The Role and Mechanisms of IL-6, IL-8 and TNF-α for Regulating Cerebral Hemodynamics in Term Infants With Hypoxic-Ischemic Encephalopathy

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Abstract

Objective: It is well known that cytokines play important role in the pathophysiological mechanisms of neonatal hypoxic-ischemic encephalopathy (HIE). Cytokines can regulate the cerebral hemodynamics, but their mechanisms have been reported rarely. This study was to explore the role and the mechanisms of cytokines on regulating cerebral hemodynamics in neonates with HIE.

Materials and Methods: The levels of peripheral blood interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-α), and endothelin-1 (ET-1), calcitonin gene-related peptide (cGRP) in 30 neonates with hypoxic-ischemic encephalopathy and 30 healthy neonates were measured on the 1st day of life using radioimmunoassay. Hemodynamic parameters of middle cerebral artery including peak systolic flow velocity (PSFV, cm/s), end-diastolic flow velocity (EDFV, cm/s), time-averaged mean velocity (TMFV, cm/s), pulsatility index (PI) and resistance index (RI) in both groups were measured by pulsed Doppler ultrasound immediately after the blood samples had been collected.

Results: In infants with hypoxic-ischemic encephalopathy, the plasma levels of IL-8 and TNF-α were increased while the serum levels of IL-6 were decreased, which correlated with disturbed cerebral hemodynamics and the changes in plasma levels of ET-1 and cGRP. Linear correlation analysis showed positive correlation of RI with IL-8 (r=0.80; p<0.01) and TNF-α (r=0.72; p<0.01) but negative correlation with IL-6 (r=-0.61; p<0.01). Furthermore, IL-8 and TNF-α were positively correlated with ET-1 (r=0.61; 0.72 respectively, p<0.01) and negatively correlated with cGRP (r=-0.51; -0.63 respectively, p<0.01), while, IL-6 was negatively correlated with ET-1 (r =-0.54; p<0.01) and positively correlated with cGRP (r=0.52; p<0.01).

Discussion: The results of this study showed that the changes of plasma levels of IL-6, IL-8 and TNF-α in neonatal hypoxic-ischemic encephalopathy may regulate cerebral hemodynamics by regulating the changes of ET-1 and cGRP so as to play an important role in the pathophysiologic mechanisms.

Keywords: newborn, hypoxic-ischemic encephalopathy, cerebral hemodynamics, interleukin-6, interleukin-8, tumor necrosis factor-alpha, endothelin-1, calcitonin gene-related peptide

Özet

Hipoxik-İskemik Ensefalopatili Term Bebeklerde, Serebral Hemodinamiğin Düzenlenmesinde IL-6, IL-8 ve TNF-α’nın Rolü ve Mekanizması


Material ve Metot: Öz hipoksik-iskemik ensefalopatili ve 30 sağlıklı yeni doğanlarda yoğunluğu gün radioimmunoessay kullanlara periferal kan interleukin-6 (IL-6), interleukin-8 (IL-8), tümör nesroz fator-alfa (TNF-α), endotelin-1 (ET-1) ve kalsi- tonin gen ilgili peptit (cGRP) seviyeleri belirlenmiştir. Ayrıca kan örneklerinin hemen alınmasıdan sonra pulsed Doppler ultra-
Methods

Participants
From January 2005 to May 2006, thirty full-term infants with HIE and thirty full-term healthy controls were enrolled into this study, and the study protocol was approved by the research committee of Beijing Obstetrics and Gynecology Hospital Affiliated to Capital University of Medical Science in China. All the HIE patients met the following criteria: (i) History of severe perinatal asphyxia; i.e. Apgar score ≤3 at 1 minute and <5 at 5 minutes after birth or umbilical arterial blood pH≤7.0. (ii) Profound metabolic academia caused by hypoxia. (iii) Neonatal neurologic manifestations, e.g. seizures, coma, hypotonia, irritation, loss of primary reflex, dilatation or diminution of pupils, intracranial hypertension. (iv) Multisystemic organ dysfunction, e.g. central respiratory failure, pulmonary hypertension, systemic hypotension, renal dysfunction, gastrointestinal abnormalities. Their mothers were all free from autoimmune disease, and all the neonates had no infectious diseases and were not treated with any medicines which could affect infantile immune function during the study.

