J Korean Med Sci 2000; 15: 203-10
ISSN 1011-8934

Effects of Decreased Cerebral Perfusion Pressure on Cerebral Hemodynamics, Brain Cell Membrane Function and Energy Metabolism During the Early Phase of Experimental *Escherichia coli* Meningitis in the Newborn Piglet

In this study, we tested the hypothesis that decreased cerebral perfusion pressure (CPP) induces cerebral ischemia and worsen brain damage in neonatal bacterial meningitis. Meningitis was induced by intracisternal injection of 10^9 colony forming units of *Escherichia coli* in 21 newborn piglets. Although CPP decreased significantly at 8 hr after bacterial inoculation, deduced hemoglobin (HbD), measured as an index of changes in cerebral blood flow by near infrared spectroscopy, did not decrease significantly. In correlation analyses, CPP showed significant positive correlation with brain ATP and inverse correlation with brain lactate levels. CPP also correlated positively with HbD and oxidized cytochrome aa_3 (Cyt aa_3) by near infrared spectroscopy. However, CPP did not show significant correlation with cerebral cortical cell membrane Na^+,K^+-ATPase activity, nor with levels of lipid peroxidation products. In summary, decreased CPP observed in this study failed to induce cerebral ischemia and further brain injury, indicating that cerebrovascular autoregulation is intact during the early phase of experimental neonatal bacterial meningitis.

Key Words: *Meningitis, Bacterial; Intracranial Pressure; Regional Blood Flow; Energy Metabolism; Animals, Newborn; Spectroscopy, Near Infrared*

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Received: 6 October 1999
Accepted: 6 December 1999

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*This study was supported in part by Samsung Biomedical Research Institute grant (#SBRI C98012) and Ministry of Health and Welfare, Republic of Korea grant (HMP-97-M-2-0019).

INTRODUCTION

Despite continuing improvements in antibiotic therapy and intensive care medicine, neonatal bacterial meningitis remains a serious disease and is still accompanied by significantly high mortality and morbidity (1, 2). The precise mechanism of neuronal injury that can result in death or neurologic sequelae is not completely understood. Therefore, better understanding of the mechanism of brain damage will be necessary to prevent this neurologic damage and reduce the mortality and morbidity caused by meningitis.

Although host inflammatory responses have been known to be primarily responsible for much of the brain injury in bacterial meningitis (3-5), abnormalities in cerebral blood flow (CBF) and metabolism may also play a role in the pathogenesis of brain damage in meningitis (6-8). In bacterial meningitis, autoregulation is usually lost (8) and CBF becomes directly dependent on cerebral perfusion pressure (CPP) (7, 9), calculated as the difference between mean arterial blood pressure (MABP) and intracranial pressure (ICP). With increasing ICP and decreasing MABP observed in bacterial meningitis, CPP can be reduced to certain threshold levels that can no longer sustain sufficient CBF for metabolic demand, and hence, cerebral ischemia may ensue. An association between cerebral ischemia and poor neurological outcome or death has been demonstrated in various studies (10-12). These findings indicate that CPP is a significant prognostic factor in bacterial meningitis. In central nervous system (CNS) infections of infancy and childhood, a striking difference in minimal CPP was found between survivors and nonsurvivors (9). In survivors, CPP could be maintained adequately by reducing ICP, but in nonsurvivors, CPP could not be maintained at levels that ensure adequate CBF, resulting in cerebral ischemia and death. These findings have important clinical implications, since continuous monitoring of CPP in children with severe CNS infections will enable rapid diagnosis and initiation of treatment when CPP is reduced to critical levels, and such treatment might improve the prognosis.

