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TNF-α antagonist attenuates systemic lipopolysaccharide-induced brain white matter injury in neonatal rats

복강 내 지질다당질 투여로 유발시킨 신생 백서의 뇌백질 손상모델에서 TNF-α 길항제 투여의 뇌백질 손상 경감 효과

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TNF-α antagonist attenuates systemic lipopolysaccharide-induced brain white matter injury in neonatal rats

By

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Abstract

TNF-α antagonist attenuates systemic lipopolysaccharide-induced brain white matter injury in neonatal rats

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Background: Systemic inflammation is an important risk factor for neurodevelopmental impairments in preterm infants. Premyelinating oligodendrocytes are main building blocks of white matter in preterm infants and vulnerable to oxidative stress and excitotoxic stress. Tumour necrosis factor-α (TNF-α) plays important roles in systemic inflammation and local inflammation leading to apoptosis of premyelinating oligodendrocytes and white matter injury (WMI) in brain tissue.

Objective: This study was conducted to investigate whether etanercept, a TNF-α antagonist, could attenuate systemic lipopolysaccharide (LPS)-induced WMI in the immature brain.

Materials: Three-day-old (P3) Sprague-Dawley rats were divided into the placebo (P) group (n=24), the L group (n=24), and the LE group (n=24). The P group was injected intraperitoneally with 100 μl of 0.9% saline followed by the same volume of PBS (phosphate buffered saline, 100 μl). LPS was administered intraperitoneally on P3 at a

dose of 2 mg/kg body weight, followed by intraperitoneal injection of 100 μl of PBS for the L group or etanercept at a dose of 5 mg/kg body weight for the LE group.

Results: We found that intraperitoneal LPS administration caused systemic and local inflammation in brain tissue. Subsequent etanercept treatment significantly attenuated LPS-induced inflammation in brain tissue as well as in systemic circulation. Intraperitoneal LPS also induced microgliosis and astrocytosis in the cingulum and etanercept treatment reduced LPS-induced microgliosis and astrocytosis. Additionally, systemic LPS-induced apoptosis of oligodendrocyte precursor cells was observed, which was lessened by etanercept treatment. The concentration of etanercept in the CSF was higher when it was administrated with LPS than when administrated with a vehicle.

Conclusion: It appears that etanercept reduce WMI in the neonatal rat brain via attenuation of systemic and local inflammation. This study provides preclinical data suggesting etanercept-mediated modulation of inflammation as a promising approach to reduce WMI caused by sepsis or necrotizing enterocolitis in preterm infants.

Keywords: newborn, white matter injury, systemic inflammation, TNF-α antagonist

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Introduction

During the neonatal period, preterm infants are at risk from several morbidities, such as sepsis and necrotizing enterocolitis, that are associated with neurodevelopment impairments (1, 2), which have been shown to be mediated by white matter injury (WMI) in the premature brain (2, 3). The pathogenesis of WMI is characterised by a loss of premyelinating oligodendrocytes (pre-OLs), which are particularly vulnerable to oxidative and excitotoxic stress, and activation of glial cells, such as microglia and astrocytes (4, 5).

Several pro-inflammatory cytokines are associated with the activation of glial cells (6). Post-mortem brain specimens from infants with periventricular WMI exhibited the expression of various cytokines, including tumour necrosis factor-α (TNF-α), during the early stages of white matter disease (7). The roll of TNF-α in WMI has been reported in several studies. TNF-α mediated the lipopolysaccharide (LPS)-triggered death of pre-OLs in an environment containing major glial cell types (8), and oligodendrocyte progenitors were particularly vulnerable to a combination of TNF-α and interferon-γ by activation of the cell death program (9). However, most of studies which reported roll of glial cells and TNF-α in the death of oligodendrocytes during the early developmental phase used in vitro methods with direct stimulation with LPS (8, 10, 11). Etanercept is a TNF-α antagonist composed of the dimeric fusion protein of the extracellular ligand-binding portion of the soluble 75-kDa TNF-α receptor II and the fragment-crystallisable (Fc) portion of human immunoglobulin (IgG) (12). Recently, etanercept has been shown to attenuate neural apoptosis, astrocytic and microglial activation, and local inflammation of the brain in several animal studies using traumatic brain injury or

ischaemic injury models (13, 14). In a study of a traumatic brain injury model, injection of intraperitoneal etanercept of 5 mg/kg reduced neuronal degeneration after 1 h, and in a study involving a rat ischemic stroke model, etanercept given intraperitoneally 30 min after insult decreased cerebral infarct (14, 15). The present study was conducted to establish a neonatal rat model of WMI corresponding to WMI in human preterm infants by inducing systemic inflammation. Furthermore, we hypothesized that administration of TNF-α antagonist following LPS-induced systemic inflammation could attenuate apoptosis of pre-OLs in the inflamed immature brain of newborn rats with subsequent preservation of myelination in the cingulum.

Materials and methods

Animals

Timed pregnant Sprague-Dawley (SD) rats arrived in the laboratory on day 19 of gestation. Animals were maintained in a room with a 12-h light/dark cycle and a constant temperature (22±2°C). The day of birth was defined as postnatal day 0 (P0). After birth, the litter size was adjusted to ten pups per litter to minimise its effect on body weight and brain size. All procedures for animal care were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the Seoul National University Hospital. Rats were euthanized by decapitation when the experimental procedure was finished.

