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Effects of Repetitive Lipopolysaccharide Exposure on Oligodendrocyte Differentiation at Different Developmental Stages: an In Vitro Study

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안 자 혜

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지도교수 김 한 석

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위 원 장 _____ 김 이 경 _____ (인)

부위원장 _____ 김 한 석 _____ (인)

위 원 _____ 최 창 원 _____ (인)

위 원 _____ 박 찬 욱 _____ (인)

위 원 _____ 박 현 경 _____ (인)

Abstract

Background: Lipopolysaccharide (LPS) exerts cytotoxic effects on brain cells, especially on those belonging to the oligodendrocyte lineage, in preterm infants. The susceptibility of oligodendrocyte lineage cells to LPS-induced inflammation is dependent on the developmental stage. This study aimed to investigate the effect of LPS on oligodendrocyte lineage cells at different developmental stages in an oligodendrocyte and microglial cell co-culture model.

Methods: The primary cultures of oligodendrocytes and microglia cells were prepared from the forebrains of 2-day-old Sprague-Dawley rats. The oligodendrocyte progenitor cells (OPCs) co-cultured with microglial cells were treated with 0 (control), 0.01, 0.1, and 1 μg/mL LPS at the D3 stage to determine the dose of LPS that induced microglia activation while did not have excessive cytotoxicity to OPCs. The co-culture was treated with 0.01 μg/mL LPS at the developmental stages D1 (early LPS group), D3 (late LPS group), or D1 and D3 (repetitive LPS group). On day 7 of differentiation, oligodendrocytes were subjected to neural glial antigen 2 (NG2)

and myelin basic protein (MBP) immunostaining to examine the number of OPCs and mature oligodendrocytes, respectively.

Results: LPS dose-dependently decreased the proportion of mature oligodendrocytes (MBP+ cells) relative to the total number of OPCs. The number of MBP+ cells in the early LPS group was significantly lower than that in the late LPS group. Compared with those in the control group, the MBP+ cell numbers were significantly lower and the NG2+ cell numbers were significantly higher in the repetitive LPS group on day 7 of differentiation.

Conclusion: Repetitive LPS stimulation during development significantly inhibited brain cell development by impairing oligodendrocyte differentiation. In contrast, brain cell development was not affected in the late LPS group. These findings suggest that inflammation at the early developmental stage of oligodendrocytes increases the susceptibility of the preterm brain to inflammation-induced injury.

Keyword: Lipopolysaccharide, Preterm, Brain, Periventricular leukomalacia, Oligodendrocyte, Microglia

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Chapter 1. Introduction

1.1. Study Background

The recent advances in perinatology have shifted the goal of neonatal intensive care from improving the survival rates of newborns to enhancing their quality of life. Neurodevelopmental outcomes of surviving high-risk infants play an important role in determining their quality of life.[1] Previous studies have reported that white matter injury of the brain in preterm infants is correlated with sequelae, such as cerebral palsy or cognitive deficit.[2] Several studies examining the neurodevelopment of preterm infants have attempted to determine the pathogenesis of white matter injury. The spectrum of periventricular white matter injury (PWMI) includes cerebral injuries ranging from focal to extensive cerebral white matter lesions.[3] Although the overall incidence of cystic necrosis and microcysts has decreased, diffuse white matter injury is commonly observed in preterm infants.[4] According to Volpe,[2] the etiology of diffuse PWMI involves less severe episodes of ischemia associated with short penetrating arteries of superficial end zones, which may account for extensive myelination impairment.

Lipopolysaccharide (LPS), a component of the gram-negative bacterial membrane, exerts cytotoxic effects on preterm brain cells, especially on those belonging to the oligodendrocyte lineage. In addition to activating the microglial cells through Toll-like receptor [4], LPS mediates brain damage by inducing the release of various cytotoxic mediators, including cytokines and reactive oxygen species.[5-7] Previous studies have investigated the maturation-dependent susceptibility of oligodendrocytes to hypoxia-ischemia insult.[8-10] Miller et al.[11] treated the primary oligodendrocyte cell cultures with LPS-activated microglial cells and reported that oligodendrocyte precursor cells (OPCs) and mature oligodendrocytes at different developmental stages exhibited differential responses to activated microglial cells. Several studies have examined the effect of perinatal and neonatal infection and inflammation on the preterm brain. Brain magnetic resonance imaging analyses have revealed that perinatal and/or neonatal infections may alter white matter development. Additionally, perinatal and/or neonatal infections can adversely affect neurodevelopmental outcomes to varying degrees in preterm infants depending on the gestational age.[12-16]

The oligodendrocyte lineage cells exhibit differential susceptibility to LPS-induced inflammation depending on the developmental stage. Diffuse white matter injury caused by infection and inflammation involves acute cell death, followed by the rapid regeneration of preoligodendrocytes (pre-OLs), which fail to mature into myelinating oligodendrocytes. A similar pattern of impaired cellular maturation has been reported in studies examining the effect of proinflammatory cytokines in preterm-equivalent rodents.[17] However, the effects of age at which inflammation or infection is induced and repeated episodes of infection and inflammation on the differentiation of oligodendrocytes throughout the critical developmental stages have not been previously reported.

1.2. Purpose of Research

This study aimed to investigate the effect of LPS on oligodendrocyte lineage cells at different developmental stages using an oligodendrocyte and microglial cell co-culture model. To establish the repetitive inflammation model, the oligodendrocyte lineage cells were exposed to LPS at two different differentiation stages

(repetitive-hit LPS insult). Furthermore, the effects of direct and repetitive LPS stimulations were evaluated at the cellular level.