Methods

In all infants, ultrasound examinations were performed at the bedside on the 1st day after birth while they were silent and all the measurements were performed by the same examiner. Infants laid in a supine position with their heads slightly elevated. Measurements of cerebral blood flow velocity were made using a 2~5 MHz convex or phased array transducer of computed sonography system (GE healthcare logiq 400 diagnostic apparatus). The transducer was placed on the temporal fontanelle to detect the hemodynamic parameters of left or right middle cerebral artery (MCA), including peak systolic flow velocity (PSFV, cm s⁻¹), end-diastolic flow velocity (EDFV, cm s⁻¹), time-averaged mean flow velocity (TMFV, cm s⁻¹), pulsatility index [PI, PI=(PSFV-EDFV)/TMFV] and resistive index [RI, RI=(PSFV-EDFV)/PSFV]. The electronic steering feature of the linear probe was used to keep the angle of insonation as low as possible, in most cases it was 0 ° or at least less than 20 ° in five complete cardiac cycles.

After the ultrasound examinations were done, 2 ml of peripheral venous blood samples in both groups were collected immediately for detecting the levels of IL-6, IL-8, TNF-α, ET-1 and cGRP simultaneously. Blood samples were placed in test tubes containing sodium citrate, mixed gently, and immediately centrifuged, and the plasma was preserved at below -20°C for later measurement by radioimmunoassay (Dongya Immunological Technology Institute in Beijing, China).

Table 1. General informations in both groups

<table>
<thead>
<tr>
<th></th>
<th>HIE Group (n=30)</th>
<th>Control Group (n=30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>27.3±2.3</td>
<td>28.1±1.9</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>38.6±0.74</td>
<td>38.3±0.60</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Birth weight (Grams)</td>
<td>3289±439</td>
<td>3413±436</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Infant age (hours)</td>
<td>10.2±5.2</td>
<td>11.1±4.8</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Apgar score, 1 minute</td>
<td>2.68±0.69</td>
<td>3.9±0.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Apgar score, 5 minutes</td>
<td>4.5±0.64</td>
<td>9.43±0.68</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Introduction

Hypoxic-ischemic encephalopathy (HIE) is one of the most common causes of neonatal death and can lead to severe long-term neurologic disability in some survivors, including cerebral palsy and neurodevelopmental delay (1-4). It is well known that loss of autoregulation of cerebral hemodynamics is the major reason of neonatal HIE (5-7), and previous studies have demonstrated that cytokines were involved in the mechanisms of hypoxic-ischemic brain damage through several ways (8-15). Although cytokines can regulate cerebral hemodynamics, data on the mechanisms of action involved appear very rarely. This study was to: 1) Determine the changes in blood levels of TNF-α (as well as the serum levels of endothelin-1 (ET-1) and calcitonin gene-related peptide (cGRP) in term infants with HIE. 2) Detect the changes of cerebral hemodynamics in the same patients. 3) Explore the role of these cytokines in regulating cerebral hemodynamic changes and the pathophysiologic mechanisms involved.

Materials and Methods

Participants

From January 2005 to May 2006, thirty full-term infants with HIE and thirty full-term healthy controls were enrolled into this study, and the study protocol was approved by the research committee of Beijing Obstetrics and Gynecology Hospital Affiliated to Capital University of Medical Science in China. All the HIE patients met the following criteria: (i) History of severe perinatal asphyxia; i.e. Apgar score ≤3 at 1 minute and <5 at 5 minutes after birth or umbilical arterial blood pH≤7.0. (ii) Profound metabolic academia caused by hypoxia. (iii) Neonatal neurologic manifestations, e.g. seizures, coma, hypotonia, irritation, loss of primary reflex, dilatation or diminution of pupils, intracranial hypertension. (iv) Multisystemic organ dysfunction, e.g. central respiratory failure, pulmonary hypertension, systemic hypotension, renal dysfunction, gastrointestinal abnormalities. Their mothers were all free from autoimmune disease, and all the neonates had no infectious diseases and were not treated with any medicines which could affect infantile immune function during the study.

