
Sequential changes in pathophysiology of systemic inflammatory response in a disseminated neonatal herpes simplex virus (HSV) infection

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Title: Sequential changes in pathophysiology of systemic inflammatory response in a disseminated neonatal herpes simplex virus (HSV) infection.

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Running title: inflammation and apoptosis in neonatal HSV infection

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ABSTRACT

Background. Disseminated neonatal herpes simplex virus (HSV) infection causes a typical systemic inflammatory response syndrome and has a high mortality rate. However, the validity of anti-inflammatory intervention against this condition remains unknown.

Objectives. We sought to demonstrate the sequential changes in the pathophysiology of disseminated neonatal HSV infections.

Study design. The HSV serum copy number as well as high-mobility group box 1 (HMGB1) and cytochrome c concentrations, which predict the severity and mortality rate of sepsis, were sequentially evaluated in a patient with disseminated neonatal HSV infection caused by HSV-2.

Results. As the patient presented with evidence of hyper-inflammation and severe illness, we empirically undertook anti-inflammatory intervention that included the administration of prednisolone, high-dose immunoglobulin, and blood exchange therapy in addition to high-dose acyclovir (ACV) therapy. The patient survived without significant neurological sequela. We found that 1) the serum concentrations of both HMGB1 and cytochrome c were extremely high, 2) temporal increases in these biomarkers were observed after admission, and 3) interestingly, the increase in HMGB1 level preceded that of cytochrome c. These results suggested that the pathophysiology of this condition changed sequentially in a dramatic manner, and the timing of our anti-inflammatory intervention was prior to the transition of pathological status from hyper-inflammation to massive apoptosis.

Conclusions. Anti-inflammatory intervention may only be effective if it is undertaken during the early phase of disseminated neonatal HSV infections.

KEY WORDS; neonatal HSV infection, sepsis, anti-inflammatory intervention, HMGB1, cytochrome c
1. Why this case is important

Neonatal herpes simplex virus (HSV) infection is a severe disease classified into three types: localized skin, eye, and mouth (SEM) disease, central nervous system (CNS) disease, and disseminated disease, which involves several organs with or without CNS involvement. Although the outlook for neonatal HSV infection has improved due to the establishment of high-dose acyclovir (ACV) therapy, the mortality rate for patients with disseminated disease remains high. The pathophysiology of disseminated disease is a typical systemic inflammatory response syndrome (SIRS); that is, viral sepsis, often leading to disseminated intravascular coagulation (DIC), shock, and multiple organ dysfunction syndrome (MODS). Recent investigations suggested that direct invasion by the pathogen as well as unregulated host-immunological responses collaboratively formed the pathology of sepsis. However, the validity and efficacy of anti-inflammatory intervention against this condition remains unknown.

Several biomarkers, such as high-mobility group box 1 (HMGB1) and cytochrome c, were found to predict the presentation of MODS and subsequent mortality in sepsis patients. However, there have been few analyses of these biomarkers in neonatal HSV infections reported. Although we used no control groups, including the analysis of healthy neonates or other infectious disease patients, we undertook a sequential analysis of these biomarkers in a single patient with disseminated neonatal HSV-2 infection, who survived without significant neurological sequelae. Here we present our observations of the dramatic sequential changes in pathophysiology, and also discuss the validity of anti-inflammatory intervention.

2. Case description

2.1. Clinical course of a case of disseminated neonatal HSV infection

A 7-day-old male baby, born at a gestational age of 36 weeks with a birth weight of 3600 g, presented with fever and not doing well. His mother showed no symptoms suggesting a prepartum genital herpes infection. The infant was taken to a nearby hospital and immediately transferred to
our institution for the provision of intensive care. He presented with high fever, tachycardia, tachypnea, and occasional apnea. Laboratory findings showed thrombocytopenia (20000/μl), prolonged coagulation time, PT; 20 sec. (normal range 9.8-12.1 sec.), APTT; 80 sec. (normal range 27.0-39.9 sec.), reduced fibrinogen level (70 mg/dl), indicating DIC, elevated aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) levels and cerebrospinal fluid pleocytosis with monocyte dominance. Mechanical ventilation to control the apnea and high-dose ACV (60mg/kg/day) were started immediately. As high serum ferritin (2900mg/dl) and urine β-2 microglobulin (16000μg/ml) concentrations suggested unregulated hyper-inflammation, high-dose immunoglobulin (1g/day) for two days and prednisolone 2mg/kg/day were administred. In addition, blood exchange therapy (BET) was carried out on the 1st and 2nd days of admission. The patient was diagnosed with disseminated neonatal HSV infection based on the detection of HSV-DNA in his cerebrospinal fluid and serum. Serum AST, ALT, LDH levels showed further increases from 690, 90, 3000 IU/L, respectively, at admission to 6000, 900, 16000 IU/L on the 3rd day of admission. Fortunately, these makers all peaked on the 3rd day, and no apparent manifestations of MODS were observed. (Figure 1.) ACV was administrated for 21 days and no relapse was observed thereafter. The patient is now 18 months old and shows normal development, although brain magnetic resonance imaging (MRI) has revealed a small cystic region in the forebrain.

