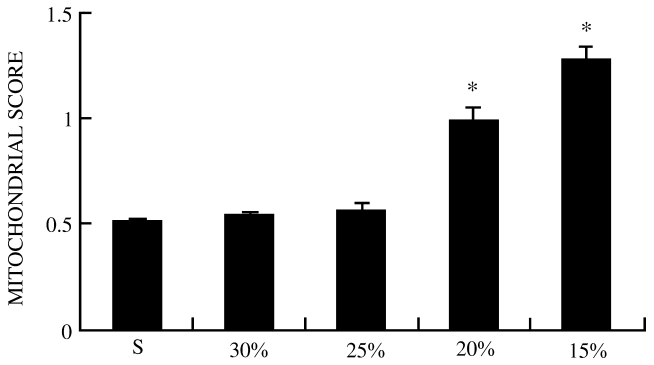

*Fig. 3. Mean mitochondrial score in the five groups after the experiment. S, sham-operated group; 30%, Hct 30% + CH group; 25%, Hct 25% + CH group; 20%, Hct 20% + CH group; 15%, Hct 15% + CH group. Values are means ± standard deviation (SD). *P < 0.05 vs. sham-operated group at the end of the experiment. CH, controlled hypotension; Hct, hematocrit.*

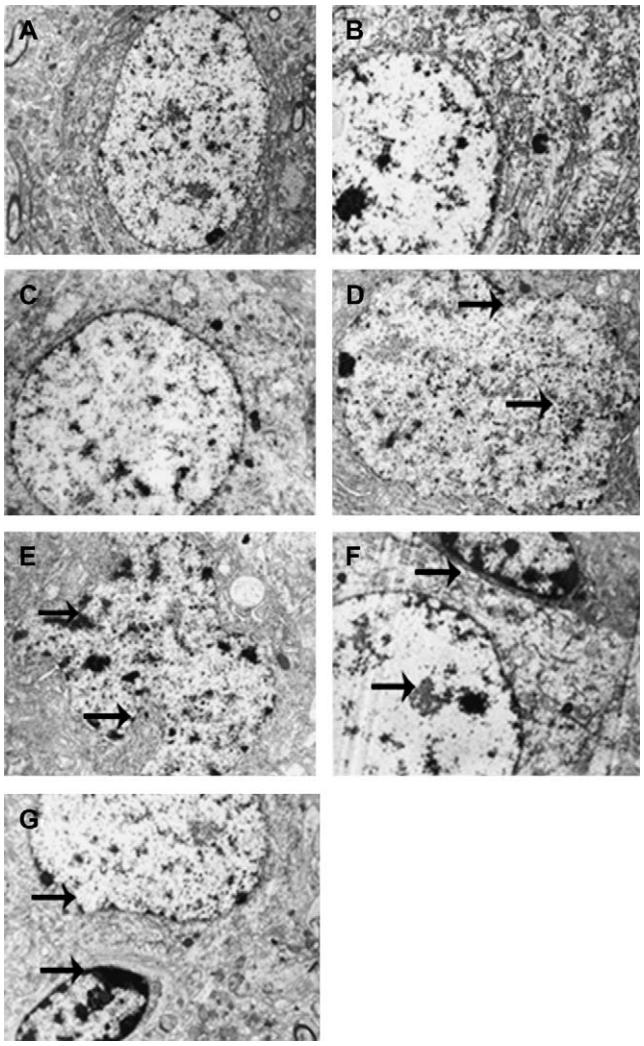

Fig. 4. Nuclear changes in the CA1 region of rat hippocampus following acute normovolemic hemodilution (ANH) combined with controlled hypotension (CH). (A) Ultrastructure of representative nucleus in the CA1 region of the hippocampus in the sham-operated group. (B–E) Ultrastructure of representative nuclei in the CA1 region of the hippocampus in the Hct 30% + CH, Hct 25% + CH, Hct 20% + CH and Hct 15% + CH groups, respectively. (F, G) Glial alteration in the Hct 20% + CH and Hct 15% + CH groups, respectively. Ultrastructural changes are shown with black arrows. Hct, hematocrit.