This study was done to determine the effects of de-

creased CPP on cerebral hemodynamics, tissue oxygenation, brain cell membrane function and energy metabolism in neonatal bacterial meningitis. We tested the hypothesis that decreased CPP induces cerebral ischemia and aggravates brain damage in neonatal bacterial meningitis. We employed near infrared spectroscopy (NIRS), a noninvasive technique, to continuously monitor cerebral hemodynamic changes and tissue oxygenation state throughout the experiment. Changes in brain cell membrane structure, function and energy stores in meningitis were determined by measuring lipid peroxidation products (conjugated dienes), Na^+,K^+-ATPase activity and concentrations of high-energy phosphate compounds in the cerebral cortex, respectively.

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. And this study 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, Korea) of less than 3 days old 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 a servo-controlled warmer (Airshields Inc., Hatboro, PA, U.S.A.) and rectal temperature was maintained between 38.0 and 39.0℃.

Bacterial preparation

E. coli strain used in this study was EC69 strain (a gift from Dr. Kwang Sik Kim, University of Southern California). EC69 strain was generated by P1 transduction of the *E. coli* K12 outer membrane protein A (Omp A) gene to Omp A^- mutant of RS218, a CSF isolate from a newborn with *E. coli* meningitis (13). The organism was cultured overnight in 10 mL of brain heart infusion broth (BHI, Difco Laboratories, Detroit, MI, U.S.A.), diluted in fresh medium, and grown for 1 hr to midlogarithmic phase. The suspension was centrifuged for 10 min at 5,000 *g* and resuspended in normal saline, and the absorbance was measured to adjust the bacterial density to the desired concentration.

Near infrared spectroscopic monitoring

To monitor the changes in cerebral blood volume and brain oxygenation, levels of cerebral oxygenated hemoglobin (HbO), deoxygenated hemoglobin (Hb), total hemoglobin (HbT) and oxidized cytochrome aa_3 (Cyt aa_3) were measured continuously using NIRS (NIR 500®, Hamamatsu Photonics KK, Hamamatsu, Japan) throughout the experiment. Details of NIRS measurements were described previously (14, 15). In this study, changes in cerebral concentrations of HbO, Hb and Cyt aa_3 were calculated from the changes in chromophore absorption spectra using modified Beer Lambert law (16), assuming an optical pathlength factor as 4.39 (17). NIRS measurements were made with a sampling time of 30 sec and stored in a computer file via RS232C port system for later analysis. The HbT, calculated as HbO plus Hb, was used as a measure of cerebral blood volume (18). HbD was calculated as HbO minus Hb, and used as an index of cerebral blood flow (19).

Experimental protocol

After surgery and a stabilization period, meningitis was induced by intracisternal injection of 10^9 colony forming units of *E. coli* in 100 μL of saline (n=21). In the control group (n=15), 100 μL of saline was injected into the cisterna magna. Continuous monitoring of NIRS, ECG, ICP, systemic blood pressure and oxygen saturation were done during the experiment. 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℃ in room air. CSF leukocyte counts were measured using hemocytometer at 4 and 8 hr after induction of

meningitis. Arterial blood gases were measured by a blood gas analyzer (Ciba-Corning Diagnostics Corp., Medfield, MA, U.S.A.). Concentrations of glucose and lactate were measured using a YSI model 2300 dual analyzer (Yellow Springs Instrument Co., Yellow Springs, OH, U.S.A.). At the end of the experiment, the brain cortex was harvested using guillotine, and rapidly frozen in liquid nitrogen and stored at -80℃ for further biochemical analyses.

Biochemical analyses of brain cortex

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 phosphocreatine (PCr) were described in detail previously (14, 15, 20). Briefly, brain cell membranes were prepared according to the method described by Harik et al. (21). 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 (22). The level of conjugated dienes was determined using the method of Recknagel and Glende (23). The concentrations of glucose and lactate in the cerebral cortex were determined spectrophotometrically using a commercially available commercial kit (Sigma). Brain concentrations of ATP and PCr were determined with a coupled enzyme assay using the method of Lamprecht et al. (24).

