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Effects of Dopamine Infusion on Cerebral Blood Flow, Brain Cell Membrane Function and Energy Metabolism in Experimental *Escherichia coli* Meningitis in the Newborn Piglet

In the present study, we tested whether maintenance of adequate cerebral perfusion pressure (CPP) by pharmacologically preventing systemic hypotension with dopamine infusion would prevent cerebral ischemia and attenuate energy depletion and neuronal injury even though intracranial pressure remains elevated in a newborn piglet meningitis model. Cerebral blood flow, measured at the end of the experiment using fluorescent microspheres, was significantly increased by dopamine infusion. The decreased cerebral cortical cell membrane Na^+, K^+-ATPase activity and increased lipid peroxidation products, indicative of meningitis-induced brain damage, were significantly attenuated by dopamine infusion. Dopamine also significantly attenuated the meningitis-induced reduction in both brain ATP and phosphocreatine levels and the increase in brain lactate level. In summary, maintenance of adequate CPP with dopamine prevented cerebral ischemia, reduced cerebral energy depletion, and attenuated brain injury in neonatal bacterial meningitis.

Key Words : *Meningitis, Bacterial; Cerebrovascular Circulation; Energy Metabolism; Infant, Newborn; Swine*

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INTRODUCTION

Bacterial meningitis still remains a serious disease at any age, and is accompanied by significantly high mortality and morbidity despite the development of highly active new antibiotics and intensive care medicine. The prognosis is particularly poor in neonates with mortality rates of 20-40% and long-term neurologic sequelae in up to 50% of survivors (1, 2). Therefore, the development of new adjuvant therapies that can modulate neuronal injury during bacterial meningitis will be necessary to improve the outcome of this disease.

Neuronal damage from bacterial meningitis is a multifactorial process. Besides the acute inflammatory responses in the subarachnoid space (3-6), alterations in cerebral blood flow (CBF) may have an additional and even critical role in the pathogenesis of brain injury that can result in neurologic sequelae or death during bacterial meningitis (3-5, 7-10). Cerebral perfusion pressure (CPP), calculated as the difference between mean arterial pressure (MAP) and intracranial pressure (ICP), has been known to be the main factor influencing CBF in bacterial meningitis (9, 11). With intracranial hypertension and systemic hypotension observed in bacterial meningitis, CPP can be reduced to a certain threshold level that can no longer sustain CBF sufficient for metabolic demand, and hence, cerebral ischemia may ensue (12). An association between cerebral ischemia and poor neurological outcome or death has been demonstrated in various studies of bacterial meningitis (13-15).

Although CPP less than 30 mmHg was strongly correlated with death or major neurological sequelae in infants and children with intracranial infections (11, 16), the degree of ICP elevation alone was not a negative prognostic factor if it was associated with a corresponding elevation in systemic MAP to maintain adequate CPP (11). In a rabbit model of pneumococcal meningitis, although ICP elevation occurred during the early stages of infection, CPP was maintained adequate for cerebral metabolic need if MAP remained normal, and cerebral ischemia and death of experimental animals were observed only with hemodynamic collapse and resultant critical reduction of CPP (12), and maintenance of MAP by high fluid therapy prevented cerebral ischemia (17). In patients with *Hemophilus influenzae* meningitis, shock at the time of admission was associated with poor outcome (18). In traumatic brain injury patients, management of CPP by maintaining optimal MAP and ICP yielded lower mortality and better clinical outcome than that achieved with traditional management solely

based on ICP (19, 20), and the avoidance of systemic hypotension was primarily responsible for this improved outcome (21, 22). Taken together, these findings suggest that maintenance of adequate CPP, primarily by manipulating MAP, would prevent cerebral ischemia, attenuate the brain damage and improve the prognosis even though ICP remains elevated in bacterial meningitis.

This study was done to determine whether maintenance of adequate CPP by pharmacologically preventing systemic hypotension with dopamine infusion would prevent cerebral ischemia and attenuate energy depletion and neuronal injury even though ICP remains elevated in neonatal bacterial meningitis. We used the newborn piglet as an animal model of neonatal bacterial meningitis because the piglet brain is comparable in vascular anatomy, energy metabolism, and maturity to human brain at birth (23). *Escherichia coli* was used to induce meningitis because it is the most common Gram-negative pathogen of neonatal meningitis (2). Adequate CPP was maintained by dopamine infusion because it is the drug of choice in the treatment of neonatal hypotension (24). Cerebral blood flow was measured using fluorescent microspheres (25, 26). Meningitis-induced alterations in brain cell membrane function, structure and energy stores were determined by measuring cerebral cortical cell membrane Na^+, K^+-ATPase activity, lipid peroxidation products (conjugated dienes), and concentrations of high energy phosphate compounds (ATP and phosphocreatine (PCr)), respectively (6, 7).