Chapter 2. Body

2. 1. Methods

Fig. 1 shows the entire timeline of the experiment.

Primary oligodendrocyte–microglial cell co–culture

The primary cultures of oligodendrocytes and microglia cells were prepared from the forebrains of 2–day–old Sprague–Dawley rats by using a shaking method of the mixed glial cultures, following the protocols of Miller et al.[11] with minor modifications. The meninges were removed and the brain tissue was triturated and dissociated mechanically. The dissociated brain tissue was maintained in basal media comprising Dulbecco's modified Eagle's medium supplemented with GlutaMAX (Gibco, Carlsbad, CA, USA) and 10% heat–inactivated fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA) at 37°C and 5% CO_2. The medium was replaced once every 3 days.

After 9 days of culture, the mixed glial culture comprised a layer of astrocytes at the bottom of the uncoated T–75 flasks with OPCs and microglial cells growing over the astrocytes. The OPCs were

isolated from the mixed glial cells by shaking for 16 hours overnight. The detached OPCs were collected by centrifugation at 50 rpm and resuspended at a density of 70,000 cells/cm^2 in oligodendrocyte culture medium to induce the maturation of OPCs into oligodendrocytes. The oligodendrocyte culture medium (insulin [I6634; Sigma, St. Louis, MO, USA], holo-transferrin [T0665; Sigma], B27 [Gibco], GlutaMAX [Gibco], 0.5% FBS [Invitrogen], and 10 μg/mL ciliary neurotrophic factor [PeproTech, Cranbury, NJ, USA]) was supplemented with 100 × oligodendrocyte-supplement (bovine serum albumin, progesterone, putrescine, sodium selenite, and 3, 3′, 5-triiodo-L-thyronine; all from Sigma). Microglial cells were purified from the astrocyte layers of the same mixed glial culture that was used for harvesting OPCs. To isolate the microglial cells, the culture was shaken for 2 hours at 200 rpm. Microglial cells were resuspended in the same basal medium as OPCs, but with insulin (I1882; Sigma). Purified OPCs contained < 5% glial fibrillary acidic protein-positive astrocytes and < 0.5% microglial cells, while purified microglial cells contained < 0.1% astrocytes and < 1% oligodendrocytes. The next day 250 μL of oligodendrocyte culture medium in the mixed glial culture (500 μL) was replaced with 250 μL of microglial cell (D0 stage) suspension at a cell density ratio of 1:1.

The cells were maintained in a humidified incubator under 8.5% CO_2 conditions.

LPS application

The OPC−microglial cell co−culture was treated with LPS at D1 and/or D3 stages. Oligodendrocyte lineage cells extracted and cultured from P2 rats are mainly OPC and preOL at the D0 (in vitro D0) stage of our study. This corresponds to 18−28 gestational week in humans.[18] In this study, the OPC differentiation status at the D3 stage was estimated as was evaluated by Miller et al. because our cell extraction and culture process was similar to theirs.[11] They immunostained oligodendrocyte lineage cells that were cultured and differentiated in oligodendrocyte culture medium on D3. As a result, it was confirmed that mature oligodendrocytes expressing neural glial antigen2 (NG2, also known as chondroitin sulfate proteoglycan 4)−, A2B5−, galactocerebroside (GalC)+ and MBP+ were mainly present. Therefore, in this study OL lineage cells on D3 are thought to correspond between 30 and 36 weeks in humans.[18]

LPS−activated microglial cells produce cytokines and reactive oxygen species, which adversely affect the maturation and survival

of immature oligodendrocytes. Our model is a modified type of an LPS-induced brain injury model proposed by Miller et al.[11] They used 0.001, 0.01, and 0.1 μg/mL dose of LPS in their experiment. There were 3 other studies which used 1 μg/mL dose of LPS,[6, 7, 19] and 1 study used 0.01 μg/mL.[20] So, in the preliminary experiment, we set the LPS doses of 0.01, 0.1, and 1 μg/mL, and decided to increase or decrease it if necessary.

To simulate repetitive-hit insult model, we needed to find a dose which sufficiently induces microglia activation without excessively strong cytotoxicity. To determine the dose of LPS, the oligodendrocyte and microglia co-culture was treated with various concentrations of LPS (0.01, 0.1, and 1 μg/mL) at the D3 stage. The LPS dose of 0.01 μg/mL was used to simulate repetitive-hit insult because this dose was evaluated to induce sufficient activation in microglia in the experiment of Miller et al.[11] but did not show cytotoxicity in our study. Next, the co-culture was treated with 0.01 μg/mL LPS on various dosing schedules. To evaluate the developmental stage-dependent effect of LPS stimulation, the early LPS group was treated with 0.01 μg/mL LPS on D1 and with vehicle (normal saline) on D3. And the late LPS group was treated with

vehicle on D1 and with 0.01 μg/mL LPS on D3. Repetitive LPS group, which was designed to simulate the repetitive-hit effect of LPS stimulation, was treated with 0.01 μg/mL LPS on both D1 and D3 (Fig. 2).

Immunostaining

The cells were fixed with 2% formaldehyde prepared from paraformaldehyde. The anti-NG2, anti-MBP, and anti-ionized calcium binding adaptor molecule 1 (IBA1, also known as allograft inflammatory factor 1) antibodies were purchased from Millipore (Burlington, MA, USA; AB5320), BioLegend (San Diego, CA, USA; 808402), and Abcam (Cambridge, UK; ab153696), respectively. The secondary antibodies were purchased from Thermo Fisher Scientific (Carlsbad, CA, USA). The antibodies were diluted in phosphate-buffered saline (pH 7.4) supplemented with 2% goat serum. The coverslips were rinsed twice between antibody applications.