Statistical analysis

Data were analyzed by unpaired t test for inter-group comparisons. To detect significant changes over time within each group, data were compared using repeated measures analysis of variance with Bonferroni correction. Relationships among parameters were compared by calculating the Pearson correlation coefficients. Statistical analysis described above was done using SAS software program version 6.04. A p-value of <0.05 was considered significant. Data were given as mean±standard deviation (SD).

RESULTS

Physiologic variables

Significant increase in ICP and decrease in CPP were observed in the meningitis group when compared to the control group at 8 hr into the experiment. Although MABP showed a tendency to decrease, it did not reach a statistical significance (Table 1).

Significant base deficit was observed in the meningitis group. No significant difference in other physiologic variables such as heart rate, arterial pH, PaO_2, $PaCO_2$, and hemoglobin concentration was observed between the two groups at the end of the experiment.

CSF bacterial titer remained elevated after intracisternal bacterial inoculation and its titer was significantly higher than blood bacterial titer throughout the experiment.

CSF leukocyte count measured at 4 and 8 hr into the experiment remained elevated in the meningitis group.

Glucose and lactate concentration in the blood, brain and CSF

No significant difference in blood and brain glucose

Table 1. Physiologic data measured at 8 hr of experiment in each group of newborn piglets

	Control	Meningitis
Heart rate (/min)	180±35	191±44
Arterial base excess (mEq/L)	4.2±2.7	-2.2±6.6*
Arterial pH	7.45±0.09	7.38±0.12
Mean arterial pressure (mmHg)	67±14	55±21
Intracranial pressure (mmHg)	4±2	11±7*
Cerebral perfusion pressure (mmHg)	64±15	45±23*
Blood glucose (mg/dL)	80.9±5.4	87.0±44.3
CSF glucose (mg/dL)	61.3±11.0	31.3±16.5*
Blood lactate (mmol/L)	1.8±0.8	2.8±1.3*
CSF lactate (mmol/L)	2.5±0.6	8.7±4.0*
Leukocyte count in CSF (/μL)	8±5	571±110*
Bacterial colony count in blood (/μL)	0±0	$5.3\times10^3\pm3.2\times10^3$*
Bacterial colony count in CSF (/μL)	0±0	$3.2\times10^5\pm1.1\times10^6$*
Hemoglobin (g/dL)	8.5±3.2	8.6±1.8

Values given represent mean±SD. *$p<0.05$ compared to control

Table 2. Biochemical data measured at 8 hr of experiment in the cerebral cortex of newborn piglets in each experimental group

	Control	Meningitis
Glucose (mmol/kg)	3.8±1.9	3.8±1.4
Lactate (mmol/kg)	3.1±1.3	5.5±4.4*
Na^+,K^+-ATPase activity (μmol Pi/mg protein/hr)	55±7	47±5*
Conjugated dienes (μmol/g protein)	0.91±0.12	1.09±0.20*
ATP (mmol/kg)	3.9±1.1	2.7±1.0*
PCr (mmol/kg)	3.5±0.6	2.4±1.2*

Values given represent mean±SD. *p<0.05 compared to control

concentration was observed between the two groups. CSF glucose concentration decreased progressively in the meningitis group and significantly reduced compared to the control group at the end of the experiment. Blood, brain and CSF lactate concentration was significantly elevated in the meningitis group compared to the control group and elevation of CSF lactate level was most prominent (Table 1, 2).

Biochemical data in cerebral cortex

Levels of lipid peroxidation products (conjugated dienes), measured as an indicator of alterations in cell membrane structure, were significantly elevated in the meningitis group. Cerebral cortical cell membrane Na^+, K^+-ATPase activity, measured as an index of brain cell membrane function, decreased significantly in the meningitis group compared to the control group. Concentrations of high-energy phosphate compounds (ATP/phosphocreatine) in the cerebral cortex were also significantly decreased in the meningitis group (Table 2).

Near infrared spectroscopic findings

Significant increase in Hb and Cyt aa_3 have been observed in the meningitis group compared to the control group at the end of the experiment. Changes in parameters of HbO, HbT and HbD between the meningitis and the control groups were not significantly different (Table 3).