MATERIALS AND METHODS

Animal preparation

The experiments described herein were reviewed and approved by the Institutional Animal Care and Use Committee of the Samsung Biomedical Research Center, Seoul, Korea. This study also followed the institutional and National Institutes of Health guidelines for laboratory animal care.

Newborn piglets of mixed strain (Yorkshire, conventional breed, purchased from Paju farm, Paju, Kyunggi-Do, Korea) less than 1 week old weighing 2.0 to 2.6 kg were used in this study. Animals inhaled ether for sedation and anesthesia was induced with 5 mg/kg intravenous sodium thiopental and supplemental doses were given as required to maintain anesthesia. After local injection with lidocaine (1%) tracheostomy was performed and the piglet was paralyzed with pancuronium 0.1 mg/kg intravenously and ventilated with neonatal pressure limited-time cycled mechanical ventilator (Sechrist Infant Ventilator, IV-100B, Sechrist Industries Co., Anaheim, CA, U.S.A.). Ventilator settings were adjusted to keep arterial PO_2 at 80-100 mmHg and PCO_2 at 35-45 mmHg. Femoral arteries and veins were cannulated for blood pressure monitoring, blood sampling, and for medication and fluid infusion, respectively. ECG, oxygen saturation, ICP, and blood pressure were continuously monitored using Hewlett Packard neonatal monitoring system (Hewlett Packard Model M1276A, Hewlett Packard Co., MA, U.S.A.). Cisternal puncture was done with a 22-gauge spinal needle (Becton Dickinson, Franklin Lakes, NJ, U.S.A.), and the needle was kept in situ for continuous ICP monitoring and intermittent cerebrospinal fluid (CSF) sampling. Throughout the experiment the piglet was placed under the servo-controlled warmer (Airshields Inc., Hatboro, PA, U.S.A.), and rectal temperature was maintained between 38.0 and 39.0°C.

Experimental protocol

After surgery and a stabilization period, 17 newborn piglets were divided into three experimental groups randomly: 5 in the control group (CG), 6 in the meningitis group (MG), and 6 in the meningitis with dopamine infusion group (DG). In both MG and DG, 10^6 colony-forming units of *E. coli* in 100 μL of saline were injected into the cisterna magna and blood, respectively. In CG, the same amount of saline was given into the cisterna magna and blood, respectively. In DG, dopamine administration was started at a dose of 5 μg/kg/min immediately after bacterial injection, and the dose was increased in increments of 2.5 μg/kg/min every 30 min up to 15 μg/kg/min to maintain CPP above 30 mmHg. Total fluid volume of 6 mL/kg/hr was given in each experimental group throughout the experiment. Continuous monitoring of ICP and MAP were done during the experiment, and CPP was calculated from these values. Arterial blood gas analyses, concentrations of glucose and lactate in the blood and CSF were measured at baseline, and every 1 hr for 8 hr after bacterial inoculation. Bacterial titers in the blood and CSF were determined by plating 10-fold dilutions on blood agar plates and incubating the plates overnight at 37°C in room air. CSF leukocyte counts were measured using hemocytometer at 8 hr after induction of meningitis. Arterial blood gases were measured on a blood gas analyzer (Ciba-Corning Diagnostics Corp., Medfield, MA, U.S.A.) and concentrations of glucose and lactate were measured using an YSI model 2300 dual analyzer (Yellow Springs Instrument Co., Yellow Springs, OH, U.S.A.). At the end of the experiment, the whole brain was harvested by using guillotine, one hemisphere was rapidly frozen in liquid nitrogen and stored at -80°C for further biochemical analyses, and the other hemisphere was dissected for regional CBF measurements.

