Cord Blood Myeloperoxidase in Preterm Infants with Periventricular Hyperechogenicity

Chien-An Wang, MD; Kuender D. Yang, MD, PhD; Chung-Bin Huang[1], MD; Hsin-Chun Huang, MD; Li-Tung Huang, MD

Background: Cytokines, adhesion molecules, and inflammatory mediators are believed to play central roles in the pathophysiologic mechanisms of brain white matter lesions. To examine the relationships of cytokines, adhesion molecules, and inflammatory mediators in the cord blood of preterm infants and neonatal cerebral ultrasound periventricular hyperechogenicity (PVH), cord blood cytokines, adhesion molecules, and inflammatory mediators were analyzed, and routine cerebral ultrasound scans were performed in all 96 premature infants.

Methods: The non-PVH group consisted of 20 infants with normal cerebral ultrasound findings during the first week of life. The PVH group consisted of 20 infants with PVH during the first week of life. Cytokines, adhesion molecules, and inflammatory mediators in cord blood including interleukin-8 (IL-8), prostaglandin E2 (PGE2), P-selectin, soluble vascular cell adhesion molecules (sVCAMs), and myeloperoxidase (MPO) were examined by enzyme-linked immunosorbent assay.

Results: There were no significant differences in IL-8, PGE2, P-selectin, and sVCAM levels between patients with and without PVH. Interestingly, MPO levels were marginally significantly higher in patients with PVH than those without PVH (7.46 ± 3.6 vs. 4.81 ± 3.5; $p = 0.024$).

Conclusions: It is concluded that MPO from leukocytes may contribute to the occurrence of PVH in premature infants.

(Chang Gung Med J 2004;27:337-43)

Key words: **premature, cytokine, adhesion molecule, inflammatory mediator, myeloperoxidase, periventricular hyperechogenicity.**

Due to the wide availability of sonography, preterm infants with periventricular hyperechogenicity (PVH) are easily recognized in clinical practice. The occurrence of PVH is believed to carry an increased risk of delayed development, i.e., clumsiness, or visual perception problems.[1-5] PVH is thought to represent white matter damage such as vascular congestion or microscopic hemorrhage.[1-6]

Cytokines, adhesion molecules, and inflammatory mediators are signaling proteins produced by stimulated immune or nonimmune effector cells. It has been well demonstrated that cytokines, adhesion molecules, and inflammatory mediators may contribute to inflammation, breakdown of the brain-

From the Department of Pediatrics, Chang Gung Children's Hospital, Kaoshiung; [1]Department of Pediatrics, St. Joseph Hospital, Hu-Wei, Taiwan.

Received: Sep. 19, 2003; Accepted: Feb. 10, 2004

Address for reprints: Dr. Li-Tung Huang, Department of Pediatrics, Chang Gung Children's Hospital. 123, Dabi Road, Niaosung Shiang, Kaohsiung, Taiwan 833, R.O.C. Tel.: 886-7-7317123 ext. 8702; Fax: 886-7-7338009; E-mail: huang_li@pie.com.tw

blood barrier (BBB), and white matter damage after infection, hypoxia, and ischemia.[7-10] Cytokines, adhesion molecules, and inflammatory mediators have been shown to be related to white matter damage in the premature brain, such as intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL).[11,12] However, there has been no study to examine the relationship between cord blood cytokines, adhesion molecules, and inflammatory mediators and the occurrence of premature PVH.

The aim of this study was to investigate whether certain cytokines, adhesion molecules, or inflammatory mediators play a role in the pathogenesis of PVH in preterm infants.

METHODS

During the study period (from July 1998 to July 2001), routine serial cerebral ultrasonographic and perinatal data were collected from all infants weighting less than 2500 g at birth admitted to the neonatal units of Chang Gung Children's Hospital at Kaohsiung. Arterial blood gas, sugar, C-reactive protein (CRP), and white blood cell (WBC) counts were immediately checked after admission to the neonatal intensive care unit. Infants with a birth weight of 2500 g or less who survived to hospital discharge were divided into 2 subgroups: patients with normal cerebral ultrasound findings, called the non-PVH group; and patients with PVH, called the PVH group. Simultaneous cord blood samples were prepared for measurement of cytokines, adhesion molecules, and inflammatory mediators including interleukin-8 (IL-8), prostaglandin E2 (PGE2), P-selectin, soluble vascular cell adhesion molecules (sVCAMs), and myeloperoxidase (MPO). Written informed consent was obtained from all parents before proceeding the study.

