OBSTETRICS

The efficacy of intravenous immunoglobulin on lipopolysaccharide-induced fetal brain inflammation in preterm rats

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OBJECTIVE: Interleukin-1 is accepted as one of the major cytokines; it is involved in inflammatory processes and systemic fetal inflammatory response that is triggered by maternal lipopolysaccharide (LPS) injection. Because it is an antiinflammatory agent, we investigated (in the brain damage of rat pups) the role of intravenous immunoglobulin (IVIG) in decreasing interleukin-1 beta (IL-1β) expression and caspase 3 activity that was induced by maternal LPS administration.

STUDY DESIGN: Dams were divided into 3 groups. Pyrogen-free saline solution (NS) was administered intraperitoneally to group 1; LPS (0.3 mg/kg) suspension in NS was administered to groups 2 and 3 at 19 days of gestation. Two hours after the first injection, a second injection of NS was administered intravenously to group 1 (NS + NS), of IVIG was administered intravenously to group 2 (LPS + IVIG), and of NS was administered intravenously to group 3 (LPS + NS). Hysterectomy was performed in one-half of the dams 2 hours after the second injection and in the other one-half of the dams 22 hours after the second injection. Pups were delivered, and the brains were extracted just after delivery. IL-1β expression and caspase 3 activity were determined in brain tissues.

RESULTS: For the pups at 4 hours, the IL-1β expression of group 2 was significantly lower than groups 1 and 3. For the pups at 24 hours, the IL-1β expression of group 2 was significantly lower than group 3 but was similar to group 1. For the pups at 24 hours, caspase 3 activity of groups 1 and 2 were significantly lower than group 3.

CONCLUSION: Maternal IVIG administration decreased IL-1β expression and caspase 3 activity in the brain tissue of rat pups, which had been induced by maternal LPS-administration.

Key words: inflammation, interleukin-1 beta (IL-1β), intravenous immunoglobulin (IVIG), lipopolysaccharide

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Fetal exposure to infection/inflammation not only results in preterm birth but also in fetal inflammatory response. It causes organ damage because of the release of proinflammatory cytokines, chemokines, and other inflammatory mediators (such as prostaglandins, leukotrienes, and complement). Fetal inflammatory response results in either multiorgan failure and intrauterine exitus or in bronchopulmonary dysplasia, intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis, sepsis, or pneumonia in the early postnatal period, which are known as fetal inflammatory response syndrome.[1-3]

The brain is one of the main affected organs in fetal inflammatory response syndrome. Data from animal studies suggest that inflammation and infection may damage the developing brain directly. Fetal inflammatory response because of maternal infection results in proinflammatory cytokine release (interleukin [IL]-1ß, tumor necrosis factor alpha [TNF-α], IL-6) and apoptosis of preoligodendrocytes in central nervous system. IL-1β is one of the main cytokines that are implicated in inflammatory processes and triggered by maternal lipopolysaccharide (LPS) injection.[4-10] Because of clinical studies, preterm infants who are exposed to chorioamnionitis or high concentrations of proinflammatory cytokines in the amniotic fluid are at increased risk of white matter injury and/or cerebral palsy.[11-14]

An important link between the innate immune system of the brain and the adaptive immune system, which are mediated by circulating B and T cells, is the family of Fc gamma receptors. They are present on the surface of microglia and other cell types, which include natural killer cells, neutrophils, and mast cells. Fc gamma receptors bind immunoglobulin G and trigger signal transduction events that leads to microglial activation.

Intravenous immunoglobulin (IVIG) is an immunomodulating agent that has a complex mode of action and may be used either to boost the patients' immunologic capabilities or to blunt the inflammatory response. In the anti-inflammatory activity, IVIG binds to

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activating Fc gamma receptors on immune effector cells, which are high-affinity receptors for immunoglobulin G, thereby blocking access of immune complexes to these receptors and inhibiting cell activation. Modulation of the production of cytokines and cytokine antagonists (IL-1 receptor antagonist) is also 1 of the major mechanisms by which immunoglobulin exerts its anti-inflammatory effects. It also contains antibodies against IL-1, IL-6, and interferon alfa, beta, and gamma. IVIG reacts with a number of membrane molecules of T cells, B cells, and monocytes that are important for self-tolerance. Interaction of IVIG with complement prevents the generation of C5b-9 membrane attack complex and complement-mediated tissue damage.[15-18]

There are some experimental and clinical studies about the administration of IVIG as an antiinflammatory and immunomodulatory agent for the treatment of autoimmune and inflammatory diseases such as Guillian-Barre syndrome, immune thrombocytopenic purpura, dermatomyositis, Kawasaki's disease, myasthenia gravis, autoimmune ancephalomyelitis, and stroke.[19-22]

We could find no study in the literature about the administration of IVIG as an antiinflammatory agent in experimental brain inflammation. The aim of this study was to investigate the effects of IVIG on LPS-induced brain inflammation in preterm rats by the determination of IL-1β expression and caspase activity.