Measurement of Cerebral Blood Flow

Regional CBF was measured sequentially at baseline and 8 h after induction of meningitis using fluorescent-labeled microspheres (Molecular Probes, Eugene, OR, U.S.A.) by a modification of the reference method for determining organ blood flow (25, 26). Approximately $0.7\text{-}1.2*10^6$ microspheres of one color were injected into the left ventricle, and the reference blood sample was withdrawn from the brachiocephalic

artery using a Harvard withdrawal syringe pump. Pressure recordings of the left ventricular catheter were done before and after each injection to verify intraventricular catheter position at the time of injection. One hemisphere of the brain obtained at the end of the experiment was dissected into the following areas: frontoparietal and temporooccipital cortex, thalamus-hypothalamus, white matter, hippocampus, brain stem, and cerebellum. Fluorescent microspheres were extracted from the tissues using the sedimentation method (26), and fluorescence was measured in a fluorescent spectrophotometer at the appropriate excitation/emission frequencies. Regional CBF were calculated from the following equation: $CBF_i=(I_i*R/I_{ref})*W_i^{-1}$ where CBF_i is regional CBF to brain tissue sample $_i$ (mL/min/g), I_i is the fluorescence intensity of sample $_i$, R is the reference blood sample withdrawal rate (mL/min), I_{ref} is the fluorescence intensity in the reference arterial blood sample, and W_i is the sample weight (g).

Biochemical analyses of brain cortex

The methods of brain cell membrane preparation and determination of cerebral cortical cell membrane Na^+, K^+-ATPase activity, levels of conjugated dienes, tissue glucose and lactate concentrations, ATP and PCr were described in detail previously (6, 7). Briefly, brain cell membranes were prepared according to the method describe by Harik et al. (27). The activity of cerebral cortical cell membrane Na^+, K^+-ATPase was determined by subtracting the enzyme activity in the presence of ouabain from the total activity in the absence of ouabain (28). The level of conjugated dienes was determined using the method of Recknagel and Glende (29). The concentrations of glucose and lactate in the cerebral cortex were determined spectrophotometrically using a commercially available kit (Sigma Chemical Co., St. Louis, MO, U.S.A.). Brain concentrations of ATP and PCr were determined with a coupled enzyme assay using the method of Lamprecht et al. (30).

Statistical analysis

Data were given as mean±standard deviation. A Kruskal-Wallis one-way analysis of variance on ranks was performed followed by the nonparametric analysis of variance with Scheffe's correction for all pairwise multiple comparisons. Statistical analyses described above were done using the SAS software program version 6.12. A p-value of <0.05 was considered significant.

RESULTS

Physiologic variables

Physiologic and laboratory data from the three experimental groups at the end of experiment are summarized in Table 1. There were no significant differences in the baseline values of these variables between the three experimental groups. The heart rate at the end of experiment was significantly increased in DG compared to corresponding values in MG. Arterial pH and base excess were significantly decreased in MG compared to CG, and these abnormalities were significantly attenuated in DG. Significant reduction of glucose and elevation of leukocyte counts and lactate level in the CSF were observed in both MG and DG compared to CG. Bacterial colony counts in the blood and CSF remained elevated in both MG and DG. There were no significant inter-group differences between MG and DG in these abnormalities.

Changes in ICP, MAP, and CPP

The changes in ICP, MAP, and CPP in the three experimental groups during the experiment are shown in Fig. 1. In CG, there were no significant changes in ICP, MAP and CPP throughout the experiment. ICP gradually increased and became significantly elevated in MG and DG when compared to CG after 4 hr of induction of meningitis, and these elevations were not significantly different between MG and DG during the experiment. In MG, MAP and CPP, calculated as MAP minus ICP, became significantly reduced compared to CG after 4 h of experiment, and these reductions were significantly attenuated in DG after 6 h of experiment.

