이학석사학위논문

신경병증성 통증에서 소교세포 *Gria1* 유전자 감소의 기능

Role of microglial *Gria1* down-regulation in neuropathic pain

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ABSTRACT

Role of microglial *Gria1* down-regulation in neuropathic pain

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Microglia is the resident immune cell in the central nervous system, and the activation of microglia is known to induce neuropathic pain. Previously, single cell microarray for spinal microglia in L4 spinal nerve transection (SNT) model revealed down-regulation of *Gria1* mRNA in ipsilateral microglia at post-operation day 7. *Gria1* gene encodes glutamate receptor 1 (GluR1) which is an AMPA receptor family. While GluR including GluR1 is known to be functionally expressed in cortical microglia, it is still unclear if *Gria1* in spinal microglia is involved in the development of neuropathic pain. Therefore, this study is aimed to determine whether the *Gria1* expression indeed decreases following microglial activation, and downregulation of *Gria1* contributes to neuropathic pain.

Primary cultured microglia from mice cerebral cortex were treated with ATP (50uM, 1hr) to induce microglial activation in vitro condition. Expression level of *Gria1* and GluR1 in ATP-activated microglia was

examined with real time PCR and immunostaining, respectively. Cytokine array (mouse cytokine array kit, panel A, R&D systems) for various cytokines was performed with ATP-treated microglia, and the change in cytokines from *Gria1* knockdown microglia was determined with ELISA. The level of phosphorylation of p38 MAPK was also confirmed in *Gria1* knockdown microglia with western blot. In addition, functional significance of *Gria1* down-regulation in microglia was confirmed in vivo. Behavior test was performed to check the pain hypersensitivity with von Frey filament by measuring paw withdrawal threshold following intrathecal injection of *Gria1* knockdown microglia to naive mice.

The expression of *Gria1* mRNA and GluR1 protein encoded by *Gria1* were significantly attenuated in the ATP-treated microglia, compared to the control, indicating the down-regulation of *Gria1* in activated microglia. Cytokine array revealed the increased level of TNF-α in activated microglia, and increased TNF-α was also mimicked in *Gria1* knockdown microglia. However, the level of phosphorylation of p38 MAPK was not changed in *Gria1* knockdown microglia. From the behavior study, it was found that paw withdrawal threshold significantly decreased after intrathecal injection of *Gria1* knockdown microglia.

Taken together, these results provide strong evidence that *Gria1* expression is decreased in activated microglia, which contributes to the neuropathic pain following peripheral nerve injury. Downregulation of *Gria1* in activated microglia is likely to be involved in the release of TNF-α which leads pain hypersensitivity in the neuropathic pain condition.

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keywords : microglia, *Gria1*, glutamate receptor1 (GluR1), TNF-α, pain

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INTRODUCTION

Microglia is the resident immune cells in central nervous system (CNS) which constitute 10~15% of the total glial cell population [1]. They are highly susceptible to the environmental change which causes morphological modification [2-4]. When injured, various chemical signals are released from neurons and other immune cells and these chemicals activate microglia [5, 6]. Among these chemicals, it is noted that adenosine triphosphate (ATP) is released during the injury and this leads to activation of microglia [4, 7-11]. Activated microglia migrate to the site of injury, where they may proliferate and release proinflammatory cytokines such as IL-1b, IL-6, and TNF-α. In pathological condition, the excessive release of proinflammatory cytokines by activated microglia is known to induce neuropathic pain [6, 12-16].

Previously, single cell microarray for microglia in L4 SNT model was carried out and down-regulation of *Gria1* mRNA was found. *Gria1* translates to the glutamate receptor 1 (GluR1) which is an AMPA receptor family. AMPA receptor has four subfamilies; GluR1, GluR2, GluR3, and GluR4 [17, 18]. Generally, it is noted that glutamate receptor in CNS mediate fast synaptic transmission [19, 20]. GluR1 on cell membrane mediate calcium influx and it causes change in intracellular calcium level which is important to the release of cytokines [21-23]. A decline of *Gria1* expression is related to neurodegenerative diseases such as Alzheimer and Parkinson's disease

[24]. However, it is still unclear if the decrease in expression level of *Gria1* induces pain [25, 26]. I hypothesized that microglia with low level of *Gria1* expression induces pain.