Chemicals

LPS was purchased from Sigma (St. Louis, MO, USA), and enzyme-linked immunosorbent assay (ELISA) kits were used for immunoassay of interleukin-1ß (IL-1ß: MyBioSource, #MBS732184) and TNF-α (MyBioSource, #MBS032310). Monoclonal mouse antibodies against O4 and glial fibrillary acidic protein (GFAP) were purchased from MyBioSource (San Diego, CA, USA) and Millipore (Billerica, MA, USA), respectively. Monoclonal mouse antibody to neural-glial antigen 2 (NG2), monoclonal rabbit antibody to platelet-derived growth factor-α receptor (PDGFα-R), rabbit antibody to TNF-α and rabbit antibody to TNF-α receptor I were purchased from abcam (Cambridge, UK). Antibody to IL-1 receptor was purchased from antibodies-online (Aachen, Germany). Antibody against myelin basic protein (MBP) and Luxol

Fast Blue (LFB) stain were purchased from ScyTek Laboratories (Logan, UT, USA). Ionised calcium-binding adapter molecule 1 (Iba1) was obtained from Wako Chemicals USA (Irvine, CA, USA), and the terminal deoxynucleotidyl transferase (TdT)-mediated uridine 50-triphosphate-biotin nick end labelling (TUNEL) staining kit was purchased from Millipore (Billerica, MA, USA). An enzyme immunoassay (Enbrel ELISA Q-ETA; Matriks Biotek Laboratories, Ankara, Turkey) was used to quantify the etanercept in cerebral spinal fluid (CSF).

Animal treatment

An intraperitoneal (IP) injection of 2 mg/kg LPS (from *Escherichia coli*, serotype 055: B5) was administered to SD rat pups of both sexes on day 3 of life (P3) followed by IP injection of etanercept at a dose of 5 mg/kg (LE group) or 100 µl of PBS (phosphate buffered saline, L group) (15, 16). The placebo group (P group) was injected intraperitoneally with 100 µl of 0.9% saline followed by the same volume of PBS (100 µl). The room temperature was maintained at 22±2°C throughout the experiment. Body weight was measured from P3 to P7, and six rats in each group were selected to measure body weight beyond P4 and were sacrificed on P7. A total of 92 rats from eight litters were used in the present study; one pup from each litter was assigned to each group. Six rats from each group were sacrificed by decapitation on P4 to collect blood and fresh cerebrum without olfactory bulb tissue for ELISA assay (16) as well as immunohistochemistry and immunofluorescence staining. Six rats from each group were sacrificed on P14 for the evaluation of changes in myelination by immunohistochemistry. A satellite experiment was performed to investigate the presence

of etanercept in the CSF. In one group (LEC group, n=10), LPS and etanercept were given intraperitoneally on P3 as above, and in the other group (PC group, n=10), 100 µl of 0.9% saline and etanercept were given on P3. On P4, under light anaesthesia with zoletil (2 mg/kg) and isoflurane (1.5%), rat pups were placed in a stereotaxic apparatus with a neonatal rat adapter, which has light ear pads (David Kopf, CA, USA). A small scalp incision was made on the skull surface, and the lambda was exposed and micropipetted to obtain 1.5 mm CSF. Then, the CSF was centrifuged at 3000 rpm for 10 s, and the supernatant was collected for analysis.

Determination of IL-1ß and TNF-α protein levels in brain tissue and blood by ELISA

IL-1ß and TNF-α expression levels were determined by ELISA. Cerebrum without olfactory bulb tissues and blood from each pup were collected on P4. Samples were homogenised by sonication in 1 ml of ice-cold PBS (pH 7.2) and centrifuged at 12,000×g for 20 min at 4°C. The supernatant was collected, and the protein concentration was determined using the BCA method. ELISA was performed following the manufacturer's instructions, and data were acquired using a 96-well plate reader (VersaMax, California, USA). The cytokine contents are expressed as pg cytokine/mg protein.

Immunohistochemistry and immunofluorescence

Six rats from each group were sacrificed by transcardiac perfusion. Immunohistochemical analysis was performed on 10-µm tissue sections prepared using

a microtome, and deparaffinisation and antigen retrieval were conducted. For immunohistochemistry staining, primary antibodies were used in an antibody dilution buffer in the following dilutions: O4, 1:2000; Iba1, 1:2000; MBP, 1:2000; PDGFα-R, 1:100; NG2, 1:500 and GFAP, 1:1000. The sections were incubated overnight at 4°C with the primary antibodies and then for 2 h with the REAL-HRP system (DaKo REAL ^{TM} EnVision ^{TM} Detection System Peroxidase/DAB+, Rabbit/Mouse K5007, CA, USA). Slides were counterstained with H&E. Photomicrographs were captured using a Leica DFC 290 microscope with a digital camera system. Immunofluorescence staining was conducted using TUNEL assay kits. Next, the sections were incubated overnight with anti-O4 antibodies at 4°C and then incubated with a secondary antibody conjugated to a fluorescent probe (Alexa Fluor 568, 1:200 anti-mouse IgM, Invitrogen, Grand Island, NY, USA) for 2 h in the dark at room temperature. Sections were washed with PBS and then mounted using VECTASHIELD mounting medium (Vector Laboratories, Inc., Burlingame, CA) for visualisation under a fluorescence microscope (Leica TCS SP8). The TUNEL-positive cells appeared green, and the O4-positive cells appeared red. Immunofluorescence staining of GFAP, Iba-1, TNF-α, TNF-α receptor, and IL-1ß receptor were also conducted as descripted above. Antigen retrieval and the composition of the antibody dilution buffer were conducted according to the manufacturer's instructions. Staining intensities were measured in brain cingulum (Bregma −1.00) using Image J software (17).