The cerebral histological investigation using electron microscopy revealed characteristic morphological damage, such as mitochondrial denaturalization, nucleus distortion and astroglial activation, in the CA1 region of the rat hippocampus on severe ANH (Hct $\leq$ 20%) combined with CH. It is clear that significant alterations in mitochondrial structure occur quickly in severely ischemic tissue, and several typical ultrastructural alterations in neurons, such as the disruption of mitochondrial cristae, severe damage to the mitochondrial membrane, and clumping and margination of chromatin in the nuclei, are related to irreversible neuronal injury (15). From these observations of the hippocampus ultrastructure, we can confirm that cerebral tissue was exposed to hypoxic–ischemic injury on ANH and CH with low Hct ($\leq$20%), which might have resulted from the combined decrease in oxygen-carrying capacity and perfusion pressure in the brain. The exact neurophysiological consequences of such ultrastructural morphological changes are unclear, and neurophysiological tests should be performed, in particular, to test the effect of the combined technique on cognitive function. In our pilot study, we chose additional similar groups of rats for neurophysiological testing, but, because of the low survival rate after anesthesia recovery in the Hct 20% + CH and Hct 15% + CH groups, we had to discontinue further testing. Nevertheless, the use of combined ANH and CH may cause certain cerebral complications, and the low recovery rate after anesthesia in these rats may have resulted, to some extent, from hypoxic–ischemic injury.

The S100 protein is a member of a large family of calcium-binding proteins. Elevated serum levels of S100 have been documented in patients with brain damage, such as stroke, head injury and global cerebral ischemia (20, 21). S100B, a subtype of S100, is found in astroglial cells and is highly specific to the brain. Its appearance in the blood indicates brain cell damage and increased permeability of the blood–brain barrier (20). In our study, the serum S100B protein level increased significantly in the Hct 20% + CH and Hct 15% + CH groups post-operatively, when compared with baseline and with the sham-operated group, which was consistent with the histological observations. One recent clinical study of ANH and CH combined has revealed an increased serum protein level of S100B after moderate ANH (Hct 28%) with CH (4), but our study showed an increased S100B level only with severe ANH (Hct $\leq$ 20%) combined with CH. The difference in results may be explained by the fact that, in our study, the animals were placed on mechanical ventilation at $F_iO_2 = 1.0$, whereas the patients in the above clinical setting were ventilated with 50% nitrous oxide in oxygen. Previous studies have demonstrated that hyperoxic ventilation may increase tolerance to anemia in both animal and human (22, 23).

Although serum S100B protein is a highly specific biochemical marker for neuronal damage, we should not rely on a single marker. Thus, we chose NSE as an additional marker for cerebral injury. However, serum level of NSE with ANH and CH combined did not differ from those at baseline or in the sham-operated group, possibly because of the time of detection. Some studies of cerebral damage induced by cardiac arrest have reported that NSE is a promising marker only at later stages (>24 h after cardiac arrest) (24, 25). The results may differ between the studies because of the different induction agents of brain damage – cardiac arrest in refs. (24, 25) and ANH and CH combined in our study. Moreover, we may have found NSE to be an effective marker had we measured it at a later stage. Because of the low survival rate after anesthesia recovery with Hct 20% + CH and Hct 15% + CH treatment, we could not determine the levels of serum NSE at a later time.

Conclusion

Our study of rats demonstrated that SNP-induced CH did not result in cerebral injury with moderate ANH, but with severe Hct ($\leq$20%), ANH with CH may result in cerebral hypoxic–ischemic injury, represented by characteristic morphological damage in the CA1 region of the hippocampus and a consistent increase in the serum S100B level. Although we did not perform further neurophysiological tests to assess functional neurophysiological outcomes, the combination of severe ANH (Hct $\leq$20%) and CH should be avoided.

References

1. Han SH, Bahk JH, Kim JH et al. The effect of esmolol-induced controlled hypotension in combination with acute normovolemic hemodilution on cerebral oxygenation. *Acta Anaesthesiol Scand* 2006; **50**: 863–8.
2. Monk TG, Goodnough LT. Acute normovolemic hemodilution. *Clin Orthop* 1998; **357**: 74–81.
3. Lim YJ, Kim CS, Bahk JH et al. Clinical trial of esmolol-induced controlled hypotension with or without acute normovolemic hemodilution in spinal surgery. *Acta Anaesthesiol Scand* 2003; **47**: 74–8.
4. Suttner SW, Piper SN, Lang K et al. Cerebral effects and blood sparing efficiency of sodium nitroprusside-induced hypotension alone and in combination with acute normovolaemic haemodilution. *Br J Anaesth* 2001; **87**: 699–5.