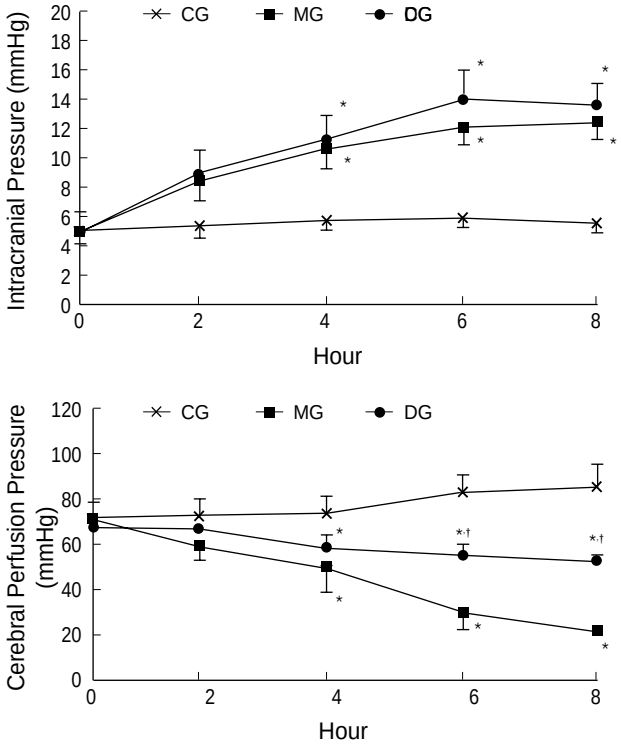
Changes in CBF

In CG, CBF measured at baseline and 8 hr of experiment did not change significantly, and no regional differences were

Table 1. Physiological and laboratory data at 8 hr of experiment in each group of newborn piglets

	CG (n=5)	MG (n=6)	DG (n=6)
Heart rate (/min)	194±36	173±31	228±19†
Arterial pH	7.37±0.04	7.30±0.05*	7.39±0.02†
Arterial O_2 (mmHg)	83±12	83±30	82±13
Arterial CO_2 (mmHg)	45±3	44±3	45±2
Arterial base excess (mEq/L)	1.2±3.6	-6.5±3.3*	-2.5±4.3
Blood glucose (mmol/L)	5.4±0.2	5.7±0.9	4.8±0.7
CSF glucose (mmol/L)	5.1±0.2	2.9±0.2*	2.5±0.6*
CSF to blood glucose ratio	0.94±0.05	0.51±0.07*	0.53±0.14*
Blood lactate (mmol/L)	1.2±0.3	2.9±1.3	2.1±0.2
CSF lactate (mmol/L)	1.7±0.2	5.7±1.3*	4.6±0.9*
CSF to blood lactate ratio	1.50±0.55	2.29±1.00*	2.16±0.53*
Leukocyte count in CSF (/μL)	47±8	1060±78*	986±88*
Bacterial colony count in blood (10^6/μL)	0	3.9±1.6*	3.6±1.8*
Bacterial colony count in CSF (10^6/μL)	0	2.7±0.4*	2.3±0.4*

Values given represent mean±standard deviation. CG, normal control group; MG, meningitis group; DG, meningitis with dopamine group; *: $p<0.05$ compared to NC; †: $p<0.05$ compared to MG.



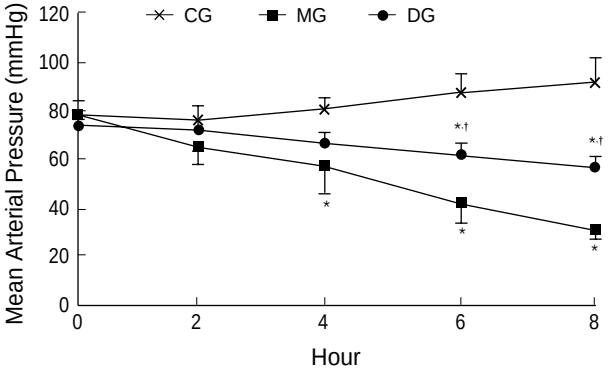


Fig. 1. Time course of changes in intracranial pressure, mean arterial pressure and cerebral perfusion pressure during the experiment in each group of newborn piglets. Data are expressed as mean ± standard deviation. CG, normal control group; MG, meningitis group; DG, meningitis with dopamine group. *: $p<0.05$ compared to NC. †: $p<0.05$ compared to MG.

Table 2. Biochemical data in cerebral cortex of the newborn piglets in each experimental group

Group	CG (n=5)	MG (n=6)	DG (n=6)
Glucose (mmol/kg)	3.7±1.3	4.4±0.9	3.4±0.6
Lactate (mmol/kg)	4.1±1.6	17.0±6.9*	4.6±1.9†
Na^+, K^+-ATPase activity (μmol Pi/mg protein/hr)	52.9±2.7	44.3±2.5*	50.6±2.6†
Conjugated dienes (μmol/g protein)	0.82±0.07	1.06±0.09*	0.93±0.11*,†
ATP (mmol/kg)	3.3±0.9	2.2±0.9*	3.3±0.6†
Phosphocreatine (mmol/kg)	3.0±0.8	1.9±0.9*	3.0±0.6†

Values given represent mean±standard deviation; CG, normal control group; MG, meningitis group; DG, meningitis with dopamine group; *: $p<0.05$ compared to NC; †: $p<0.05$ compared to MG.

observed (Fig. 2). In MG, CBF at 8 hr showed a tendency to decline without statistical significance from the corresponding values in CG at 8 hr in all brain regions. In DG, CBF at 8 hr was significantly increased compared to MG and CG in all brain regions except white matter.