Cerebral ultrasonography (Acuson 128XP, CA, USA) using a 7-MHz probe was performed within 1 week after birth and every 2-4 weeks thereafter until patients were discharged. All examinations included scanning of the brain on coronal view from front to back and on sagittal view from the periphery of 1 side to that of the other. PVH was defined as 1 or more areas (frontal, parietal, or occipital) of increased echodensity, with at least 1 dimension greater than 1 cm at the periventricular region, observed in both the coronal and sagittal planes.[12] After the disappearance of these echodensities, either cystic lesions or focal ventricular dilatation were observed.

Levels of IL-8, PGE2, P-selectin, sVCAMs, and MPO were determined by enzyme-linked immunosorbent assay (ELISA; with the following detection limits: PGE2, 36.2 pg/mL; sVCAMs, 2.0 ng/mL; P-selectin, 0.5 ng/mL; and IL-8, 10 pg/mL from R&D Systems, Minneapolis, MN, USA; and MPO, 1.5 ng/mL from OXIS Research, Portland, OR, USA) according to the manufacturer's instructions.

Statistical analysis

The cytokines, adhesion molecules, and inflammatory mediators in the cord blood of infants with PVH were compared with those of the control group. Data were analyzed using Student's *t*-test and Chi-square or Fisher's exact test. Calculations were performed using the Statistical Package for the Social Sciences (SPSS, vers. 10; Chicago, IL, USA). A p value of less than 0.05 was considered significant.

RESULTS

During the 2-year period, 96 neonates of less than 37 weeks of gestational age and weighing 2500g or less were admitted to our neonatal ICU. Twenty infants weighing 2500g or less at birth who had normal cranial ultrasound findings were randomly selected as the control. Twenty infants weighing 2500g or less at birth, who had PVH during the first week of life, and who were followed-up with normal cranial ultrasound findings were included in this study. Infants with histories of neonatal seizures or hypoxic-ischemic encephalopathy were excluded. There were no signs or symptoms of hypoxic-ischemic encephalopathy in these infants during the admission period or any sequelae of hypoxic-ischemic encephalopathy after 2 years of follow-up.

There was no significant difference in maternal and intrapartum factors between the 2 groups, including gestational age, maternal age, mode of delivery, premature rupture of the membrane, pre-eclampsia, or maternal $MgSO_4$ usage (Table 1).

There were no significant differences in factors of the preterm babies between the 2 groups, including birth body weight, gender, prenatal use of steroids and antibiotics, delay of initial crying, 1-min

Table 1. Comparison of Maternal Data between the PVH and Non-PVH Groups

	Brain sonography at 1 week old		
	PVH	Non-PVH	
Number	20	20	*p* value
Gestational age (weeks)	32.35±2.3	32 ±2.38	0.639
Maternal age (years)	29.55±5.73	31.15±3.73	0.302
Cesarean delivery	15	15	NS
Toxemia	6	2	0.114
PROM	8	6	0.507
Prenatal steroid use	5	2	0.212
Prenatal antibiotics use	10	4	0.525
Maternal MgSO4 use	8	4	0.168

Abbreviations: PROM: premature rupture of membrane; NS: not significant.
Data are presented as the mean±SD.

Table 3. Comparison of Cytokines, Adhesion Molecules, and Inflammatory Mediator Levels between the PVH and Non-PVH Groups

	Brain sonography at 1 week old		
	PVH	Non-PVH	
Number	20	20	*p* value
IL-8 (pg/ml)	113.26±174.2	46.96±61.4	0.122
sVCAM (ng/ml)	620.85±178.8	652.15±225	0.629
PGE2 (pg/ml)	1322.5±2137.5	1242.3±1712.6	0.896
P-selectin (ng/ml)	86±71.3	83.52±41	0.892
MPO (ng/ml)	7.46±3.6	4.81±3.5	0.024*

Data are presented as the mean±SD.
* $p<0.05$ compared to the normal brain ultrasonography group.

Table 2. Comparison of Infant Data between the PVH and Non-PVH Groups

	Brain sonography at 1 week old		
	PVH	Non-PVH	
Number	20	20	*p* value
BBW (g)	1521.75±279	1479.75±272	0.633
Gender male	7	9	NS
female	13	11	NS
DOIC	3	5	0.429
1-min Apgar score	5.25±1.6	5.45±2.0	0.729
5-min Apgar score	7.6±1.27	7.4±1.6	0.665
Serum pH	7.29±0.09	7.25±0.07	0.144
Sugar (mg/dl)	61.7±40.5	57±47.7	0.739
CRP (mg/l)	3.4 ±0.48	3.7±0.91	0.258
WBC (1000/mm^3)	9424±4145	9201±7777	0.911
ANC (1000/mm^3)	4361.73±3205.7	4425.43±6591	0.969
Hospitalization days	43.3±23.3	38.9±15.5	0.487
Mechanical ventilation (days)	4.45±7.76	3.55±4.44	0.655
Surfactant treatment	4	3	0.677