Table 3. Changes in near infrared spectroscopy (NIRS) parameters measured at 8 hr of experiment in newborn piglets

	Control	Meningitis
Δ HbO_2 (μmol/L)	-1.8±3.1	-1.9±7.2
Δ Hb (μmol/L)	-0.8±3.7	3.2±4.3*
Δ HbT (μmol/L)	-2.2±4.5	1.3±9.3
Δ HbD (μmol/L)	-3.0±9.6	-4.6±7.4
Δ Cyt aa_3 (μmol/L)	0.2±0.8	0.6±0.8*

Values given represent mean±SD
Δ: Changes from baseline values
*p<0.05 compared to control

Correlation coefficients analyses

In correlation analyses, CPP showed significant positive correlation with MABP and negative correlation with ICP. CPP more closely correlated with MABP than with ICP (Fig. 1). No significant correlation was observed between MABP and ICP. CPP and MABP, but not ICP,

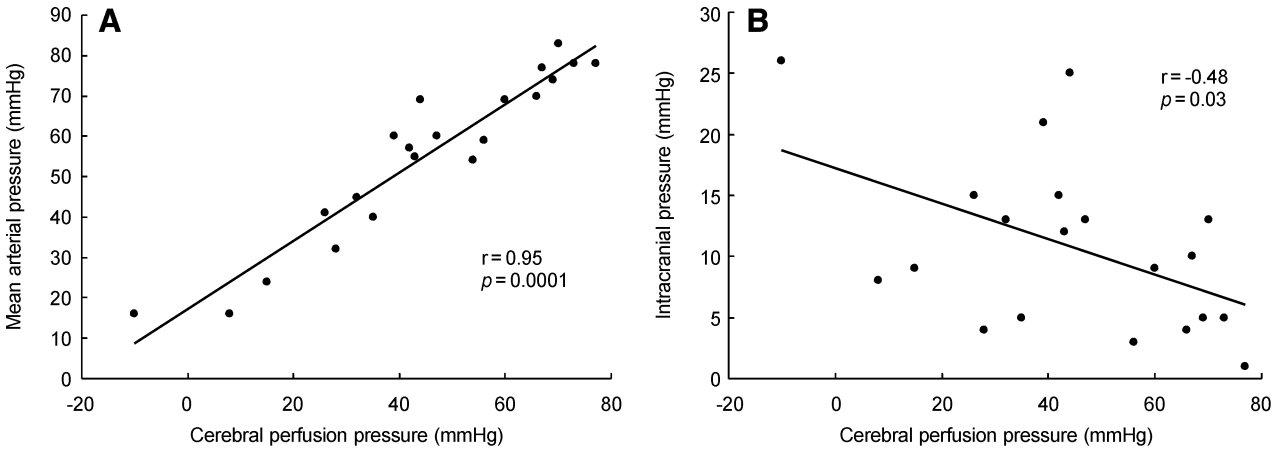

Fig. 1. The relationship between cerebral perfusion pressure and mean arterial blood pressure (A), and between cerebral perfusion pressure and intracranial pressure (B) measured at 8 hr into experiment of meningitis induced newborn piglets. The solid lines represent the best fit by linear regression analysis with y=17.2+0.85×(A), y=17.3−0.15×(B).