Biochemical data in the cerebral cortex

In MG, the cerebral cortical cell membrane Na^+, K^+-ATPase activity and the levels of lipid peroxidation products (conjugated dienes), measured as an index of alterations in brain cell membrane function and structure, were significantly reduced and elevated, respectively, when compared to CG, and these abnormalities observed in MG were significantly attenuated in DG (Table 2). The cerebral lactate concentration in MG was markedly increased, and this abnormality was significantly attenuated in DG. The reduced concentrations of high-energy phosphate compounds (ATP/PCr) in the cerebral cortex observed in MG were also significantly improved in DG.

DISCUSSION

In the present study, maintenance of adequate CPP by pharmacologically preventing systemic hypotension with dopamine infusion prevented cerebral ischemia, and significantly attenuated cerebral energy depletion and the secondary neuronal injury even though ICP remained elevated in the newborn piglet model of bacterial meningitis. In our previous study of experimental neonatal bacterial meningitis (7), CPP was more dependent on MAP than on ICP. In severely head injured patients, the vast majority of falls in CPP were caused by reduced MAP (20, 22), and the CPP was a better predictor of poor outcome than ICP (21). These findings suggest that isolated ICP elevation alone is not usually severe enough to interfere with cerebral perfusion, and that CPP based management is simply acting as a proxy for the avoidance of systemic hypotension in neonatal bacterial meningitis (11, 12, 17, 18).

There may be a threshold for cerebral hypoperfusion, above which a CPP must be maintained to meet metabolic demand and prevent cerebral ischemia and the ensuing brain injury in neonatal bacterial meningitis (7, 11, 16). These minimum