I had experiments in following procedures. I examined (1) whether the level of *Gria1* mRNA and protein was reduce in activated primary cultured microglia, (2) whether *Gria1* knockdown microglia release TNF-α, (3) whether p38 MAPK in *Gria1* knockdown microglia will be phosphorylated, (4) whether *Gria1* knockdown microglia induce pain in vivo.

MATERIALS AND METHODS

All Surgical and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the School of Dentistry, Seoul National University (Seoul, Korea). Animal treatments were performed according to the ethical guidelines of the International Association for the Study of Pain [27] and the Guide for the Care and Use of Laboratory Animals (National Research Council (US), 2011). Day1 C57BL/6J wild-type mouse and Adult C57BL/6J wild-type male mouse (Daehan, Sungnam, Korea) were used. Animals were housed in a conventional facility with a 12:12 hr light cycle (lights on 8.00am) and ad libitum access to water and chow. Mice were acclimatized for at least one week prior to experiments.

Primary microglia culture

Mixed glial cultures were prepared from cerebral cortex of 1-2 days old postnatal mice. Briefly, mixed glial cells were cultured and maintained for 14-16 days in high-glucose DMEM medium including 10% fetal bovine serum, 1% penicillin streptomycin, and 1% fungi zone. Microglia were harvested from the mixed glia by shaking T75 flasks. The media including floating cells were collected and microglia were plated at 6-well plates at the density of 2×10^6cells/ml for subsequent ATP treatments or used for transfection.

Real time PCR

Total RNA was extracted by miRNeasy Mini kit (Qiagen), and 0.5-1.0 μg was processed for cDNA synthesis using M-MLV Reverse Transcriptase (Invitrogen) according to the manufacturer's instructions. Real time PCR was performed with cDNA obtained from primary cultured microglia as a template using a 7500 Real-Time PCR system (Applied Biosystems). The threshold cycle (Ct) of the GAPDH or siRNA negative control was used as a reference control to normalize the expression level of the target gene (ΔCt) to correct for experimental variation. Relative mRNA levels were calculated according to the $2^{-\Delta\Delta Ct}$ method. Real-time RT-PCR experiments were performed at least three times, and the means ± SEM. Values are presented unless otherwise noted. The PCR primer sequences used in this study are as follow; *GAPDH*, 5'-TCCATGACAACTTTGGCATTG-3' and 5'-CAGTCTTCTGGGTGGCAGTGA-3'; *TNF-α*, 5'-AGCAAACACCAAGTGGAGGA-3' and 5'-GCTGGCACCACTAGTTGGTTGT-3'; *Gria1*, 5'-TCAATGAAGCCATACGGACA-3' and 5'-GCTGACCACTCTGCCATTCT-3'.

Immunocytochemistry

Cultured mouse primary microglial cells on cover slip were incubated 2 days in 37℃, 5% CO_2 incubator. When the microglial cells became resting condition, ATP (Sigma A2383-5G) 50μM was treated on primary microglial cells. After this, the cells initially rinsed three times with Phosphate Buffered Saline (PBS; Sigma). Cells were fixed with 4%

paraformaldehyde (millipore) in PBS for 10 mins at room temperature. Following three times with PBS-T (0.1% Triton X-100), incubated for 1hr in a blocking solution containing 5% normal goat serum (NGS; ImmunoResearch) and FBS (Gibco) in PBS-T. The cells were stained in 4℃ during overnight with primary antibody. The primary antibody diluted in blocking solution and the titer was rabbit anti GluR1 C-terminus(1:500) (Millipore AB1504) and rabbit anti Iba1 C-terminus(1:1000) (Wako 019-1974). The next day, cells were rinsed three times for 10mins with PBS and the incubated for 1hr with secondary antibody solution in PBS-T. Secondary antibody used were Alexa fluor 488 goat anti-rabbit(1:200) (Jackson ImmunoResearch 115-545-003) and cy3 fluor goat anti rabbit(1:200) (Jackson ImmunoResearch 111-165-003). Following further wash of three times with PBS, slide glasses were mounted in the vectashield mounting media (Vector). Fluorescense images were obtained using a confocal laser-scanning microscopy (LSM700; Zeiss).

