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

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

Amaç: Sitokinlerin, neonatal hipoksik-iskemik ensefalopatisinin (HIE) fizyopatolojik mekanizmalarında önemli bir rolü olduğu bilinmektedir. Ancak, sitokinlerin serebral hemodinamiği düzenleyip düzenlemedikleri ve mekanizmalarına dair bilgiler çok azdır. Bu çalışmanın amacı, sitokinlerin HIE'li yenidoğanlarda serebral hemodinamiği düzenlemedeki rolünü ve mekanizmalarını araştırmaktır.

Materyal ve Metot: Otuz hipoksik-iskemik ensefalopatili ve 30 sağlıklı yenidoğanda doğdukları gün radioimmünoessey kullanılarak periferal kan interlökin-6 (IL-6), interlökin-8 (IL-8), tümör nekrozis faktör-alfa (TNF-α), endotelin-1 (ET-1) ve kalsitonin gen ilişkili peptit (cGRP) seviyeleri belirlenmiştir. Ayrıca kan örneklerinin hemen alınmasından sonra pulsed Doppler ultra-

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son ile iki gruptaki pik sistolik akım hızı (PSFV, cm/s), son-diyastolik akım hızı (EDFV, cm/s), zaman-ortalamalı ortalama akım hızı (TMFV, cm/s), pulsatilite endeksi (PI) ve rezistans endeksi (RI) dahil olmak üzere orta serebral arterin hemodinamik parametreleri ölçülmüştür.

Sonuçlar: Bulgular, hipoksik-iskemik ensefalopatisi olan çocuklarda IL-8, TNF-α plazma seviyeleri artarken, IL-6 serum seviyelerinin azaldığını göstermiştir. Bu sonuçlar, bozulmuş serebral hemodinami ve ET-1 ve cGRP plazma seviyelerindeki değişikliklerle korele idi. Lineer korelasyon analizi, RI'nın IL-8 (r=0.80, p<0.01) ve TNF-α (r=0.72, p<0.01) ile pozitif, IL-6 (r=-0.61, p<0.01) ile negatif korelasyonunun bulunduğunu göstermiştir. Ayrıca, IL-8 ve TNF-α ile ET-1 (sırasıyla r=0.61, 0.72) arasında pozitif bir korelasyon (p<0.01) ve cGRP (sırasıyla r=-0.51, -0.63; p<0.01) arasında negatif korelasyon bulunduğu görülmüştür. Buna karşılık, IL-6'nın ET-1 (r=-0.54, p<0.01) ile korelasyonu negatif, cGRP (r=0.52, p<0.01) ile korelasyonu pozitif olarak belirlenmiştir.

Tartışma: Bu çalışmadan elde edilen sonuçlar, yenidoğan hipoksik-iskemik ensefalopatisinde IL-6, IL-8 ve TNF-α plazma seviyelerinin ET-1 ve cGRP değişikliklerini düzenleyerek serebral hemodinami kontrolünde ve fizyopatolojik mekanizmalarında önemli bir rolü olabileceğini göstermektedir.

Anahtar sözcükler: yenidoğan, hipoksik-iskemik ensefalopati, serebral hemodinami, interlökin-6, interlökin-8, tümör nekrozis faktör-alfa, endotelin-1, kalsitonin gen ilişkili peptid

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 interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis-α (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 scores ≤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 can 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-TEDFV)/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 several infants, and all the indeces were measured over three to 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

	HIE Group (n=30)	Control Group (n=30)	*p* value
Maternal age (years)	27.3±2.3	28.1±1.9	>0.05
Gestational age (weeks)	38.6±0.74	38.3±0.60	>0.05
Birth weight (Grams)	3289±439	3413±436	>0.05
Infant age (hours)	10.2±5.2	11.1±4.8	>0.05
Apgar score, 1 minute	2.68±0.69	9.30±0.70	<0.01
Apgar score, 5 minutes	4.58±0.64	9.43±0.68	<0.01

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

	HIE Group (n=30)	Control Group (n=30)	*p* value
PSFV (cm/s)	34.9±9.1	42.4±7.2	<0.01
EDFV (cm/s)	8.97±2.01	16.33±3.11	<0.01
TMFV (cm/s)	17.7±3.9	26.9±4.9	<0.01
PI	1.47±0.27	1.09±0.08	<0.01
RI	0.75±0.03	0.61±0.03	<0.01

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

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

Statistical analysis

Statistical analysis was performed with SPSS for Windows (Release 12.0, SPSS Inc, Chicago, IL). The results are reported as mean±SD and assessed by Student's *t*-test and linear correlation analysis.

Results

General information

The general information on both groups are listed in Table 1. Two patients died after this study.

Cerebral hemodynamic changes in the first day of life in infants with and without HIE; see Table 2.

It can be seen from Table 2 that there was significant decrease in blood flow velocity including PSFV, EDFV and TMFE and marked increase in PI and RI in infants with HIE compared with the healthy controls ($p<0.01$).

Changes of blood levels of IL-6, IL-8, TNF-α, ET-1 and cGRP in the first day of life in infants with and without HIE; see Table 3.

It can be seen from Table 3 that the serum concentration of IL-8, TNF-α, ET-1 and cGRP in infants with HIE were increased markedly ($p<0.01$), while the serum level of IL-6 was increased significantly ($p<0.01$).

The correlation between IL-6, IL-8 and TNF-α and the changes of RI in infants with HIE

By linear correlation analysis, it was found that RI was positively correlated with IL-8 and TNF-α levels (r=0.80, 0.72, respectively, $p<0.01$) and negatively correlated with IL-6 (r=-0.61, $p<0.01$).

The correlation between IL-6, IL-8 and TNF-α and the changes of ET-1 and cGRP in infants with HIE

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 phagocytosity 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 A_2, a key enzyme to hydrolyze brain cell membrane phospholpid. 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 chemotaxic 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

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 coagulin by endothelial cells and promotes anticoagulin activity and thrombosis in the vessels. TNF-α can damage vascular endotheliocytes, 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-α 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.

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