Statistical analysis

SPSS version 22.0 (SPSS for Windows Inc., Chicago, IL, USA) was used for the

statistical analysis. ANOVAs with Bonferroni comparison tests were used to compare the groups. The results are expressed as the mean ± standard deviation.

Results

All rats survived until experimental sacrifice. Compared with the P group, rats in both the L and LE groups experienced a slight decrease in body weight on P4 (Fig. 1). Despite the subsequent increase, the body weight of the L group was significantly lower than that of the P group from P5 to P7 ($p<0.01$), and rats in the LE group showed no significant differences in weight during P5 and P7 compared with the P group ($p=0.206$).

LPS-induced inflammatory responses were attenuated by etanercept

Systemic exposure to LPS resulted in inflammatory responses in the brain and serum on P4 (Fig. 2 A, IL-1ß and Fig. 2 B, TNF-α). Serum IL-1ß and TNF-α concentrations were increased in LPS-exposed rats compared with the placebo rats ($p<0.01$ and $p<0.05$, respectively), and the levels in the brain tissue were also significantly increased in LPS-exposed rats ($p<0.05$ and $p<0.01$, respectively). The LE group showed lower IL-1ß levels in serum ($p<0.05$) and lower TNF-α levels in serum and brain tissue than the L group ($p<0.05$ and $p<0.01$, respectively), but attenuation of IL-1ß in the brain was not significant in the LE group ($p=0.223$).

Etanercept preserved premyelinating oligodendrocytes during LPS exposure

LPS exposure resulted in decreased PDGFα-R and NG2 immunostaining in the brain tissue on P4 (both $p<0.01$) (Fig. 3 A-B, D-E, P, Q), but these were significantly increased in the LE group compared to the L group ($p<0.01$ and $p<0.05$, respectively)

(Fig. 3 C, F, P, Q). Decreased distribution of pre-OLs in the LPS-exposed rat brains was also evidenced by decreased O4 immunofluorescence staining ($p<0.01$), and the O4+ cell was increased in the LE group compared to the L group ($p<0.05$) (Fig. 3 G-I, R). TUNEL immunofluorescence staining also was increased in the L group on P4 ($p<0.01$), and this increase was attenuated in the LE group ($p<0.01$) (Fig. 3 J-L, S). Double-labelling showed that many TUNEL-positive cells were positive for O4 ($p<0.01$) (Fig. 3 N), and the cells that were doubly stained by O4 and TUNEL were decreased in the LE group (Fig. 3 M-O, T). Double immunofluorescence staining of TNF-α receptor and IL-1ß receptor with O4 in the L group showed that each receptor was well expressed on the O4+ cells (Fig. 4).

Etanercept attenuated LPS-induced microgliosis and astrocytosis resulting in improved myelination

A significant increase in the number of reactive astrocytes showing hypertrophy of cellular processes was found in the LPS-exposed rat brains on P4 ($p<0.01$), and this increase was attenuated by etanercept treatment ($p<0.01$) (Fig. 5 A-C, M). Iba-1 immunostaining was also increased in the L group ($p<0.01$), and this increase was attenuated in the LE group ($p<0.01$) (Fig. 5 -D-F, N). Astrocytosis and microgliosis were still more prominent in the LE group than the control group on P4. Double immunofluorescence labelling in the L group showed that GFAP or Iba-1 positive cells were expressed with TNF-α (Fig. 5 G-L). Immunohistochemistry using MBP and LFB on P14 showed that LPS exposure resulted in decreased MBP and LFB (both $p<0.01$)

(Fig. 6 A-B, D-E, G, H) and these were significantly increased in the LE group compared to the L group ($p<0.01$ and $p<0.05$, respectively) (Fig. 6 C, F, G, H).

Etanercept levels in the central nervous system

CSF obtained on P4 was analysed by ELISA to detect etanercept, and the concentration was higher in the LEC group than in the PC group (<0.01) (Fig. 7).