5. Fenger-Eriksen C, Hartig Rasmussen C, Kappel Jensen T et al. Renal effects of hypotensive anaesthesia in combination with acute normovolaemic haemodilution with hydroxyethyl starch 130/0.4 or isotonic saline. *Acta Anaesthesiol Scand* 2005; **49** (7): 969–74.
6. Crystal GJ, Rooney MW, Salem MR. Regional hemodynamics and oxygen supply during isovolemic hemodilution alone and in combination with adenosine-induced controlled hypotension. *Anesth Analg* 1988; **67** (3): 211–8.
7. Martens P, Raabe A, Johnsson P. Serum S-100 and neuron-specific enolase for prediction of regaining consciousness after global cerebral ischemia. *Stroke* 1998; **29**: 2363–6.
8. Lynch JR, Blessing R, White WD et al. Novel diagnostic test for acute stroke. *Stroke* 2004; **35**: 57–63.
9. Selakovic V, Raicevic R, Radenovic L. The increase of neuron-specific enolase in cerebrospinal fluid and plasma as a marker of neuronal damage in patients with acute brain infarction. *J Clin Neurosci* 2005; **12**: 542–7.
10. Shaaban Ali M, Harmer M, Vaughan R. Serum S100 protein as a marker of cerebral damage during cardiac surgery. *Br J Anaesth* 2000; **85**: 287–98.
11. Rosen H, Rosengren L, Herlitz J et al. Increased serum levels of the S-100 protein are associated with hypoxic brain damage after cardiac arrest. *Stroke* 1998; **29**: 473–7.
12. Wunderlich MT, Ebert AD, Kratz T et al. Early neurobehavioral outcome after stroke is related to release of neurobiochemical markers of brain damage. *Stroke* 1998; **30**: 1190–5.
13. Gross JB. Estimating allowable blood loss corrected for dilution. *Anesthesiology* 1983; **58**: 277–80.
14. Somogyi P, Tagaki H. A note on the use of picric acid paraformaldehyde–glutaralaldehyde fixative for correlated light and electron microscopic immunocytochemistry. *Neuroscience* 1982; **7**: 1779–83.
15. Flameng W, Borgers M, Daenen W et al. Ultrastructural and cytochemical correlates of myocardial protein by cardiac hypothermia in man. *J Thorac Cardiovasc Surg* 1980; **79**: 413–24.
16. Yildirim E, Solaroglu I, Okutan O et al. Ultrastructural changes in tracheobronchial epithelia following experimental traumatic brain injury in rats: protective effect of erythropoietin. *J Heart Lung Transplant* 2004; **23**: 1423–9.
17. Hare GM, Mazer CD, Mak W et al. Hemodilutional anemia is associated with increased cerebral neuronal nitric oxide synthase gene expression. *J Appl Physiol* 2003; **94**: 2058–67.
18. Sadahiro M, Haneda K, Mohri H. Experimental study of cerebral autoregulation during cardiopulmonary bypass with or without pulsatile perfusion. *J Thorac Cardiovasc Surg* 1994; **108**: 446–54.
19. Fukusaki M, Kanaide M, Inadomi C et al. Human middle cerebral artery flow velocity during controlled hypotension combined with hemodilution – transcranial Doppler study. *J Clin Anesth* 2005; **17**: 177–81.
20. Bottiger BW, Mobes S, Glatzer R et al. Astroglial protein S-100 is an early and sensitive marker of hypoxic brain damage and outcome after cardiac arrest in humans. *Circulation* 2001; **103** (22): 2694–8.
21. Hachimi-Idrissi S, Van der Auwera M, Schiettecatte J et al. S-100 protein as early predictor of regaining consciousness after out of hospital cardiac arrest. *Resuscitation* 2002; **53** (3): 251–7.
22. Weiskopf RB, Feiner J, Hopf HWm et al. Oxygen reverses deficits of cognitive function and memory and increased heart rate induced by acute severe isovolemic anemia. *Anesthesiology* 2002; **96**: 871–7.
23. Meier J, Kemming G, Meisner F et al. Hyperoxic ventilation enables hemodilution beyond the critical myocardial hemoglobin concentration. *Eur J Med Res* 2005; **10**: 462–8.
24. Rosen H, Sunnerhagen KS, Herlitz J et al. Serum levels of the brain-derived proteins S-100 and NSE predict long-term outcome after cardiac arrest. *Resuscitation* 2001; **49** (2): 183–91.
25. Rech TH, Vieira SR, Nagel F et al. Serum neuron-specific enolase as early predictor of outcome after in-hospital cardiac arrest: a cohort study. *Crit Care* 2006; **10** (5): R133.

Address:
Dr *Ran Lv*
Department of Anesthesiology
Sir Run Run Shaw Hospital
3 East Qingchun Road
Hangzhou 310016
China
e-mail: nulvran@yahoo.com.cn