Abbreviations: BBW: birth body weight; DOIC: delay of initial crying; CRP: C-reactive protein; WBC: white blood cell; ANC: absolute neutrophil count; NS: not significant.
Data are presented as the mean±SD.

and 5-min Apgar scores, serum pH, sugar, CRP, WBC, the need for a surfactant, the incidence of respiratory distress syndrome, the need for mechanical ventilation days, and hospital days (Table 2).

Analysis was performed to compare differences of cytokines, adhesion molecules, and inflammatory mediators in cord blood (Table 3). There were no significant differences in P-selectin ($p=0.892$), IL-8 ($p=0.122$), PGE2 ($p=0.896$), or sVCAM ($p=0.629$) levels. However, MPO levels were marginally significantly higher in patients with PVH than in those without PVH ($p=0.024$).

DISCUSSION

The major finding of this study suggests that cord blood MPO is associated with the occurrence of premature PVH. At present, there are few data

regarding the roles of cytokines, adhesion molecules, and inflammatory mediators in premature PVH. Our results add information about the underlying pathophysiologic mechanisms of premature white matter injury. Because lumbar puncture to collect cerebrospinal fluid (CSF) is slightly invasive for preterm infants, we measured cord blood cytokines instead. Therefore, we cannot exclude that there might be certain undetected correlations between CSF cytokines, adhesion molecules, and inflammatory mediators and PVH. Moreover, relationships between cord blood cytokines, adhesion molecules, and inflammatory mediators and long-term neurological outcomes deserve further study.

The immune system is activated by invasive organisms and by stress.[13] Chemotactic substances such as interleukins may be activated first. Neutrophils are attracted to the inflammatory site by chemotactic substances. Endothelial cells of the vascular wall express adhesion molecules to bind with the neutrophils. Adhesion molecules include P-selectin and sVCAMs. P-selectin is expressed on endothelial cells within a few minutes,[14] whereas sVCAMs are expressed within a few hours. Neutrophils migrate to inflammatory sites and release myeloperoxidase, which kills invasive microorganisms.[13]

The results of this study demonstrate that elevated cord blood MPO levels were marginally significantly higher in preterm infants with PVH than those without PVH. Because of the difficulty of collecting patients, we believe that if we were to increase the sample size, then there would be a more-significant result indicating that MPO plays a major role in the pathogenesis of PVH. MPO levels in the cord blood may originate from neutrophils or inflammatory tissue. This finding suggests that neutrophils might migrate and release MPO, which consequently damages cerebral white matter. Furthermore, increased MPO activity has been shown after hypoxic-ischemia injury, and this also causes brain damage.[15] In support of this idea, allopurinol through its mechanism of blocking MPO production has been proposed to reduce hypoxic-ischemic brain injury. Hudome et al.[16] concluded that neutrophils contribute to hypoxic-ischemic brain injury in neonatal rats, and that neutrophil depletion before the insult is neuroprotective. Darakchiev et al.[25] used an experimental anti-inflammatory drug (FL1003, butyrolactone) to show that brain tissue water content and MPO activity in a mini-pig model were significantly lower after receiving butyrolactone in severe brain injury.

Other evidence supports myeloperoxidase's contribution to ischemic brain injury in hypertensive rats with middle cerebral artery occlusion. Barone et al.[23] found an increase in PMN infiltration and MPO activity in the ischemic cortex. That biochemical and histological study strongly suggested that PMNs adhere within blood vessels and infiltrate into brain tissue due to focal ischemia and that the associated inflammatory response might contribute to focal ischemic injury. Other evidence from Miljkovic-Lolic et al.[24] showed a neuroprotective effect of hyperbaric oxygen treatment before middle cerebral artery occlusion. They also found that a reduced cerebral infarct size was associated with decreased MPO activity in the pretreated group. Neurologic outcomes correlated better with MPO activity than with the infarct volume.

MPO truly plays a role in brain edema and injury in animal models. Our findings in cord blood also support this idea. Blocking MPO production or adding anti-inflammatory drugs might have neuroprotective effects in animal models. In the future, PVH preterm infants might receive these anti-inflammatory drugs to prevent further neurologic sequelae.