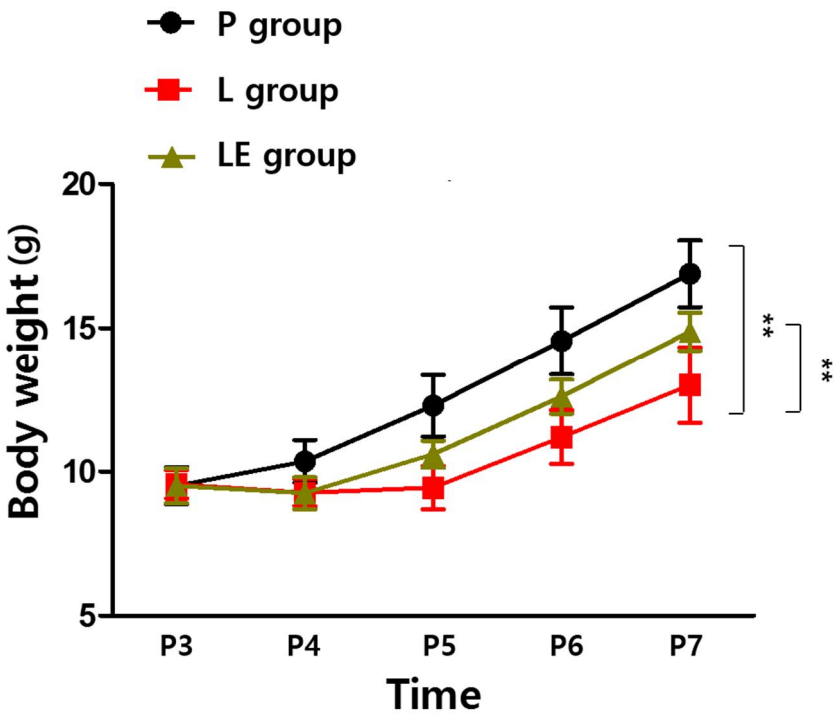

Fig. 1. Etanercept-attenuated poor body weight gain in LPS-treated rats. ** represents a significant difference between groups (P<0.01).

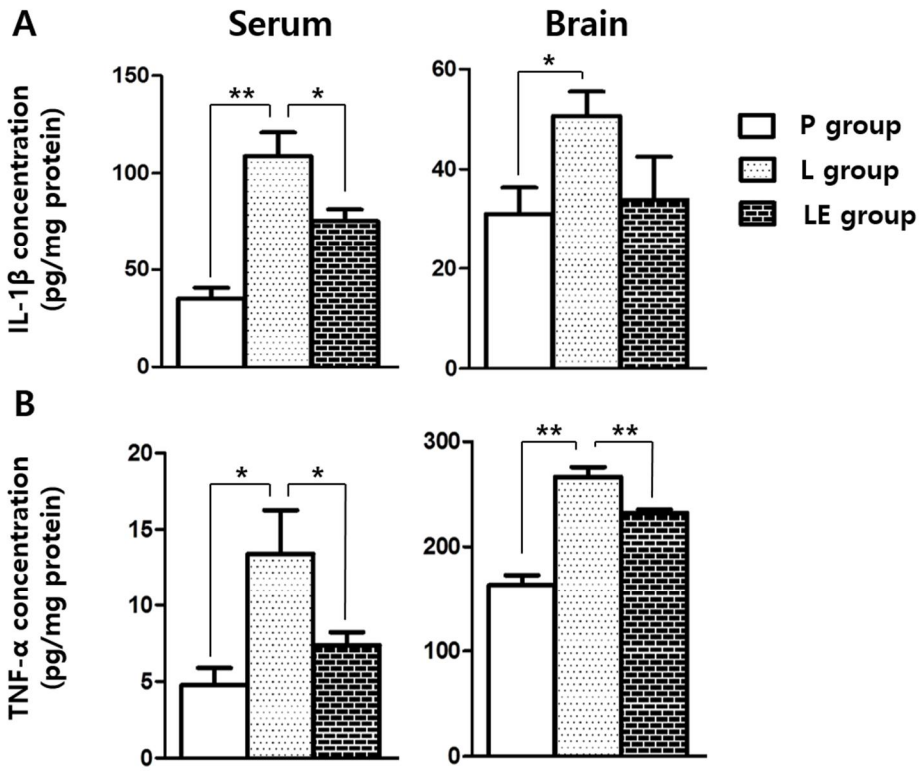

Fig. 2. The LPS-induced changes in the levels of inflammatory cytokines in circulation and the brain tissue of rats on P4 (A, IL-1ß and B, TNF-α). ** and * represent significant differences between groups ($P<0.01$ and $P<0.05$, respectively). ANOVA with a Bonferroni test was conducted. There were six rats per group.