Proteome profiler cytokine assay

Briefly, relative expression of cytokines was quantified using the Proteome Profiler Mouse Cytokine Array kit, Panel A (R&D Systems). The array was performed according to manufacturer's exact specifications using 700 μl supernatant of control group (not treat) and ATP treated group, respectively.

Transfection

AccuTarget™ *Gria1* siRNAs (Bioneer 1365968) and negative control siRNA were purchased from Bioneer (Daejeon, Korea). The sequences of si*Gria1* is antisense 5'-UUGGAAUCCAAGUUACCUC(dTdT)-3'. Primary cultured microglia were transfected with *Gria1* siRNA (10nM) by electroporation using a microporator™ (MP-100; Digital Bio Technology) following the manufacturer's instructions. Transfected microglia were seeded at 1.0×10^6 cells per well of 6-well plates. Real-time PCR was used to determine knockdown efficacy of siRNA. After 48hrs for *Gria1*, transfected microglia (1.0×10^6cells, 10μl) were used for intrathecal(i.t) injection.

ELISA

After transfection, primary microglia cells were plated 1.5×10^6cells/well in 6-well plates and the cell supernatant collected after 48hrs of transfection. The levels of TNF-α in supernatant were quantified using the mouse TNF-α DuoSet ELISA kit (R&D Systems). ELISA was performed by adding 100μl of each sample to wells in a 96-well plate. The samples were tested in triplicate. TNF-α ELISA was performed according to the manufacturer's instructions.

Western blot

Protein lysates were prepared using pro-prep (Invitrogen life technologies) buffer. These were sonicated 30secs and incubated at 4℃

for 10mins. Samples centrifuged 13000rpm for 5mins and quantified with BCA assay kit (Thermo). Samples were electrophoretically separated on 4% to 15% precast gels (Bio-rad) and protein bands were electrically transferred to polyvinylidene difluoride membranes (Bio-rad). Membranes were blocked for 1hr at room temperature in Tris-buffered saline-Tween 20 (TBS-T) containing 5% BSA. And membranes were incubated overnight at 4℃ with antibodies against phospho-p38 (Cell signaling; 1:1000) and p38 (Cell signaling; 1:1000) in TBS-T, β-actin (Sigma; 1:10000) in TBS-T supplemented with 5% skim milk. Membranes were incubated for 1hr at room temperature with horseradish peroxidase-conjugated anti-rabbit or anti-mouse antibodies (Santa cruz biotechnology; 1:5000) in TBS-T supplemented with 5% skim milk. Bands were visualized with Image lab (Bio-rad) using ECL supersignal west pico chemiluminescent substrate (Thermo) as per the suppliers' instructions. Between all incubation steps, membranes were washed 3 times for 10mins with TBS-T.

Behavior test

Gria1 knockdown microglia were injected intrathecally in normal mice as described previously. Briefly, under isoflurane anesthesia, mice were injected using a 50μl Hamilton syringe fitted with a 31 gauge needle by direct lumbar puncture between the L5 and L6 level. Control animals were injected with microglia transfected with negative control siRNA. Paw withdrawal threshold was tested 1, 3, and 5hrs after injection.

Statistical Analyses

All data are presented as mean ± SEM and analyzed using GraphPad Prism version 5.0 (GraphPad). One-way ANOVA followed by Bonferroni's post-hoc test and non-paired t-test for real time PCR analysis. The data for behavioral tests were analyzed with Mann-whitney test. Differences were considered to be significant when P value was less than 0.05. The numbers of cells tested are indicated in parentheses where applicable.

RESULTS

Level of *Gria1* and GluR1 protein of primary microglia decreased after ATP treatment

In vitro validation of *Gria1* down-regulation in activated microglia, based on single-cell transcriptome assay where *Gria1* was found to be down-regulated in POD7 activated microglia. To mimic injury condition, I applied ATP to primary microglia. I monitored whether activated microglia have low mRNA level of *Gria1*. Figure.1A shows that ATP (50μM, for 1hr) application decreased *Gria1* mRNA level of primary microglia compare to control (n=4).