2.2. Sequential analysis of serum HSV-DNA copy number, and HMGB1 and cytochrome c concentrations

Serum HSV-DNA copy number was quantified by real-time PCR using TaqMan® probes (Applied Biosystems) that could differentiate between HSV-1 and HSV-2, as described previously. Serum concentrations of HMGB1 and cytochrome c were assayed by use of an enzyme-linked immunosorbent assay (ELISA) at the Shinotest Science Laboratory (Kanagawa) for HMGB1, and at the SRL Laboratory (Tokyo) for cytochrome c. All specimens were obtained with informed consent from the parents, in accordance with the World Medical Association’s Declaration of
Helsinki. The serum HMGB1 and cytochrome c values in healthy adults are 0.6-1.5 ng/ml and <0.1 ng/ml, respectively.\textsuperscript{5-6}

Results showed that HSV-2 DNA was detected (4.0 x 10\textsuperscript{3} copies/ml) in his serum at admission, before decreasing to undetectable levels within 18 hours. Serum concentrations of HMGB1 and cytochrome c were 31.9 ng/ml and 16.0 ng/ml, respectively, at admission. The concentrations of both markers increased temporarily after admission, with the peak concentration of HMGB1 preceding that of cytochrome c. The peak values of HMGB1 and cytochrome c were 71.2 ng/ml (on the 2\textsuperscript{nd} day) and 217 ng/ml (on the 3\textsuperscript{rd} day), respectively. (Figure 1.)

3. Other similar and contrasting cases in the literature

One previous publication reported certain evidence for hyper-inflammation in a case of disseminated neonatal HSV infection successfully treated with anti-inflammatory intervention combined with high-dose ACV.\textsuperscript{8} Unfortunately, they did not carry out any pathophysiological analyses.

4. Discussion

HMGB1 is a novel cytokine, and originally a nuclear DNA-binding protein, that plays a critical role in the activation of the inflammation response to tissue damage as an ‘alarmin’.\textsuperscript{9} Its release into extracellular fluid from necrotic cells or certain activated leukocytes induces an innate immune response and the production of other proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-\textalpha).\textsuperscript{10} Cytochrome c is an intramitochondrial protein that translocates into the cytoplasm and extracellular space during the apoptotic process.\textsuperscript{6} It is already known that apoptosis in endothelial cells and parenchymal organs plays a critical role in the development of MODS in sepsis patients.\textsuperscript{11}

In our patient, the levels of biomarkers reflecting the severity of the sepsis were extremely high. In addition, these levels temporally increased after admission in spite of the administration of
high-dose ACV and a rapid decrease in serum HSV-DNA copy number. Although normal values for neonates have not yet been established for these biomarkers, the high serum concentrations of HMGB1 and cytochrome c in our patient indicated that he was at high risk for developing MODS. Interestingly, the increase in HMGB1 level preceded that of cytochrome c. It is already known that some proinflammatory cytokines, and TNF-α in particular, initially cause inflammation via the NF-κB pathway and persistent stimulation leads to subsequent apoptosis in the target cells. Therefore, it is possible that our observations reflect the sequential inflammation process in this patient.

While it is important to note the possible impact on these findings from the blood exchange therapy (BET), which presumably reduce the serum levels of these biomarkers in a similar manner to those of AST, ALT and LDH, it is estimated that a severe HSV infection led to an excessive release of proinflammatory cytokines, and subsequent HMGB1 secretion from necrotic cells enhanced the inflammation and allowed it to develop to systemic and pathological levels, with massive apoptosis thereafter observed on the release of cytochrome c into the serum.

Kamei et al, reported a retrospective analysis of the effect of corticosteroid therapy in addition to ACV in cases of adult HSV encephalitis and demonstrated that it improved the neurological outcome. However, in neonatal HSV infection, the efficacy of anti-inflammatory intervention against disseminated neonatal HSV infection remains unclear. We empirically undertook anti-inflammatory intervention in addition to ACV administration. It may be possible that its effectiveness in this patient was due to the fact that it was started in the early phase of the disease, prior to the progression of hyper-inflammation into massive apoptosis. In addition, sequential monitoring of HMGB1 and cytochrome c concentrations may be beneficial in detecting this physiological phase.

To date, this is the only case presented as demonstrating a good course, and further study, including meta-analysis, is needed to confirm our hypothesis.
Funding

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Conflict of Interest

None.

Declared Ethical approval

This study was performed in accordance with the World Medical Association's Declaration of Helsinki.

Acknowledgements

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Acknowledgements

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Figure legend

Figure 1.

The course after admission from the 1\textsuperscript{st} to 7\textsuperscript{th} day of admission is shown. Therapeutic agents and treatments, sequential data of serum Herpes simplex virus (HSV)-2 copy number (shown as open circles in graph A), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) (shown as filled black triangles, filled gray triangles, and open triangles, respectively in graph B), serum concentrations of high-mobility group box 1 and cytochrome c (shown as filled rectangles and open rectangles, respectively in graph C) are shown. AST, ALT, LDH, HMGB1, and cytochrome c showed temporary increases after admission, despite appropriate ACV administration and a rapid decrease in serum HSV-2 DNA copy number, with the increase in HMGB1 preceding that of cytochrome c.
Figure 1.

- Mechanical ventilation
- ACV (60mg/kg/day)
- PSL (2mg/kg/day)
- High-dose gamma globulin (1g/kg)
- Blood exchange therapy
- Nafamostat mesilate

### Figure A

HSV-2 Copy number

### Figure B

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### Figure C

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