For NG2 immunostaining, the cells were permeabilized with 0.3% Triton X-100 (Sigma) for 10 minutes and incubated with anti-NG2 antibody (1:500) for 2 hours at room temperature, followed by incubation with goat anti-rabbit Alexa 488 (1:200; Thermo Fisher

Scientific) for 2 hours. To perform immunostaining of MBP and IBA1, the cells were permeabilized with 0.3% Triton X-100 for 10 minutes and incubated with anti-MBP antibodies (1:500) for 1 hour, followed by incubation with goat anti-rabbit Alexa 488 or anti-goat cy3 (1:100; Sigma) for 1 hour. The cells were counted by an investigator blinded to the experimental conditions using a Zeiss Axioplan 2 microscope (Carl Zeiss, White Plains, NY, USA). Five random, consecutive fields were counted in each coverslip under 200 × magnification. More than 1,000 cells were counted in the control group. On day 7 of differentiation, oligodendrocytes were subjected to NG2 (green) and MBP immunostaining (red) to identify OPCs and mature oligodendrocytes, respectively. To stain the nuclei, the cells were incubated with 10 μg/mL 4′ , 6-diamidino-2-phenylindole (DAPI, blue) for 5 minutes. The cells were mounted on glass slides with Immu-Mount (Thermo Fisher Scientific, Pittsburgh, PA, USA) after antibody application. The numbers of NG2+ and MBP+ cells relative to the total number of cells in the control, early LPS, late LPS, and repetitive LPS groups were quantified.

Statistical analysis

All data are expressed as mean ± standard error of mean (n = 4–

5/group). The means of different groups were analyzed using one-way analysis of variance analysis of variance with Bonferroni corrections or Mann-Whitney U testing (nonparametric) based on the homogeneity of variance testing (Levene's test) using SPSS 25 statistical analysis software (SPSS Inc., Chicago, IL, USA). The differences were considered significant at $P < 0.05$.

Ethics statement

The procedures used and the care of animals were approved by the Institutional Animal Care and Use Committee in Hanyang University (approval No. 14-0050).

2. 2. Results

To simulate the dose-effect of LPS on oligodendrocytes and microglial cells, the effects of different doses of LPS on oligodendrocyte differentiation in vitro on day 3 were examined by subjecting the oligodendrocyte-microglia co-culture to immunostaining for MBP and IBA1. Representative images of oligodendrocytes, which were treated with different doses of LPS on D3 and on D7 of differentiation are shown in Figs. 3 and 4. The

number of MBP+ cells in the LPS (0.01 μg/mL)−treated group was non−significantly lower than that in the control group on D7. LPS dose−dependently decreased the proportion of mature oligodendrocytes (MBP+ cells) relative to the total number of OPCs. The number of microglial cells in the LPS (0.1 and 1 μg/mL)−treated groups was significantly lower than in the control group ($P < 0.001$ and $P = 0.007$, respectively). However, the number of microglial cells in the 1 μg/mL LPS−treated group was significantly higher than that in the 0.1 μg/mL LPS−treated group ($P = 0.009$) (Fig. 4).

Based on these results, 0.01 μg/mL LPS was used to simulate repetitive−hit inflammation. To develop the repetitive LPS exposure model for developing oligodendrocytes, the oligodendrocyte−microglia co−culture was treated with 0.01 μg/mL LPS on D1 (early LPS group), D3 (late LPS group), or D1 and D3 (repetitive LPS group). The 0.01 μg/mL LPS in the late LPS group did not affect significantly both the MBP+ cell numbers and the NG2+ cell numbers. The number of MBP+ cells in the early LPS group was significantly lower than that in the control group and late LPS group (all $P < 0.001$). On D7 of differentiation, compared with those in the control group and the late LPS group, the MBP+ cell numbers were significantly

lower (all $P < 0.001$) and the NG2+ cell numbers were significantly higher (all $P < 0.001$) in the repetitive LPS group (Figs. 5 and 6). The decreased MBP+ cell numbers and the increased NG2+ cell numbers were significant in the repetitive LPS group compared to the early LPS group ($P = 0.004$ and $P < 0.001$, respectively).

2. 3. Discussion

To the best of our knowledge, this is the first study to demonstrate the effect of repeated LPS exposure on oligodendrocyte differentiation using the oligodendrocyte and microglial cell co-culture model. Some animal studies have demonstrated developmental stage-dependent susceptibility or LPS-hypoxic ischemia-induced brain injury. However, the effect of LPS exposure at the early and late developmental stages on the induction of OPC differentiation and oligodendrocyte injury in vitro has not been previously reported. Repetitive LPS stimulation during the developmental stage significantly inhibited brain cell development and consequently impaired oligodendrocyte differentiation. In contrast, single LPS exposure at the late developmental stage did not affect brain cell development. This may be due to the difference in

susceptibility to the LPS insult according to the oligodendrocyte differentiation stage. In the study of Miller et al., LPS-activated microglia induced OPC death, but reduced apoptosis of mature oligodendrocytes.[11] In this context, Nicholas et al. suggested that products secreted by microglia such as insulin-like growth factor-2 (IGF-2) may have protective effect to oligodendrocytes from potentially toxic soluble factors produced by microglia.[21] In the present study, we used cell survival rate as a variable to compare cell susceptibility. But to speculate the molecular mechanism of these different responses of oligodendrocyte lineage cells in different differentiation states, examining of molecular mechanism of apoptosis of oligodendrocyte lineage cells and also the degree of microglial activation which is thought to induce the apoptosis is needed.