As *Gria1* translates to glutamate receptor 1 (GluR1), immunostaining was performed to test if GluR1 protein expression decreased when ATP was treated on primary cultured microglia (Figure.1B). Microglia cells were visualized by anti-Iba1 (green) immunostaining and they were costained with anti-GluR1 (red). In addition, ATP application, which followed the same protocol as figure.1A, caused decreased expression of GluR1 compared to the control. These results indicate that treatment of ATP on microglia resulted in diminished *Gria1* and GluR1 protein expression.

Cytokine screening was performed on supernatant of ATP-treated primary microglia

Next, I examined cytokines in the supernatant of ATP (50μM, for 1hr) treated primary cultured microglia. Figure.2 shows that various cytokines were increased or decreased. In supernatant of ATP-treated primary microglia, TNF-α increased by 36%, C5/C5α increased by 70%, M-CSF increased by 296%, and IFN-γ increased by 31%. On the other hand, IL-1α decreased by 21% and TIMP-1 decreased by 65%. I targeted TNF-α because it can be the pain inducing cytokine.

Gria1 knockdown primary microglia release TNF-α

Next, I tried to identify the mechanism how down-regulated *Gria1* in microglia induces pain. *Gria1* was knockdown specifically by siRNA and I checked for the variation in release. I confirmed a degree of *Gria1* knockdown with real time PCR previously and also checked *TNF-α* mRNA level (Figure.3A, upper panel). 48hrs after si*Gria1* transfection, *Gria1* mRNA level significantly decreased by 50%±16%. Whereas, mRNA level of *TNF-α* increased 46±23% (Figure.4A, lower left panel). After 48hr si*Gria1* transfected on microglia, the level of TNF-α in supernatant measured. TNF-α increased in si*Gria1* group compared to siRNA negative control group by 41±24%(n=3) (Figure.3B, lower right panel).

The level of phosphorylation of p38 MAPK in *Gria1* knockdown microglia did not change

When microglia activate, its p38 MAPK is phosphorylated. To evaluate

whether *Gria1* knockdown microglia implied active condition, I checked pp38 expression in *Gria1* knockdown microglia by western blot. However, the level of pp38 was not different compared to control (Figure 4, n=3).

Intrathecal injection of primary microglia with *Gria1* knockdown induced pain hypersensitivity

Furthermore, in vivo study was performed to identify the function of *Gria1* knockdown microglia. I intrathecally injected *Gria1* knockdown microglia to naive mice and measured mechanical allodynia (Figure.5). As I expected, *Gria1* knockdown microglia elicited pain hypersensitivity. With this experiment, I can conclude that *Gria1* knockdown microglia induced allodynia in vivo.