Methods

In all infants, ultrasound examinations were performed at the bedside on the 1st day after birth while they were silent and all...
Increased expression of IL-6 after infarction had several other actions of TNF-α, antagonizing the neurotoxicity of excitatory amino acids (23,24), and so on. The results of this study showed the blood levels of IL-8 increased significantly in infants with HIE. IL-8 is one of the strongest chemotactic factors of neutrophilic leukocytes, which could mediate ischemic-reperfusion injury by inducing neutrophils accumulating in the lesion regions, i.e. it could obstruct those small vessels and capillaries and lead to ischemia of local brain tissues (17,18). We also found the serum levels of TNF-α were elevated markedly in HIE infants, which could enhance the phagocytosis and cytotoxicity of neutrophilic granulocyte, and modulate the expression of many other proteins, such as IL-8 and other hazardous substances to induce over-inflammatory reactions in local brain tissue (19-21). Other actions of TNF-α involved in the pathogenesis include: (i) Injuring the vascular endotheliocytes, damaging the cell membrane and intracellular organs, leading to morphological changes of blood vessels, and increasing permeability of endotheliocytes. (ii) Damaging blood-brain barrier and increasing its permeability. (iii) Activating cerebral phospholipase A2, a key enzyme to hydrolyze brain cell membrane phospholipid. The hydrolysis of nerve cell membrane phospholipids may impair the function and structure of cells and lead to brain cell injury. (iv) Improving the synthesis and release of chemotactic factors such as interleukin-1 (IL-1) and IL-8, inducing leukocyte accumulation and “breath outbreak” to accelerate over-inflammatory and lead to brain cell injuries. Cytokines may also have a toxic effect, causing increased production of nitric oxide synthase, cyclooxygenase and free radical release (19-21). All these data support a relationship between increasing levels of cytokines (such as IL-8 and TNF-α in this study) and inflammation and/or tissue destruction.

Animal experiments showed that there was no expression of IL-6 in normal rat brain cells, but in infracted areas its expression was increased markedly, and IL-6 expression gradually increased with the prolonged infarction period in the local ischemic cerebral tissues (22). Increased expression of IL-6 after infarction had a protective role for brain tissues by improving the excretion of neural growth factors, inhibiting the synthesis of IL-1β and TNF-α, antagonizing the neurotoxicity of excitatory amino acids (23,24), and so on. The results of this study showed the blood levels of IL-6 was decreased markedly in patients with HIE, which may prevent its protective effects on ischemic cerebral tissues and accelerate hypoxic-ischemic injuries.

Study on the fetus of high-risk pregnancy by Dubiel M et al. (25) has showed abnormal MCA PI significantly correlated with TNF-α and IL-6 levels. This might suggest TNF-α and IL-6 may

### Table 2. The comparing of cerebral hemodynamic changes in the first day of life in infants with and without HIE (x±s)

<table>
<thead>
<tr>
<th></th>
<th>HIE Group (n=30)</th>
<th>Control Group (n=30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSFV (cm/s)</td>
<td>34.9±2.1</td>
<td>42.4±7.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EDFV (cm/s)</td>
<td>8.97±2.01</td>
<td>16.33±3.11</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TMFV (cm/s)</td>
<td>17.7±3.9</td>
<td>26.9±4.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PI</td>
<td>1.47±0.27</td>
<td>1.09±0.08</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RI</td>
<td>0.75±0.03</td>
<td>0.61±0.03</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

### Table 3. The comparing of IL-6, IL-8, TNF-α, ET-1 and cGRP in infants with and without HIE (x±s)

<table>
<thead>
<tr>
<th></th>
<th>HIE Group (n=30)</th>
<th>Control Group (n=30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (ng/L)</td>
<td>52.6±23.3</td>
<td>80.7±21.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL-8 (µg/L)</td>
<td>0.67±0.15</td>
<td>0.46±0.12</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TNF-α (µg/L)</td>
<td>1.18±0.27</td>
<td>0.90±0.22</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ET-1 (ng/L)</td>
<td>57.1±13.9</td>
<td>34.7±9.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>cGRP (ng/L)</td>
<td>290±145</td>
<td>191±99</td>
<td>&lt;0.01</td>
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</tbody>
</table>

The linear correlation analysis showed that IL-8 was positively correlated with ET-1 levels (r=0.62, p<0.01) and negatively correlated with cGRP levels (r=-0.55, p<0.01), TNF-α positively correlated with ET-1 levels (r=0.73, p<0.01) and negatively correlated with cGRP levels (r=-0.62, p<0.01). IL-6 was negatively correlated with ET-1 (r=-0.54, p<0.01) and positively correlated with cGRP (r=0.52, p<0.01).