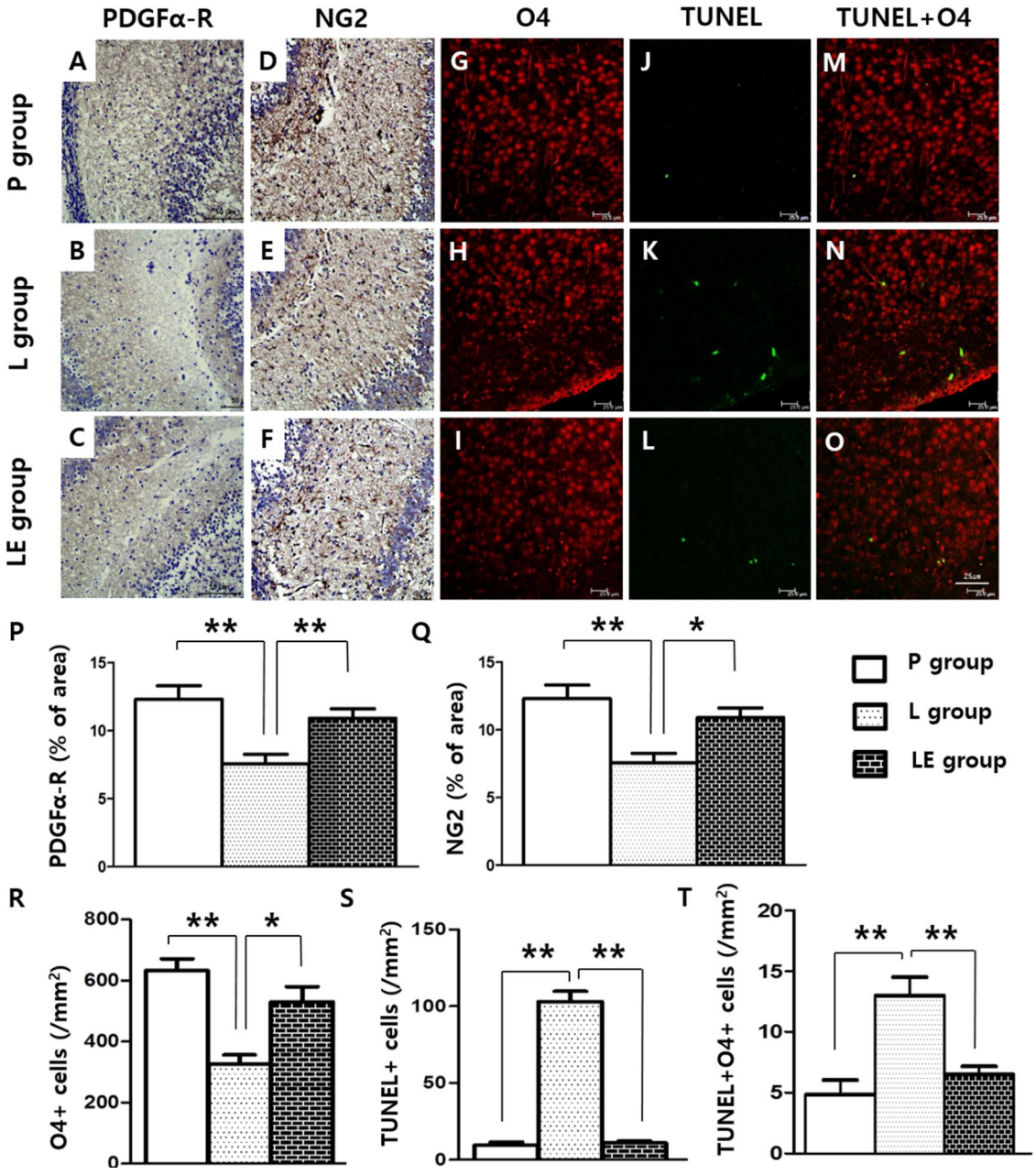

Fig. 3. Representative photomicrographs of pre-OLs (A-F) by immunohistochemistry using PDGFα-R and NG2 and O4+ oligodendrocyte (G-I) and TUNEL staining cells (J-L) by immunofluorescence (IF) staining of the cingulum (Bregma -1.0) on P4. Pre-OLs were attenuated in the L group and preserved in the LE group (P-Q). Double-labelling showed that many TUNEL-positive cells were also positive for O4 (M-O). O4+ oligodendrocytes were decreased in the L group with increased TUNEL-positive cells in

the IF staining, and O4+ oligodendrocytes were recovered in the LE group (R-T). ** and * represent significant differences between groups ($P<0.01$ and $P<0.05$, respectively). ANOVA with a Bonferroni test was conducted. There were six rats per group.

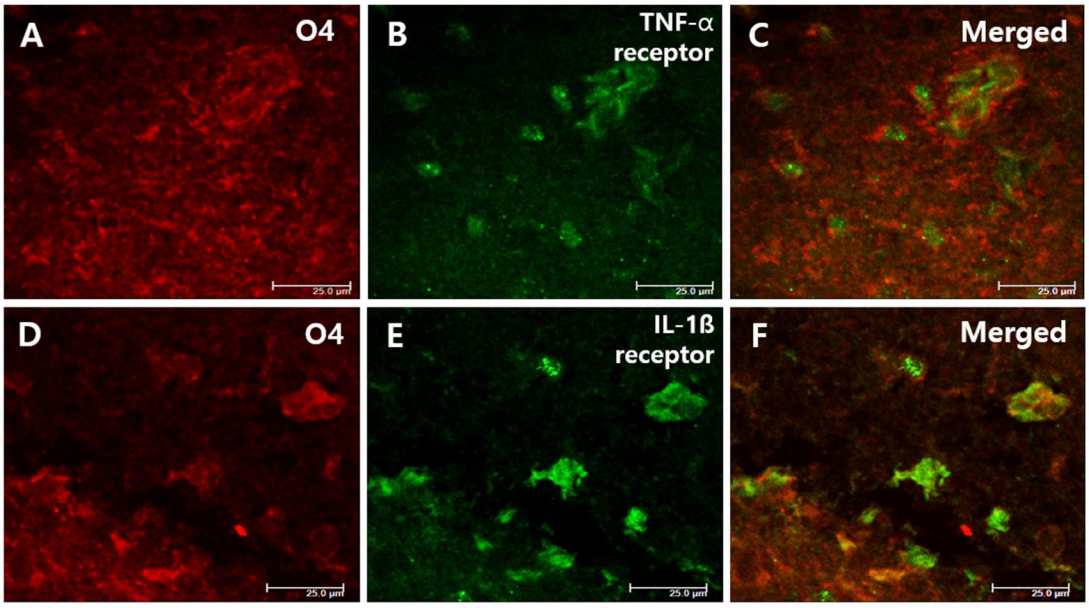

Fig. 4. Representative photomicrographs of O4+ oligodendrocyte (A, D), TNF-α receptor (B) and IL-1ß receptor (E) immunofluorescence staining of the cingulum (Bregma -1.0) in the L group on P4. Double-labelling showed that many O4+ oligodendrocyte also were positive for TNF-α receptors and IL-1ß receptors (C, F).