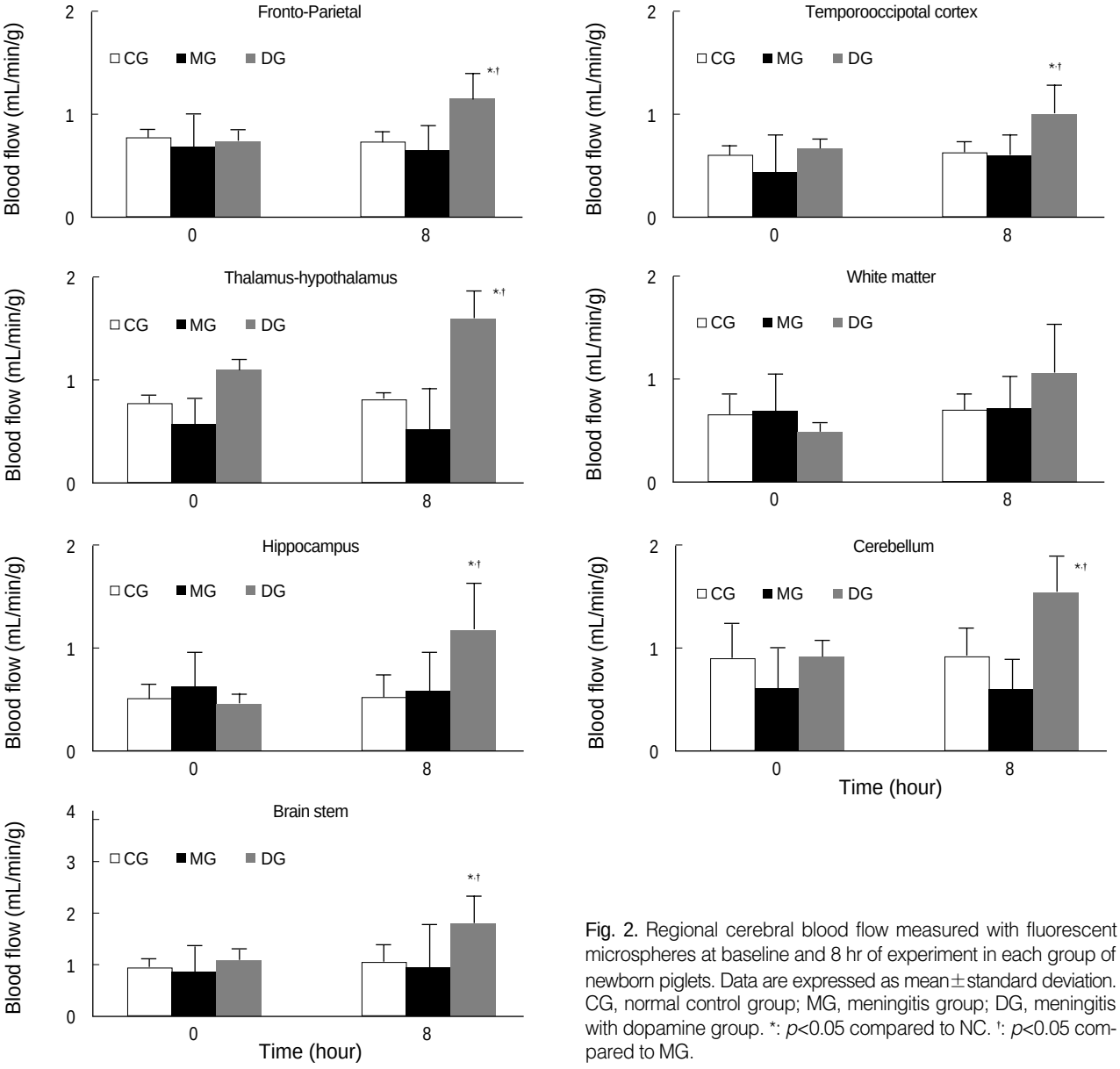


Fig. 2. Regional cerebral blood flow measured with fluorescent microspheres at baseline and 8 hr of experiment in each group of newborn piglets. Data are expressed as mean±standard deviation. CG, normal control group; MG, meningitis group; DG, meningitis with dopamine group. *: $p<0.05$ compared to NC. †: $p<0.05$ compared to MG.

threshold CPP values have been suggested as 55 mmHg and 40-45 mmHg in the adults and children, respectively (21, 22). However, the threshold CPP level in neonates has not been known. In children and infants with central nervous system infections, this value has been reported to be 30 mmHg (11, 16). In newborns, normal CPP has been reported to be 30 mmHg (31), but this value does not necessarily represent the minimal adequate amount of CPP required. In our previous study of neonatal meningitis (7), profound increases in the brain lactate level and decrease in ATP concentration were observed when CPP was reduced to levels below 25 mmHg. In the present study, maintenance of CPP above 30 mmHg prevented cerebral ischemia and secondary brain damage in experimental neonatal bacterial meningitis. Further studies will be necessary to clarify this.

Our data of cerebral energy depletion and anaerobic glycolysis due to cerebral ischemia as evidenced by reduced cerebral ATP/PCr levels and elevated brain lactate levels in MG at CBF levels comparable to the corresponding values in CG suggest that metabolic need during bacterial meningitis are increased (9), and increased CBF will be necessary to meet this increased metabolic demand. Further studies of cerebral metabolic rate during bacterial meningitis will be necessary to clarify this. Significant attenuation of these abnormalities and increase in CBF at 8 hr in DG implicates that maintenance of minimal adequate CPP by pharmacologically avoiding systemic hypotension with dopamine infusion could prevent cerebral ischemia, energy depletion, and anaerobic glycolysis in neonatal bacterial meningitis.

No significant differences in the extent of ICP elevation

between MG and DG throughout the experiment in this study also suggest that pharmacologic elevation of MAP using dopamine could improve CBF without aggravating intracranial hypertension during the early phase of neonatal bacterial meningitis.

In this study, although elevation of CSF lactate level was prominent, it neither showed any significant differences between MG and DG, nor correlated with the brain lactate level. These results suggest that the mechanism of lactate increase in the brain and CSF is different, and that the elevated CSF lactate level originate primarily from acute inflammation in the subarachnoid space rather than as a consequence of cerebral ischemia, energy depletion, and lactic acidosis (6).

In the present study, the reduced cerebral cortical cell membrane Na^+, K^+-ATPase activity and the increased lipid peroxidation products (conjugated dienes) observed in MG were significantly improved in DG. Preservation of cerebral energy stores by preventing cerebral ischemia in DG might be responsible for the significant attenuation of the secondary brain damage in neonatal bacterial meningitis (6, 7).

In summary, critical reduction of CPP insufficient to maintain adequate CBF worsened brain injury, and maintenance of adequate CPP by preventing systemic hypotension with dopamine infusion prevented cerebral ischemia, and attenuated the ensuing brain damage even though ICP remained elevated in neonatal bacterial meningitis.

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