In this study, an LPS-induced brain injury model proposed by Miller et al.[11] was modified. In this model, 1 μg/mL of LPS exerted deleterious effects on OPC survival and proliferation. This study examined the response of oligodendrocytes to various concentrations of LPS in the oligodendrocyte-microglial cell co-culture model. At concentrations of 1 and 0.1 μg/mL, LPS at the D3 stage significantly

decreased the number of oligodendrocytes in the oligodendrocyte-microglial cell co-culture. A similar effect was not observed upon treatment with 0.01 μg/mL LPS (Fig. 4). 0.01 ug/ml was confirmed to be a sufficient dose to induce microglial activation in a study by Miller et al.,[11] but it did not show cytotoxicity when used to stimulate cells on D3 in our study. However, 0.1 and 1 μg/mL doses showed a significant decrease in cell survival rate compared to the control group, suggesting the possibility of a dose-effect with microglia-induced cell toxicity. Miller et al. have shown that LPS induced the production of tumor necrosis factor-α (TNF-α) in a dose dependent manner in their experiments. In addition, in the same study it was also confirmed that LPS alone without microglia did not show toxicity that could cause OPC death.[11] Therefore, also in this study, the decrease in OPC viability according to the increase in LPS dose is thought to be due to the over-activation of microglia according to the LPS dose. However, in order to confirm this, it would be required to examine the molecular mechanism of activated microglia-induced OPC death.

One peculiar phenomenon is that, in the case of IBA1+ cells, 0.1 μg/mL dose LPS showed a significant decrease in cell number

compared to the control group, but with 1 μg/mL dose, cell number was increased compared to the control group and the 0.1 μg/mL dose group. Liu et al. conducted an experiment to validate the LPS effect on the viability of microglia with microglia single culture. When stimulated with LPS at a concentration ranging from 0.001 μg/mL to 1 μg/mL, microglia viability showed a dose-dependent decreasing curve for up to 6 hours of response time with LPS doses of 0.01 μg/mL or higher.[22] In our study, even after exposure to LPS stimulation for more than 6 hours, the cell number at 1 μg/mL was higher from that of the 0.1 μg/mL stimulation group. In order to understand the mechanism of this phenomenon, it would be necessary to verify the activation level of microglia through evaluation of the morphology, size, number, and marker expression of microglia.[23]

The susceptibility of the brain white matter to systemic inflammation is dependent on the presence of pre-OLs at the gestational age of 23-32 weeks in humans. A similar pre-OL phenotype is reported in newborn rats.[24] The effects of LPS exposure at D1 (early LPS group) and D3 (late LPS group) developmental stages, which correspond to 23-32 weeks of gestational age, on OPCs and oligodendrocyte injury were analyzed

in vitro. LPS exposure at the D3 stage did not significantly affect the numbers of MBP+ and NG2+ cells. In contrast, LPS exposure at the D1 stage significantly decreased the number of MBP+ cells (Fig. 6). This suggests that OPCs and oligodendrocytes exhibit differential responses to LPS at different developmental stages and that these cells exhibit maturation-dependent susceptibility to LPS. Miller et al.[11] reported that the oligodendrocytes exhibited differential responses to the activated microglial cells in vitro depending on the developmental stage. Activated microglial cells promote cell death of OPCs but simultaneously enhance survival and downregulate apoptosis in mature oligodendrocytes. This result may be because OPCs, which are susceptible to LPS-activated microglia, form the majority of cells in the co-culture at the D1 stage. At the D3 stage, a large proportion of OPCs can develop into mature oligodendrocytes, which are resistant to LPS-activated microglial cells. This explains the decreased number of MBP+ cells in the early LPS group and the preserved number of MBP+ cells in the late LPS group. However, this study did not confirm the accurate developmental stages of MBP+ cells at the D1 and D3 stages.

Another notable finding of our study is that LPS exposure at the

early developmental stage (D1) exacerbated the detrimental effects of the subsequent LPS exposure (on D3) on OPCs and oligodendrocyte injury (Fig. 6). Two mechanisms explain this result. The first mechanism is based on the susceptibility of OPCs to LPS insults. In the repetitive LPS group, most cells are OPCs that are susceptible to LPS-induced inflammation at the D1 stage. The first (early) LPS stimulation promoted OPC damage and a minimal number of OPCs can mature into MBP+ oligodendrocytes. However, the surviving OPCs are more susceptible to the next inflammatory insult. Thus, the second LPS insult on D3 further decreases the number of MBP+ cells at the D7 stage. The second mechanism involves LPS delivered through infections or in vitro injection arresting the maturation of OPCs. Hence, OPCs may lose their ability to develop into MBP+ mature oligodendrocytes in the repetitive LPS group.

Next, we focused on the number of NG2+ cells. Compared with that in the control group, the number of NG2+ cells were significantly higher in the repetitive LPS group. NG2+ cells are mainly OPCs, whose numbers were intuitively expected to decrease in the repetitive LPS group. However, the increased number of NG2+ cells after repetitive LPS insult suggests an increase in the population of

OPCs. In this experiment, an evaluation of the cell state of D3 after applying the early LPS stimulation on D1 would be required to estimate the process that occurs after late LPS stimulation on D3 until D7 by repetitive insult. Then the mechanism of paradoxical increase in the number of NG2+ cells might have been elucidated.