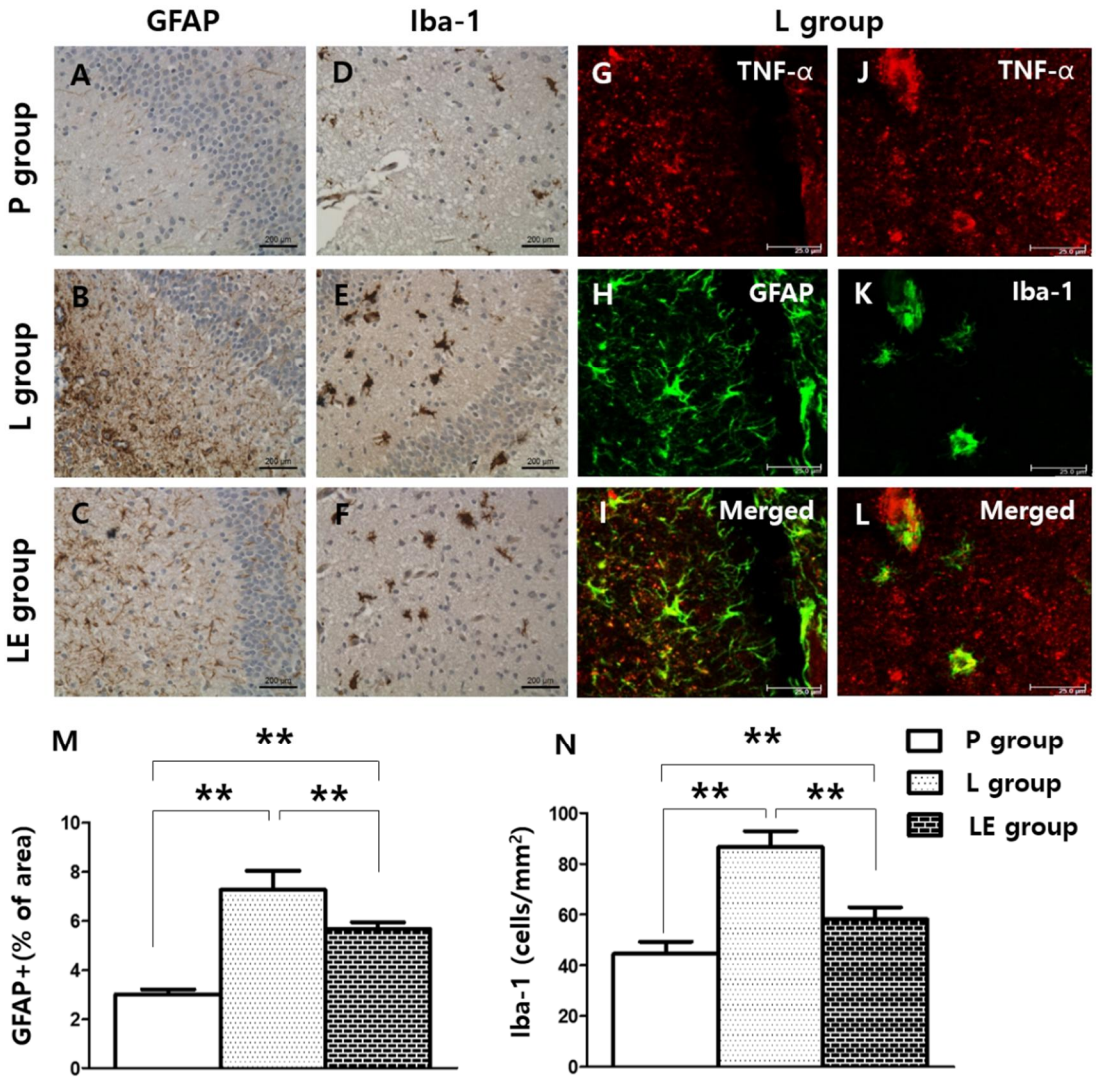

Fig. 5. Representative photomicrographs of astrocytosis (A-C) and microgliosis (D-F) by immunohistochemistry and TNF-α (G, J), GFAP (H) and Iba-1 (K) by immunofluorescence staining of the cingulum (Bregma -1.0) on P4. Significant increases in the number of reactive astrocytes and microgliosis were observed in the cingulum white matter following neonatal LPS exposure, which was attenuated in the LE group (M, N). In the L group, GFAP or Iba-1 positive cells were also positive for TNF-α (I, L). ** represents significant differences between groups ($P<0.01$). ANOVA with a Bonferroni test was conducted. There were six rats per group.