In this study, although IL-8 in PVH patients was higher than that of the controls, it did not reach a significant difference ($p = 0.122$). IL-8 is a chemotactic substance and an inflammatory mediator that can induce neutrophil aggregation and damage brain cells. A previous study showed that IL-8 levels in blood are higher in cerebral palsy neonates.[10] Likewise, neonatal bacterial sepsis with higher levels of IL-8-positive monocytes indicates a poorer outcome.[17] Here, we did not find increased IL-8 in patients with periventricular hyperechogenicity. This may be explained by the temporal effect of IL-8. If the blood was not collected at the peak level, we may have missed the possible contribution of IL-8 to premature PVH.

Both P-selectin and sVCAMs are adhesion molecules.[13] Selectin is mainly involved in leukocyte rolling and adhesion,[18] while sVCAMs are mainly involved in leukocyte adhesion and migration. P-selectin and sVCAM expressions increase after cerebral ischemia.[14] Previous studies have shown that

monoclonal antibodies against P-selectin and sVCAMs reduce reperfusion injury after ischemic brain injury.[19] However, our results do not support that P-selectin and sVCAMs are involved in the occurrence of PVH.

Prostaglandin E2 in CSF can cause damage to the BBB and result in cerebral edema in animals under hypoxic-ischemia injury and reperfusion.[20,21] However, in this study, there was no significant difference in serum PGE2 levels between PVH patients and the controls.

The primary source of cytokines, adhesion molecules, and inflammatory mediators might come from the uterus, amniotic fluid, placenta, or fetus.[9] Previous studies have demonstrated that during maternal infection, amniotic fluid cytokines, such as IL-6, IL-1beta, and tumor necrosis factor-alpha, all contributed to neonatal brain white matter lesions with subsequent cerebral palsy.[12] These cytokines, adhesion molecules, and inflammatory mediators might increase the permeability of the BBB and facilitate the passage of microbial products and cytokines into the brain. Together, they lead to endothelial damage with subsequent microhemorrhage or edematous change of the periventricular white matter.[22] We measured cytokines, adhesion molecules, and inflammatory mediators from cord blood in this study. This study may bear a more-direct causal relationship with neonatal white matter lesions.

Results from this study suggest that cord blood MPO levels are correlated with PVH in preterm infants. The possible correlation between cytokines, adhesion molecules, and inflammatory mediators and PVH raises much interest in searching for the underlying mechanism of white matter injury in preterm infants and may open an avenue for the development of new therapies for premature white matter diseases.

Acknowledgements

The study was supported in part by grant NHRI-EX90-9026SP to Dr. Li-Tung Huang and Dr. Kuender D. Yang.

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臍帶血Myeloperoxidase造成早產兒之腦室周圍高超音波值

王建安 楊崑德 黃崇濱[1] 黃新純 黃立同

背 景： 在腦白質病變的病理生理機轉中，細胞素 (cytokines)、沾粘分子 (adhesion molecules) 和發炎介質 (inflammatory mediators) 認爲扮演中心的角色。爲了研究早產兒臍帶血中細胞素、沾粘分子和發炎介質和腦部超音波中腦室周圍高超音波值 (periventricular hyperechogenicity-PVH) 之間的關係，從96位早產兒裡檢查臍帶血中細胞素、沾粘分子、發炎介質和腦部超音波。

方 法： 非PVH組爲20位早產兒第一周大時爲正常之腦部超音波，PVH組爲20位早產兒第一周大時爲腦室周圍高超音波值之腦部超音波。早產兒出生後用酵素螢光免疫分析法檢查臍帶血中之細胞素、沾粘分子和發炎介質包括IL-8、PGE2、P-selectin、sVCAM和MPO。

結 果： IL-8、PGE2、P-selectin和sVCAM的值在兩組中並沒有顯著差異。有趣的是唯一有顯著差異爲在有腦室周圍高超音波值中之MPO值比沒有腦室周圍高超音波值高 (7.46 ±3.6 vs. 4.81 ±3.5; $p = 0.024$)。

結 論： 我們認爲中性顆粒球中之MPO在早產兒腦室周圍高超音波值之出現或許有相關性。
(長庚醫誌 2004;27:337-43)

關鍵字：早產兒，細胞素，沾粘分子，發炎介質，myeloperoxidase，腦室周圍高超音波值。

長庚兒童醫院 高雄院區 兒童內科；[1]天主教若瑟醫院 虎尾 兒科
受文日期：民國92年9月19日；接受刊載：民國93年2月10日。
索取抽印本處：黃立同醫師，長庚兒童醫院 兒童內科。高雄縣833鳥松鄉大埤路123號。Tel.: (07)7317123轉8702; Fax: (07)77338009; E-mail: huang_li@pie.com.tw