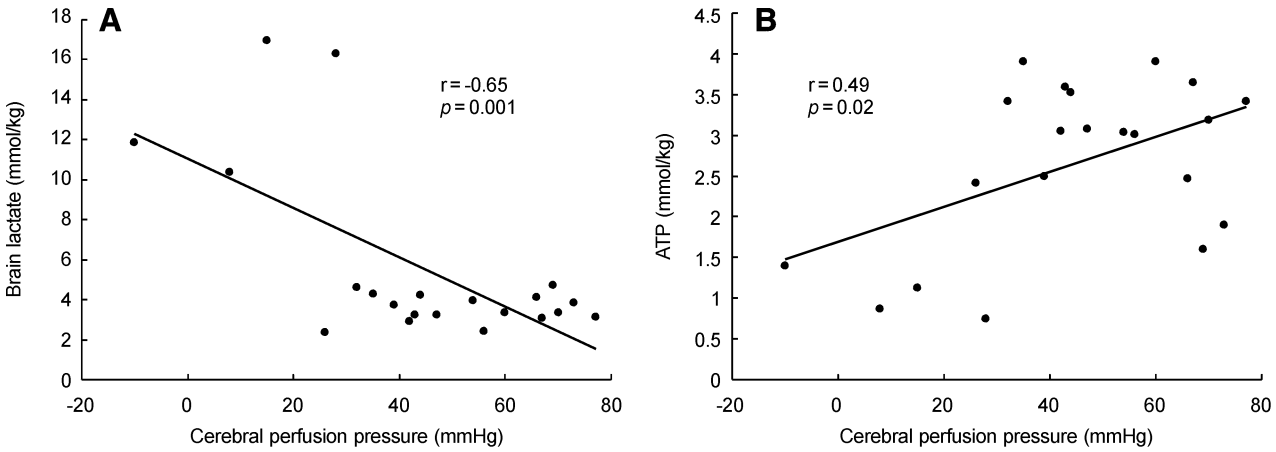

Fig. 2. The relationship between cerebral perfusion pressure and concentration of cerebral lactate (A), and between cerebral perfusion pressure and the level of cerebral ATP (B) measured at 8 hr into experiment of meningitis induced newborn piglets. The solid lines represent the best fit by linear regression analysis with y=11.1−0.12×(A), y=1.7+0.02×(B).

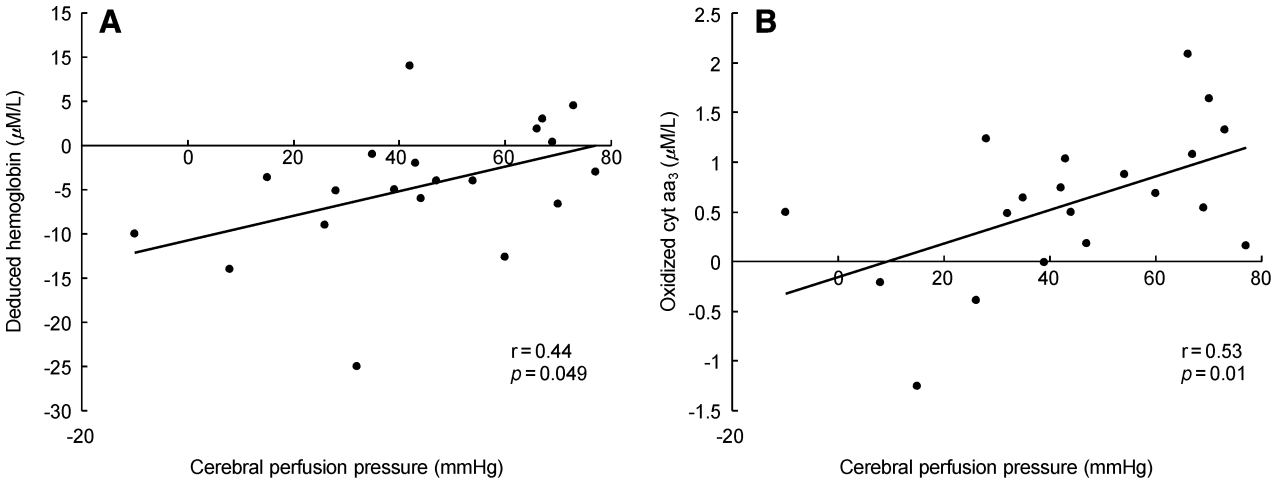

Fig. 3. The relationship between cerebral perfusion pressure and concentrations of deduced hemoglobin (A), and between cerebral perfusion pressure and concentrations of oxidized cytochrome aa_3 (B) measured at 8 hr into experiment of meningitis induced newborn piglets. The solid lines represent the best fit by linear regression analysis with y=−10.8+0.14×(A), y=−0.16+0.02×(B).