There are several studies that confirmed that OPC became more proliferative after stimulation. Segovia et al.[31] reported that pre-OL degeneration induced by hypoxic-ischemia was offset by a robust regenerative response that resulted in a several-fold expansion in the pool of surviving pre-OLs in chronic lesions. Interestingly, these regenerated pre-OLs exhibited persistent maturation arrest and did not differentiate or generate myelin. Billiards et al.[32] demonstrated that in contrast to mature oligodendrocytes, OPCs underwent proliferation to generate new oligodendrocytes compensatively in response to multiple insults based on white matter analysis in periventricular leukomalacia. Pang et al.[7] reported that hypomyelination can be resulted from decreased number or dysfunction of mature oligodendrocytes. Various studies have demonstrated the development failure of oligodendrocyte lineage after insults, such as LPS or hypoxic-ischemia.[33, 34]

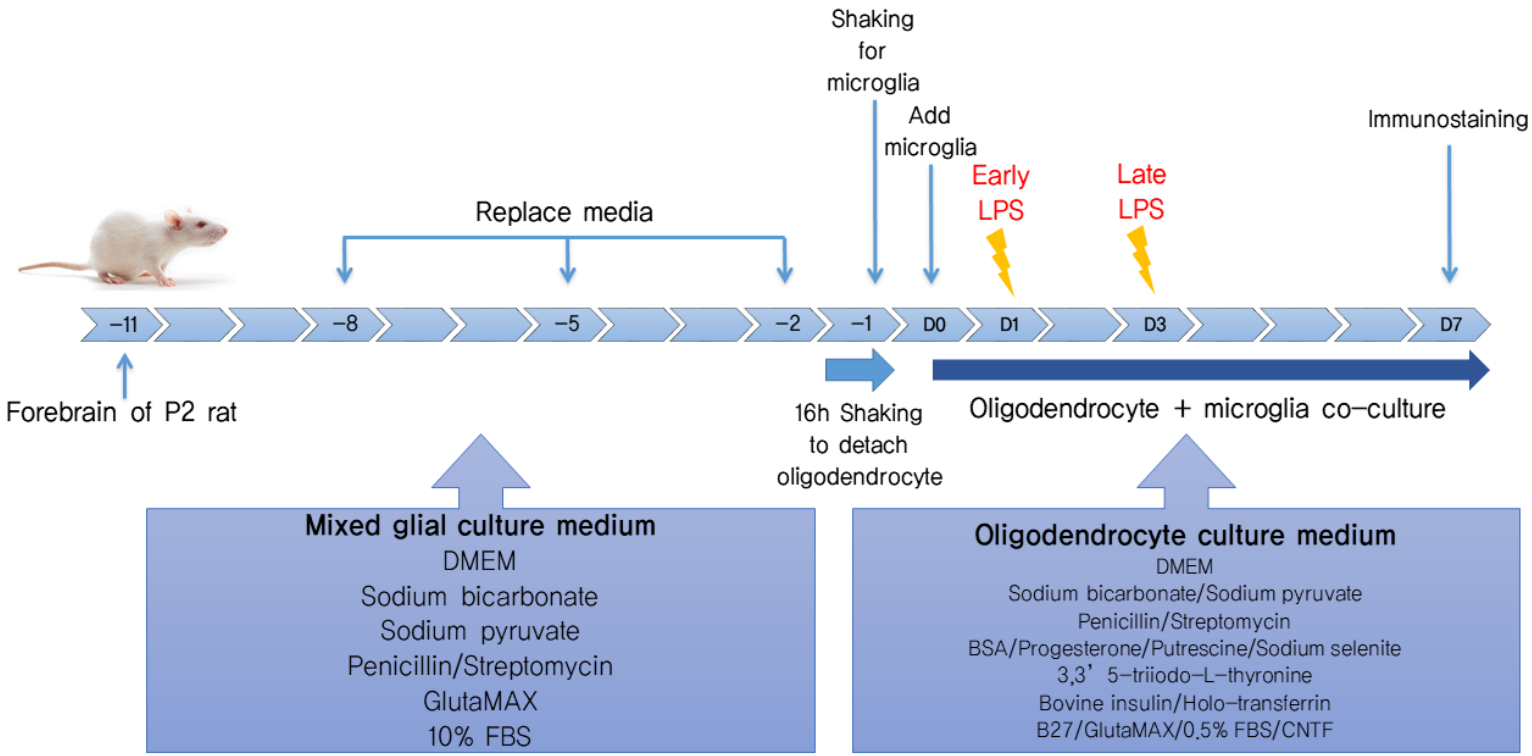

Figure 1. Timeline of the experiment.