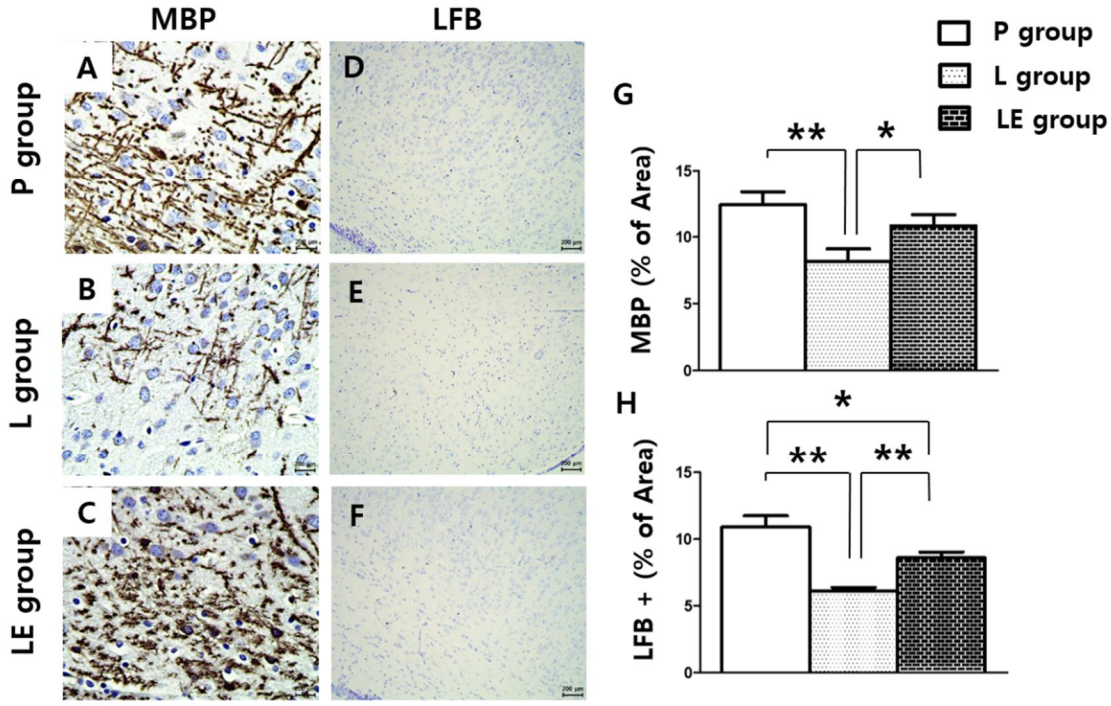

Fig. 6. Representative photomicrographs of myelination by immunohistochemistry (A-F). Cells with MBP and LFB staining were attenuated in the L group and preserved in the LE group (G-H) of the cingulum (Bregma -1.0) on P14. ** and * represent significant differences between groups ($P<0.01$ and $P<0.05$, respectively). ANOVA with a Bonferroni test was conducted. There were six rats per group.

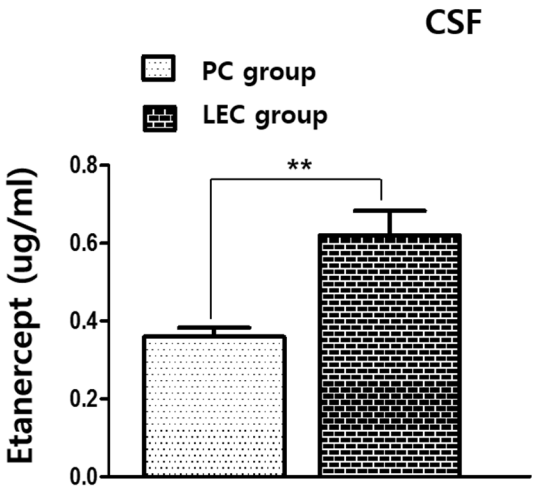

Fig. 7. ELISA of etanercept levels in the CSF. The concentration of etanercept in CSF was higher in the LEC group than in the PC group (n=10 for each group). A Wilcoxon rank-sum test was conducted, and ** represents a significant difference between groups ($P<0.01$).

Discussion

A postnatal newborn rat model of systemic inflammation was reproduced from the study by Fan et al., in which LPS was administered to rats intraperitoneally on day 5 of life (16). The vulnerability of white matter during systemic inflammation has been related to the presence of pre-OLs at the gestational age of 24–32 weeks in humans, which is equivalent to the same pre-OL phenotype in a newborn rat (18). Previous studies have suggested that white matter vulnerability at postnatal day 4 in a rat or mouse would correspond to that in preterm infants (19, 20). In this study, the WMI model was successfully reproduced by IP injection of LPS on P3, and the TNF-α antagonist etanercept attenuated the LPS-induced inflammatory processes with subsequent improvement in myelination.

There was significant systemic inflammation after LPS administration in the newborn rats, which subsequently exhibited poor weight gain during the first week of life. Systemic inflammation not only induced local inflammation of the brain but subsequent injury as well, as indicated by increased TUNEL-positive cells in the cingulum white matter. Apoptosis occurred in the O4-positive pre-OLs with decreased myelination in the white matter and was accompanied by microgliosis and astrocytosis in the cingulum, which was consistent with the study by Fan et al. (16). More immature form of pre-OLs containing NG2 and PDGFα-R also were influenced by systemic LPS and were preserved by TNFα inhibition in this study.

TNF-α was shown to play a critical role in the pathogenesis of WMI because TNF-α signalling is required in LPS-triggered pre-OL death (8). Pre-OLs do not undergo apoptosis when major glial cell types are absent, which emphasises the importance of

intercellular communication in the mechanism of inflammatory pre-OL death. Through this communication, activation of microglia and astrocytes has detrimental effects on neural precursor cells via released TNF-α (21, 22). The pathogenesis of local brain inflammation during systemic inflammation has not yet been well elucidated, but both direct and indirect pathways of triggering local inflammation have been suggested. Circumventricular organs in the brain might play important roles in the mediation of systemic inflammation and the expression of TNF-α in the brain with CD14 expression (23). On the other hand, circulating TNF-α has the ability to directly activate the endothelium of the brain's blood vessels, producing soluble molecules such as prostaglandins with subsequent local inflammation (24). Many studies used the in vitro methods with direct stimulation with LPS to demonstrate the effect of glial cells and TNF-α on the death of oligodendrocytes during the early developmental phase (8, 10, 11). In the present study, systemic inflammation by LPS in vivo successfully induced apoptosis of Pre-OLs with the expression of receptors for TNF-α and IL-1β on the Pre-OLs. These changes were accompanied by increased glial cells such as microglia and astrocytes in the brain with the expression of TNF-α (Figure 5).