showed a significant positive correlation with cerebral high-energy phosphate compounds and negative correlation with brain lactate concentration (Fig. 2). Profound increase in brain lactate and decrease in brain ATP concentration was also observed when CPP was reduced to less than 25 mmHg. In NIRS parameters, CPP showed significant positive correlation with Cyt aa_3 and HbD (Fig. 3). CPP did not show significant correlation with cerebral cortical cell membrane Na^+,K^+-ATPase activity nor with levels of conjugated dienes.

DISCUSSION

In this study, we used the newborn piglet as an animal model of neonatal meningitis because the piglet brain is comparable in brain growth velocity to human brain at birth (25, 26). The rigid confines of the skull without fontanel enabled us to monitor changes in ICP and CBF more accurately. It is also suitable in size for continuous monitoring by NIRS. *E. coli* was used to induce meningitis because it is the most common gram-negative pathogen of neonatal meningitis (2).

In the intact brain, adequate CBF to meet metabolic demand is maintained by autoregulatory mechanisms that induce changes in cerebral vascular resistance in response to changes in CPP (27, 28). In bacterial meningitis, these regulatory mechanisms fail (8), and CBF becomes directly dependent on CPP (7, 9). In this study, although significant decrease in CPP were observed after induction of meningitis, MABP was not significantly decreased, and HbD, measured as an index of cerebral

blood flow, was not significantly reduced. These results indicate that autoregulation of CBF is not dysfunctional at this early phase of bacterial meningitis. Thus, cerebral ischemia was not actually induced at this level of reduction in CPP.

Although low CPP was strongly correlated with death or neurologic injury in infancy and childhood infections of CNS, isolated ICP elevation when accompanied by a corresponding increase in MABP, which maintained CPP, was not predictive of poor outcome (9). Rosner & Daughton (29) reported that in traumatic brain injury patients, in which cerebral ischemia is the most important event determining outcome, management of CPP by maintaining optimal MABP and ICP yielded lower mortality and better clinical outcome than that achieved with traditional ICP only based management. In this study, CPP showed significant correlation with both ICP and MABP though, CPP was more closely correlated with MABP than with ICP, suggesting CPP is more dependent on MABP than on ICP. Further studies will be necessary to determine whether maintenance of adequate CPP, such as by pharmacologically increasing MABP, would ameliorate brain damage and improve prognosis even though ICP is increased in bacterial meningitis.

Some clinical data indicate that reducing CPP below a certain threshold insufficient to maintain metabolic demand causes cerebral ischemia and is strongly associated with death or major neurological sequelae in children with intracranial infections (9, 30). In children, this value has been reported to be 30 mmHg (9, 30). In newborns, normal CPP has been reported to be 30 mmHg (31), but this value does not necessarily represent minimal amount of CPP required. In this study, profound increases in brain lactate level and decreases in ATP concentration were observed when CPP was reduced to levels below 25 mmHg. Further studies will be necessary to clarify this.

Significant decrease in brain ATP and PCr and increase in brain lactate level observed in this study suggests anaerobic glycolysis. In our previous study of bacterial meningitis (20), we demonstrated that this anaerobic glycolysis primarily results from inflammatory responses in the subarachnoid space. However, in this study, CPP showed significant positive correlation with ATP, PCr and inverse correlation with brain lactate levels. When CPP was reduced to levels below 25 mmHg, profound increases in brain lactate level and decreases in ATP concentration were also observed. These results suggest that reducing CPP to below a certain threshold level that cannot meet metabolic demand may induce cerebral ischemia and potentiate anaerobic glycolysis and energy depletion.