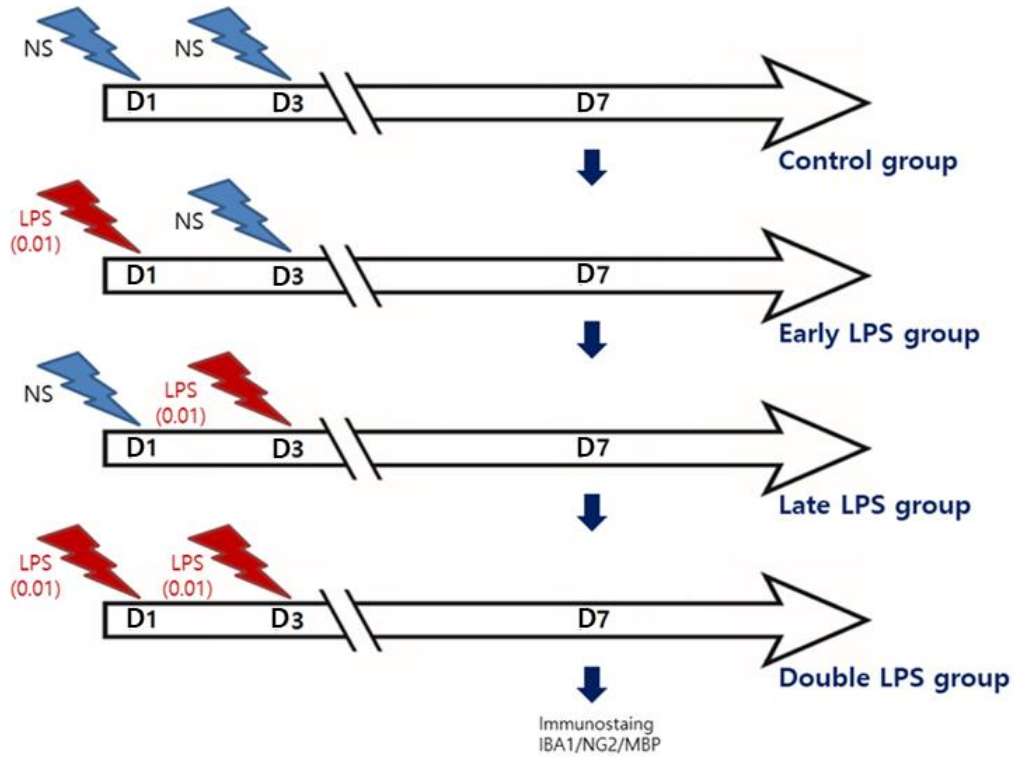

Figure 2. Schematic outline of the experimental protocol. The oligodendrocyte−microglial cell co−culture was treated with 0.01 μg/mL dose of LPS which induced sufficient activation in microglia but did not show cytotoxicity in our study, at the developmental stages D1 (early LPS group), D3 (late LPS group), or D1 and D3 (double LPS group).

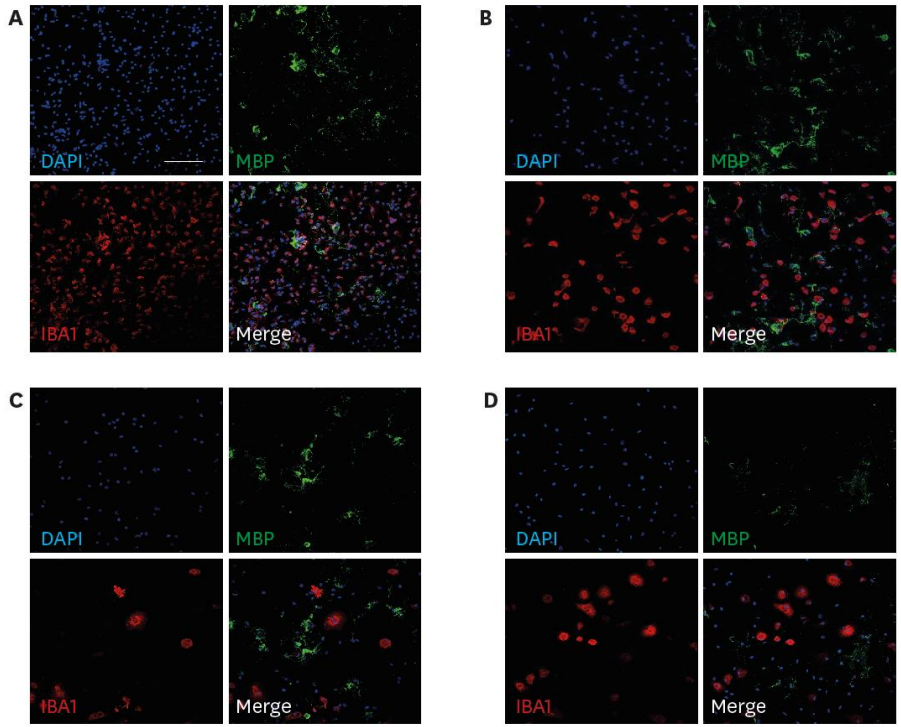

Figure 3. Effect of various doses of LPS on oligodendrocyte differentiation. On day 3, the co-culture was treated with 0.01, 0.1, and 1 μg/mL LPS. On day 7 of differentiation, the nuclei were counterstained with 4′ ,6-diamidino-2-phenylindole (blue). The oligodendrocyte-microglia co-culture was subjected to MBP (green; mature oligodendrocyte marker) and IBA1 (red; microglia marker) immunostaining. The co-culture was treated with vehicle (control) (A) and 0.01 (B), 0.1 (C), and 1 μg/mL LPS (D). Scale bar = 100 μm (200 × magnification).