Etanercept is a TNF-α antagonist that has already been developed and approved for the treatment of rheumatoid arthritis and several rheumatic diseases, and treatment with etanercept suppressed systemic inflammation and local inflammation in the brains of LPS-treated rats. It decreased microgliosis and astrocytosis and preserved myelination in white matter, subsequently decreasing apoptosis of O4-positive pre-OLs in the LPS- and etanercept-treated newborn rats compared to the LPS-exposed rats that did not receive etanercept. It is unclear whether the effect of etanercept was via suppression of systemic inflammation or direct suppression of local inflammation in the brain or by

both mechanisms. However, brain TNF-α levels were strongly suppressed in the LE group compared to the L group, suggesting a role for etanercept in modulating local inflammation. Interestingly, etanercept was more detectable with systemic inflammation (LEC group) than without (PC group). Etanercept is such a large molecule that it is usually unable to penetrate the blood-brain barrier (BBB) (25), but the several studies have demonstrated that the BBB could be disrupted by LPS exposure (26, 27). This functional change in BBB during systemic inflammation might widen the therapeutic range in the WMI induced by systemic inflammation in preterm infants.

Conclusion

Although preterm infants are at higher risk of conditions such as sepsis, meningitis and necrotizing enterocolitis during the neonatal period (28), an evidence-based strategy for reducing WMI has not yet been established in this population. Clinical studies have accumulated evidence of an association between serious postnatal systemic inflammation and WMI or neurodevelopmental outcomes in preterm infants (28, 29). Therefore, controlling undue inflammation in the brain during these episodes might help reduce WMI and subsequent neurodevelopmental impairment in preterm infants. As TNF-α antagonists have already been used to treat a number of neurodegenerative and autoimmune diseases, clinical trials would be worthwhile for this potential treatment for WMI in preterm infants.

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요약 (국문초록)

복강 내 지질다당질 투여로 유발시킨 신생 백서의 뇌백질 손상모델에서 TNF-α 길항제 투여의 뇌백질 손상 경감 효과

목적

미숙아에서 전신의 염증반응은 신경학적 발달이상의 중요한 위험인자로 알려져 있다. 수초화전단계의 희소돌기아교세포는 뇌백질을 구성하는 주요 세포로 산화 스트레스나 흥분독성 스트레스에 대해 취약한 것으로 알려져 있다. 알파종양괴사인자(TNF-α)는 전신 염증반응과 국소 염증 반응에서 중요한 역할을 하며 이를 통해 수초화전단계의 희소돌기아교세포의 세포자멸사와 뇌백질 손상 발생을 유발하게 된다. 본 연구에서는 TNF-α 길항제인 이타너셉트가 전신 지질다당질 투여로 유발된 뇌백질 손상을 경감시키는 여부를 살펴보고자 한다.

방법

생후 3일된 신생쥐를 P군, L군, LE군으로 나눈다 (각 24마리). P군에는 복강내에 0.9% 식염수를 100 μl주입한 후에 인산염완충식염수 100 μl를 주입한다. L군과 LE군에서는 지질다당질 2m/kg을 복강내에 주입하고, 이후에 L군은 PBS 100μl을 복강내 주입하며 LE군은 이타너셉트 5mg/kg를 복강내에 주입한다. 생후 4일 째 혈액을 채취하고 뇌조직을 얻어 효소면역검사와 면역조직화학염색, 면역형광염색을 시행한다. 생후 14일 째 뇌조직을 얻어 면역조직화학염색을 시행한다. 효소면역검사를 통해 혈

액과 뇌조직에서의 IL-1ß 와 TNF-α의 농도를 측정하고, 면역조직화학염색과 면역형광염색을 통해 O4, Iba1, MBP, PDGFα-R, GFAP, TUNEL 염색을 시행한다.

결과

신생쥐에서 복강내 지질다당질 투여는 전신 염증과 뇌에서의 국소 염증을 유발하였다. 이타너셉트를 투여한 결과 이러한 전신과 국소 염증은 감소하였다. 또한 지질다당질 투여로 인해 뇌백질에서 미세아교세포의 활성도와 별아교세포증이 발생하였으며 이타너셉트를 투여하였던 군에서 이러한 반응이 감소하는 것으로 나타났다. 지질다당질 투여는 희소돌기아교세포의 전구세포의 세포자멸사를 유발하였고, 이는 이타너셉트 투여로 약화되었다. 이타너셉트의 뇌척수액 내 농도는 지질다당질과 함께 투여 하였을 경우에 높게 측정되었다.

결론

이타너셉트는 신생쥐에서 지질다당질로 유도된 전신 염증과 뇌의 국소 염증을 약화시키고 뇌백질의 손상을 감소시키는 것으로 나타났다. 본 연구를 통해 패혈증이나 괴사성장염으로 발생하는 뇌백질 손상 치료의 이론적 배경을 제시할 수 있었다.

주요어: 신생아, 뇌백질 손상, 전신 염증, TNF-α 길항제

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