Methods

All experiments were performed with the approval of The Animal Care and Ethics Committee of Akdeniz University, School of Medicine (05.01.2010. Evaluation no:10-01/01, decision no:01). Wistar albino rats that weighted 200-280 g were used in the study (all animals were obtained from the Experimental Animal Center of Akdeniz University). LPS (*Escherichia coli* serotype 0.55B5) was obtained from Sigma Chemical Co (St. Louis, MO); IVIG (Tegeline flacon 5% 2.5 g 50 mL) from Er-Kim Co (France). The Western blot kit for rat IL-1β was obtained from R&D Systems Inc (MAB5011; Minneapolis, MN); the caspase 3 kit for colorimetric method was obtained from Invitrogen Corporation (Camarillo, CA).

Animals and experimental protocol

This study was conducted on the basis of 30 live rat pups in the 4- and 24-hour groups. Ten dams were included in each group at the beginning of the study. However, the number of dams changed according to the number of live pups that were delivered.

For mating purposes to start estrous cycle, female rats were induced with sawdust of male rats for 3 days. They were housed overnight with the males, starting at 8:00 PM. Females were checked for pregnancy by 8:00 AM the next morning. The presence of a vaginal plug was designated as gestational day 0. Pregnant rats were allowed free access to food and water at all times and were maintained on a 12-hour light/dark cycle in a controlled temperature (20-25°C) and humidity (50%) environment until used. On gestational day 19, they were divided randomly into 3 groups.

Group 1 (NS + NS)

Ten dams were injected intraperitoneally with normal saline solution (NS) at the same volume with the LPS dose of 0.3 mg/kg. After 2 hours, a second dose of NS (same volume with the IVIG dose of 1 g/kg) was injected at the tail vein; ether anesthesia was used for sedation. Two hours after the second NS injection and 24 hours after the second NS injection, 5 dams in each group were anesthetized with halotane (4% induction and 1-1.5% maintenance). Laparotomy was performed; fetal sacs were isolated, and the fetuses were removed. The fetal brains were dissected on ice, frozen in liquid nitrogen immediately, and stored at −80°C until used (30 rat pups for each 4-hour and 24-hour group).

Group 2 (LPS + IVIG)

Ten dams were injected intraperitoneally with LPS (0.3 mg/kg; *Escherichia coli* 055:B5). Two hours after LPS injection, IVIG was administered to all of them at a dose of 1 g/kg from the tail vein; ether anesthesia was used. Two hours after IVIG administration and 24 hours after IVIG administration, 5 dams from each group were anesthetized with halotane (4% induction and 1-1.5% maintenance). Laparotomy was performed; fetal sacs were isolated, and the fetuses were removed. The fetal brains were dissected on ice, frozen in liquid nitrogen immediately, and stored at −80°C until used (30 rat pups for each 4-hour and 24-hours group).

Group 3 (LPS + NS)

Ten dams were injected intraperitoneally with LPS (0.3 mg/kg). Two hours after LPS, NS was administered to all of the dams (same volume with the IVIG dose of 1 g/kg) from the tail vein; ether anesthesia was used. Two hours after NS administration and 24 hours after NS administration, 5 dams from each group were anesthetized with halotane (4% induction and 1-1.5% maintenance). Laparotomy was performed; fetal sacs were isolated, and the fetuses were removed. The fetal brains were dissected on ice, frozen in liquid nitrogen immediately, and stored at −80°C until used (30 rat pups for each 4-hour and 24-hours group).

Detection of IL-1β by Western blot

Brain tissues from the rat pups in the 4- and 24-hour groups were homogenized in 0.1 mol/L phosphate buffer that contained 8 mol/L urea. Before being used for electrophoresis, tissue homogenates were denaturated by boiling with an equal amount of sample loading buffer (125 mmol/L Tris-Hcl, pH 6.8, 20% glycerol, 0.002% bromophenol blue, 10% β-mercaptoethanol, 4% sodium dodecylsulfate) at 100°C for 5 minutes. Homogenate samples were subjected to sodium dodecylsulfate-polyacrylamid gel (electrophoresis sulfate-polyacrylamid gel electrophoresis, 10% gel, 20 μg of protein per lane) and then transferred on to polyvinylidene difuoride (RPN303F; Amersham, Buckinghamshire, UK) membrane and probed with IL-1β antibodies and beta-actin sc-56055 (Biotech Inc., Santa Cruz, CA). Bound antibody was detected by incubation for 1 hour with horseradish peroxidase-conjugated rabbit antigoat secondary antibodies (1:3,000 dilution; Sigma Chemical Company); the signal