Figure 1

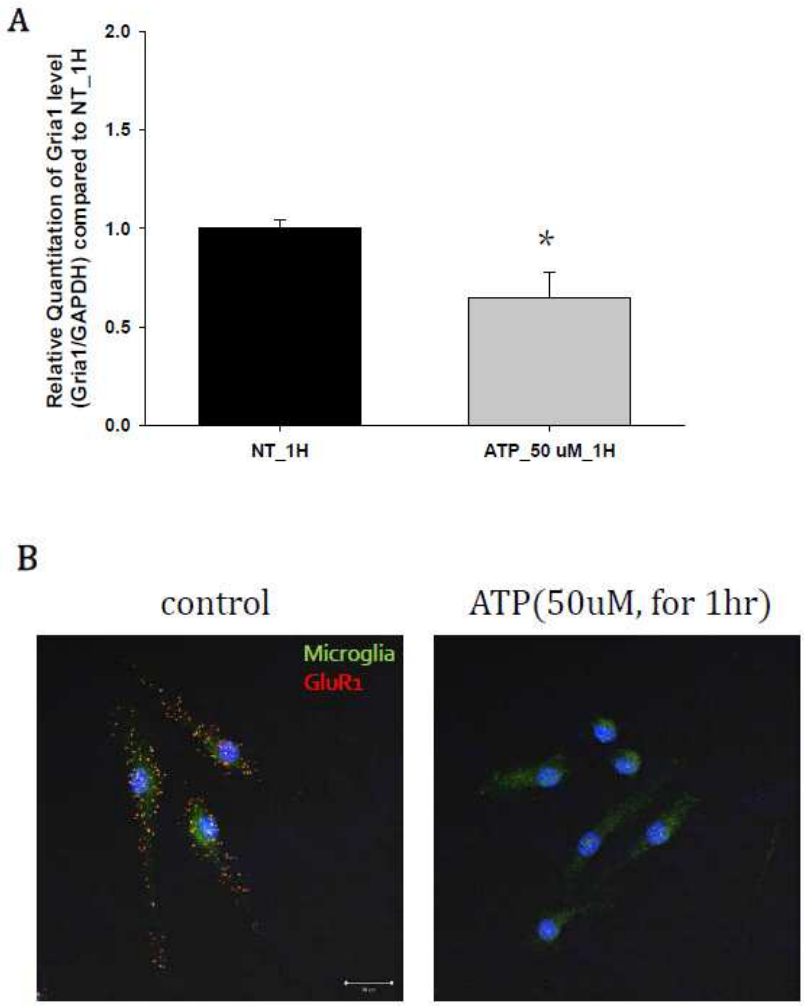


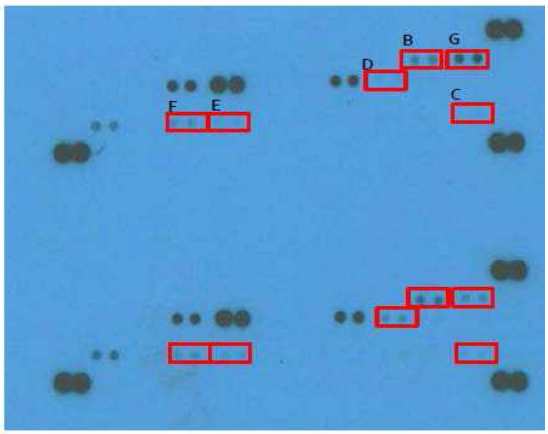
Figure 1. Expression of *Gria1* mRNA and GluR1 in primary microglia activated by ATP treatment decreased.

(A) Quantitative real time PCR was performed to detect *Gria1* in primary cultured microglia were treated with ATP (50μM, for 1hr). *Gria1* mRNA levels are normalized with GAPDH (n=4, non-paired t test, * indicate a significant difference ($p<0.05$) between treated means compared to control. Results are presented as the mean ± SEM) (B)

Immunocytochemistry was used to assess GluR1 in primary cultured microglia exposed to ATP (50μM, for 1hr). Immunofluorescences presented microglia (green; Alexa488), GluR1 (red; cy3), and nucleus (blue; DAPI). Scale bar= 20μm.