### Discussion

It has been demonstrated that cytokines play an important role in the pathophysiological mechanisms of neonatal brain damage (8-15). Savmam et al. (16) have found elevated IL-8 in cerebrospinal fluid in neonates after birth asphyxia. The results of this study showed that the blood levels of IL-8 increased significantly in infants with HIE. IL-8 is one of the strongest chemotactic factors of neutrophilic leukocytes, which could mediate ischemic-reperfusion injury by inducing neutrophils accumulating in the lesion regions, i.e. it could obstruct those small vessels and capillaries and lead to ischemia of local brain tissues (17,18). We also found the serum levels of TNF-α were elevated markedly in HIE infants, which could enhance the phagocytosis and cytotoxicity of neutrophilic granulocyte, and modulate the expression of many other proteins, such as IL-8 and other hazardous substances to induce over-inflammatory reactions in local brain tissue (19-21). Other actions of TNF-α involved in the pathogenesis include: (i) Injuring the vascular endotheliocytes, damaging the cell membrane and intracellular organs, leading to morphological changes of blood vessels, and increasing permeability of endotheliocytes. (ii) Damaging blood-brain barrier and increasing its permeability. (iii) Activating cerebral phospholipase A2, a key enzyme to hydrolyze brain cell membrane phospholipid. The hydrolysis of nerve cell membrane phospholipids may impair the function and structure of cells and lead to brain cell injury. (iv) Improving the synthesis and release of chemotactic factors such as interleukin-1 (IL-1) and IL-8, inducing leukocyte accumulation and “breath outbreak” to accelerate over-inflammatory and lead to brain cell injuries. Cytokines may also have a toxic effect, causing increased production of nitric oxide synthase, cyclooxygenase and free radical release (19-21). All these data support a relationship between increasing levels of cytokines (such as IL-8 and TNF-α in this study) and inflammation and/or tissue destruction.
regulate fetal cerebral blood flow in some cases. The results of present study showed that: (i) There was significant cerebral hemodynamic disturbance in infants with HIE. (ii) The serum concentration of IL-8, TNF-α in infants with HIE were increased and IL-6 was decreased. (iii) The hemodynamic parameter RI was positively correlated with IL-8 and TNF-α levels and negatively correlated with IL-6. (iv) IL-8 and TNF-α were positively correlated with ET-1 levels and negatively correlated with cGRP levels while IL-6 was negatively correlated with ET-1 and positively correlated with cGRP. It suggested that the lower the IL-6 level, the higher the levels of IL-8 and TNF-α, and the more decrease in cerebral perfusion. Hence, IL-6, IL-8 and TNF-α might play a role in the changes of cerebral hemodynamics in neonatal hypoxic-ischemic encephalopathy. As the most effective chemotactic factor on the neutrophilic leukocytes, IL-8 can decrease cerebral blood flow by inducing leukocytes to accumulate in the microvessels of injury area and block the blood vessels. It also induces the production of coagulant by endothelial cells and promotes anticoagulant activity and thrombosis in the vessels. TNF-α can damage vascular endothelialcytes, and lead to morphological and functional changes, such as necrosis, scaling, cell space enlargement, and thus reduce cerebral blood flow. The effects of IL-6 on cerebral blood flow may be achieved indirectly by suppressing the synthesis of IL-8 and TNF-α. Cytokines may also influence intravascular cell adhesion, coagulation and thrombosis (26). It can activate the endothelium and stimulate its procoagulant properties, while inhibiting its anticoagulant and fibrinolytic effects (27). This might also explain why blood flow changes in the cerebral artery were related to IL-6, IL-8 and TNF-α levels.

Endothelin-1 (ET-1), a 21 amino acid polypeptide, produced by vascular endothelial cells, is a potent vasoconstrictor peptide that has an important role in the maintenance of basal vasomotor tone, interacting with other vasoactive agents and potentiating their vasoactive effect (28,29). Calcitonin gene-related peptide (cGRP) is one of the most potent vasodilator peptides known (30), having a potency of ~10-fold greater than prostaglandins and ~100-1000-fold greater than classical vasodilators, such as acetylcholine, adenosine and 5-HT (31). So, the balance of serum levels of ET-1 and cGRP is important in maintaining the tissue and organ blood supply by regulating the counterpoise in vascular tension. The results of this study have shown that the serum levels of ET-1 and cGRP were significantly increased in infants with HIE, thus the elevated cGRP can antagonize the vasoconstrictive effects of the elevated ET-1 in blood serum.

Our study has also shown that IL-8 and TNF-α were positively correlated with ET-1 and negatively correlated with cGRP while IL-6 was negatively correlated with ET-1 and positively correlated with cGRP. So, we believe that the changes of IL-6, IL-8 and TNF-6 in term infants with HIE influence cerebral hemodynamics by regulating the changes of serum ET-1 and cGRP, and accordingly exert a marked effect in the pathophysiologic mechanisms underlying hypoxic-ischemic encephalopathy.

Acknowledgments

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References