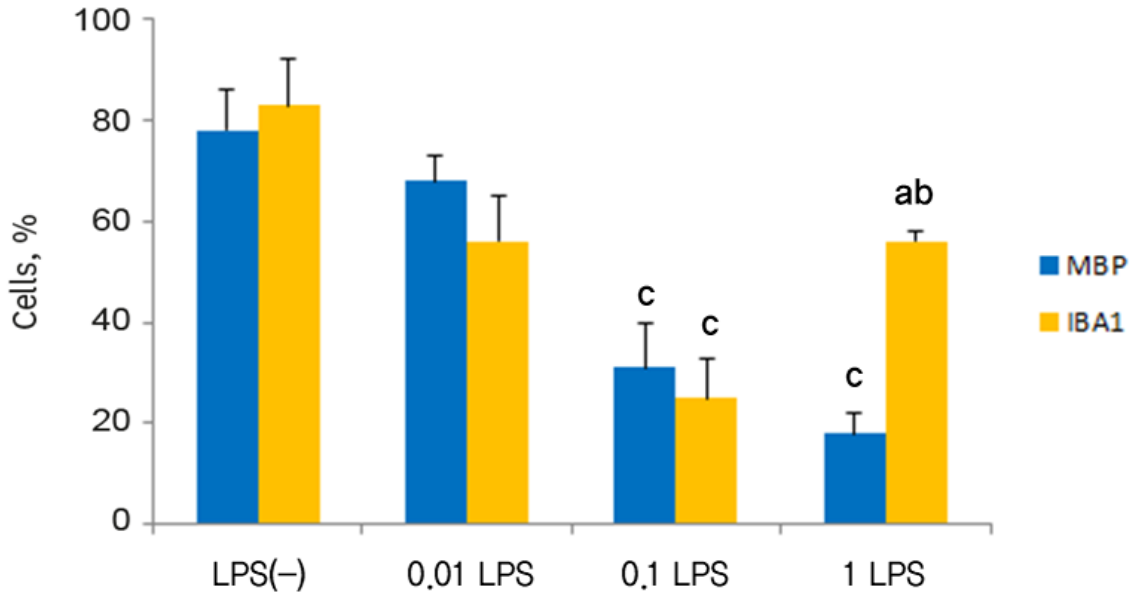

Figure 4. Representative image of oligodendrocytes and microglia in the co–culture treated with various doses of LPS on day 7 of differentiation (n = 4/group). *P* values from Mann–Whitney U test: [a]0.007 vs. control; [b]0.009 vs. LPS 0.1. *P* values from one–way analysis of variance analysis of variance with Bonferroni: [c]< 0.001 vs. control.

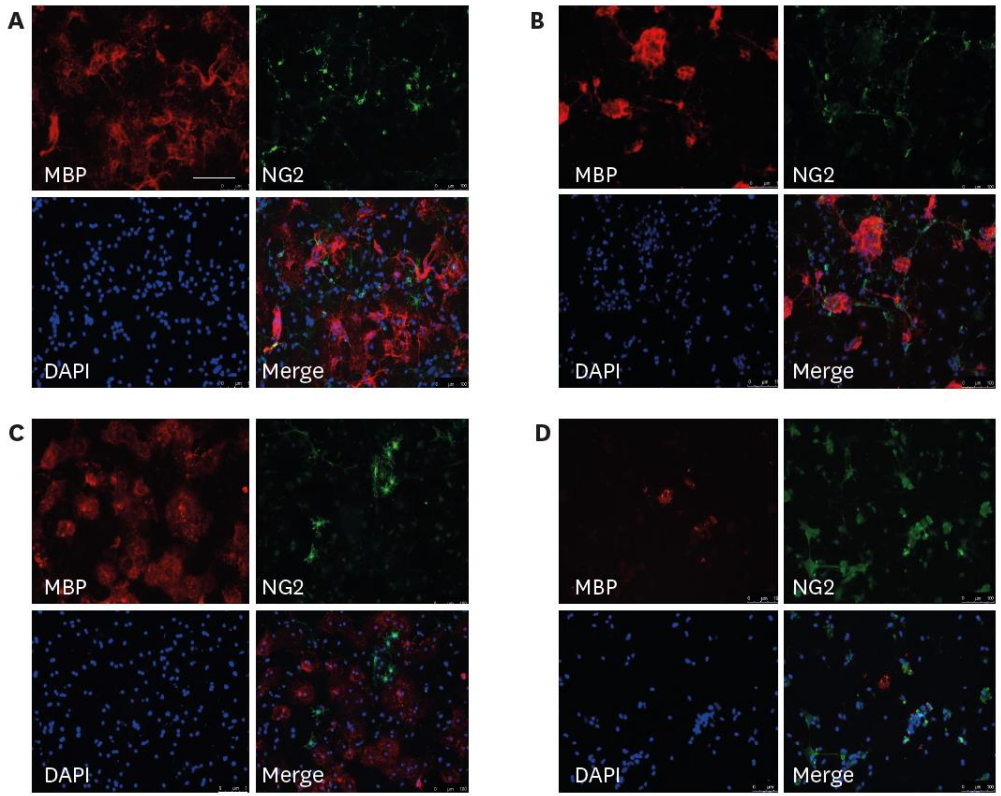

Figure 5. Representative images of the oligodendrocyte−microglia co−culture subjected to immunostaining after in vitro treatment with vehicle (control) (A) or 0.01 μg/mL LPS at D1 (early LPS group) (B), D3 (late LPS group) (C), or D1 and D3 (double LPS group) stages. Scale bar = 100 μm (200 × magnification).