Using a newborn piglet model, Tsuji et al. (19) showed that cerebral HbD measured by NIRS significantly correlated with MABP, CBF velocity measured by Doppler ultrasound and CBF measured by the radioactive microsphere technique. These findings indicate that NIRS monitoring is a very useful technique in detecting cerebral hemodynamic changes such as cerebral ischemia, and HbD is the most sensitive indicator in detecting these changes in CBF. Significant correlation of CPP with HbD observed in this study also supports this assumption. However, no significant changes in HbD in the presence of significant decreases in CPP observed in this study indicate that cerebrovascular autoregulation is still intact, and CBF is not actually reduced to this decreased level of CPP during this early stage of bacterial meningitis.

In rabbits with pneumococcal meningitis, Tureen et al. (32) observed a significant reduction in CBF measured by microsphere technique, decrease in cerebral oxidized cytochrome aa_3, a relative increase in Hb and a decrease in HbO without significant changes in HbT measured by NIRS compared with uninfected control rabbits at 18 and 22 hr after induction of meningitis. Comparable HbT and increased Hb, despite decreased CBF, might be attributable to an increase in blood on the venous side of the capillary bed to compensate for the reduction in blood on the arterial side. However, a significant increase in Hb, without significant changes in HbO, HbT, or HbD, observed in this study at 8 hr after bacterial inoculation suggests the possibility that cerebral venous vasodilatation and engorgement might occur primarily during the early phase of bacterial meningitis, rather than as a secondary compensatory phenomenon for reduced CBF.

In contrast to the significant reduction of Cyt aa_3 observed in the study of Tureen et al. (32), increased Cyt aa_3 was observed in this study. The reasons for this discrepancy are difficult to explain. Ogata et al. (33) reported increased Cyt aa_3 after endotoxin administration in dogs and this finding seems to act as a compensatory neuroprotective effect in response to cerebral oxygen deprivation indicated by the decrease in blood pressure, cerebral blood volume and oxyhemoglobin. Using young rabbits, Takashima et al. (34) demonstrated that Cyt aa_3 decreased only in the terminal stage of prolonged hypoxia. Given that Cyt aa_3 redox changes occur late only when oxygen and substrate delivery is extremely compromised, Cyt aa_3 monitoring with NIRS would not be a clinically useful technique for early detection of impending tissue hypoxia-ischemia and energy depletion. These findings also suggest that increased Cyt aa_3 observed in this study may represent an early compensatory neuroprotective phenomenon against a lack of oxygen, and decreased Cyt aa_3 observed in the study of Tureen et al. (32) may represent a late severe oxygen and energy

deprivation state. Taken together, these findings suggest that continuous monitoring of Cyt aa_3 using NIRS is not a sensitive method for the early detection of cerebral oxygen and energy depletion state.

Our data of decreased Na^+,K^+-ATPase activity and increased lipid peroxidation products (conjugated dienes) indicate meningitis induced neuronal dysfunction and brain injury. No significant correlation was observed in this study between CPP and these parameters of changes in brain cell membrane structure and function. These results indicate that decreased CPP does not worsen brain damage at least during this early phase of neonatal bacterial meningitis.

In conclusion, decreased CPP observed in this study failed to induce cerebral ischemia and further brain injury, indicating that cerebrovascular autoregulation is intact during the early phase of experimental neonatal bacterial meningitis. Further studies will be necessary to determine whether decreased CPP below a certain threshold level insufficient to maintain adequate CBF aggravates brain damage and maintenance of adequate CPP, primarily by manipulating MABP, ameliorates brain injury in neonatal bacterial meningitis.

ACKNOWLEDGEMENT

We thank Young Joo Hong and Saem Kang for their technical assistance. This study was supported in part by Samsung Biomedical Research Institute grant (#SBRI C98012) and Ministry of Health and Welfare, Republic of Korea grant (HMP-97-M-2-0019).

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