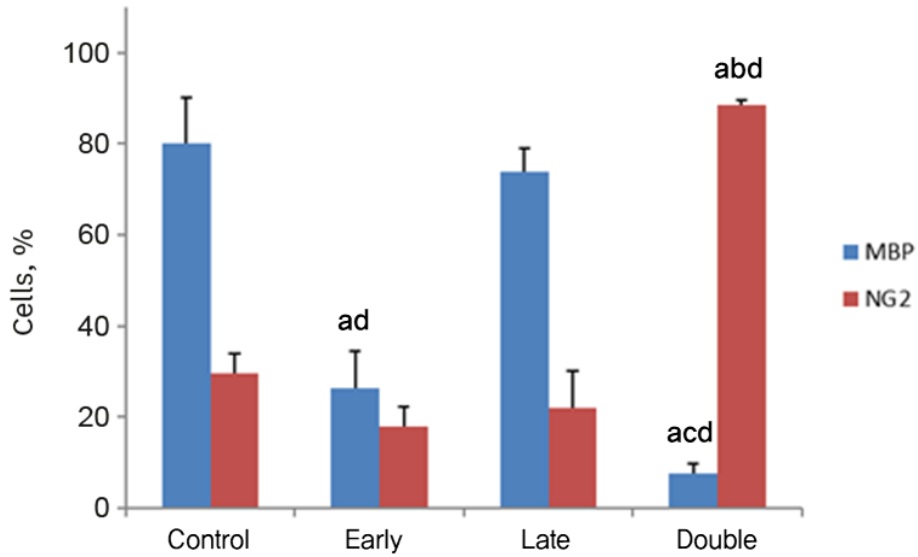

Figure 6. Quantification of the relative numbers of NG2+ and MBP+ cells. Relative numbers of NG2+ and MBP+ cells were quantified after treatment with vehicle (control) or 0.01 μg/mL LPS at D1 (early LPS group), D3 (late LPS group), or D1 and D3 (double LPS group) stages. The number of MBP+ cells in the early LPS group was significantly lower than that in the control group. Compared with those in the control group, the MBP+ cell numbers were significantly lower and the NG2+ cell numbers were significantly higher in the double LPS group at day 7 of differentiation (n = 5/group). All *P* values from one-way analysis of variance analysis of variance with Bonferroni; [a]< 0.001 vs. control; [b]< 0.001 vs. early LPS; [c]0.004 vs. early LPS; [d]< 0.001 vs. late LPS.

Chapter 3. Conclusion

This study demonstrated that LPS dose-dependently decreased the viability of oligodendrocyte lineage cells using the oligodendrocyte-microglial co-culture system. Additionally, repetitive LPS stimulation adversely affected OPCs, which can be attributed to the vulnerability of OPCs to LPS insult and the LPS-induced developmental arrest of OPCs or pre-OLs. These findings provide useful insights into the development and severity of periventricular leukomalacia in some preterm infants and the potential targets for protecting vulnerable premature brains from damage and subsequent developmental impairment.

This study has several limitations. Firstly, we decided to examine the dose-effect of LPS at the D3 state because we thought that the dose which did not affect the D1 state cell would not have any effect on the D3 state cells which was more mature than cells on D1. However, if the cytotoxicity of LPS was confirmed at both D1 and D3 state, it would have been a more sophisticated design. Next, the morphology of activated microglial cells, which can dose-

dependently exacerbate the oligodendrocyte injury, was not delineated in this study. And the correlation between the morphological changes in LPS-activated microglial cells and the response of OPCs was not determined. Furthermore, the exact numbers of OPCs or mature oligodendrocytes classified according to the morphology at D1 and D3 stages, which will enable the determination of the developmental stage of oligodendrocytes, were not determined. If immunostaining was performed in various ways using antibodies such as A2B5, GalC, O4 and O1, it would have been possible to check the cell maturation status of OPC, preOL, immature oligodendrocyte, and mature oligodendrocyte in more detail and the proportion of each cell type.

The findings of this study highlight the potential correlation between susceptibility to neuroinflammation at different developmental stages and the underlying neurobiological processes in preterm infants with periventricular leukomalacia. Thus, understanding the triggered and synergistic effects of early exposure to inflammation, even at low doses, will improve our understanding of impaired white matter development in premature infants.

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국문 초록

본 연구는 희소돌기아교세포 (oligodendrocyte)와 미세아교세포 (microglia cell)의 공동 배양 환경에서 발달 단계에 따른 희소돌기아교세포의 분화에 대한 지질다당질 (Lipopolysaccharide; LPS)-유발 감염 및 염증의 영향과 반복 자극(repetitive-hit) 모델에 대한 연구로서 진행되었다. 생후 2일된 쥐의 뇌에 있는 희소돌기아교세포와 미세아교세포를 추출하여 통제 집단, 1일차 LPS노출 집단(초기), 3일차 LPS 노출 집단(후기), 1일 및 3일차 LPS 노출 집단(초기 및 후기; repetitive)의 네 가지 조건을 만들어 MBP와 NG2 수준을 분석하였다. 그 결과 통제 집단과 비교했을 때, 초기 LPS 노출은 MBP 수준을 낮추고 희소돌기아교세포 분화에 영향을 미쳐 뇌세포의 발달을 저해한 반면, 후기 LPS 노출만으로는 뇌세포 발달에 영향을 미치지 않은 것을 확인하였다. 또한 초기 및 후기 모두 LPS 자극을 받은 경우, NG2 세포의 비율이 크게 증가하여 희소돌기아교세포 계통의 발달이 더 심하게 저해된 것을 알 수 있었다. 이를 통해 희소돌기아교세포 초기 발달 단계에서의 LPS 노출이 뇌의 염증 취약성을 유발시킬 수 있으며, 반복되는 LPS 자극에 의해 염증 자극에 대한 감수성이 증가될 수 있음을 추측할 수 있다.

주요어: 지질다당질, 미숙아, 뇌, 뇌실주위백질연화증, 희소돌기아교세포, 미세아교세포

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