Figure 2

A. Cytokine array



Control

ATP 50μM

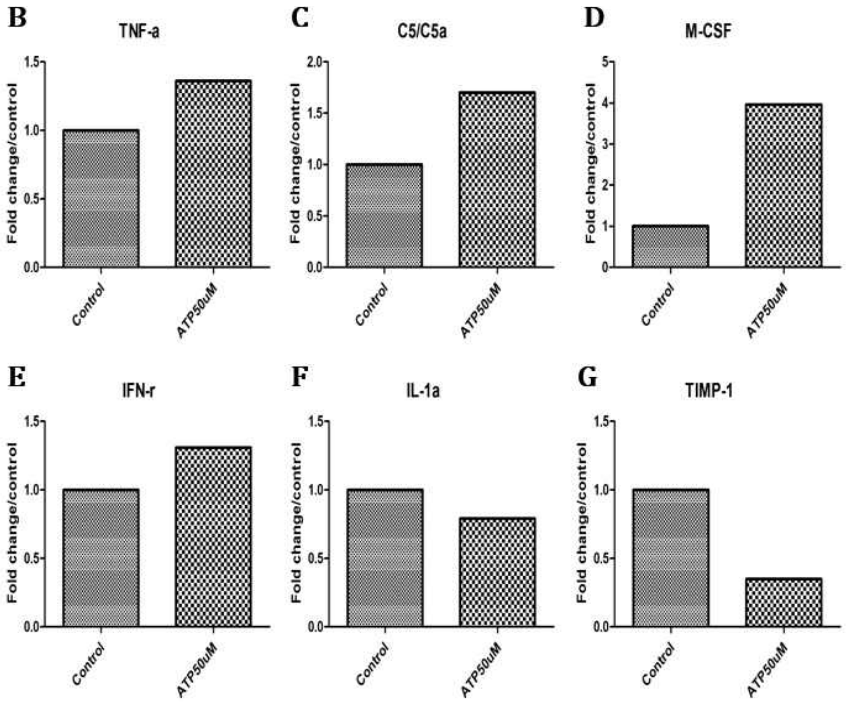


Figure 2. Change level of cytokines in ATP-treated primary microglia confirmed by cytokine array.

They are the level of cytokines in supernatant. (A) Film of cytokine array. (B) TNF-α (C) C5/C5α (D) M-CSF (E) IFN-γ (F) IL-1α (G) TIMP-1. Cytokines are presented as fold change in expression relative to control.

Figure 3

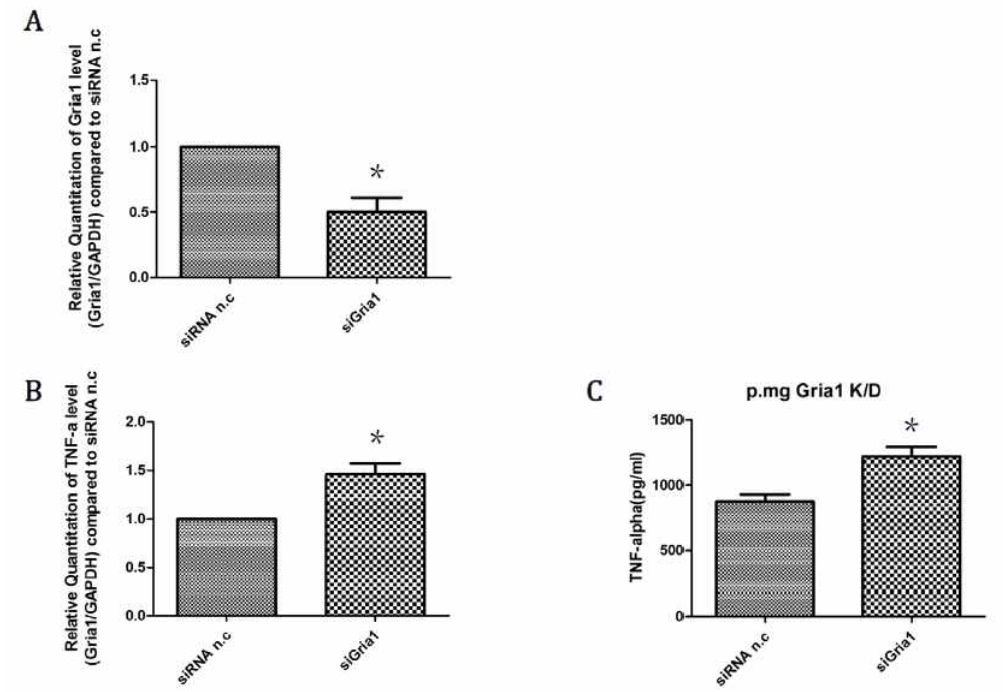


Figure 3. *Gria1* knockdown in primary microglia resulted increase of TNF-α levels in supernatant.

(A) After *Gria1* knockdown, *Gria1* had significantly decreased (upper panel, n=3) and (B) TNF-α mRNA level increased (lower left panel, n=3). (C) TNF-α levels were significantly increased when *Gria1* was knockdown in primary cultured microglia (lower right panel, n=3). siRNA negative control (10nM) and si*Gria1* (10nM) used. *$p<0.05$ compared with siRNA negative control transfected primary microglia. non-paired t test. Data are represented as mean ± SEM.

Figure 4

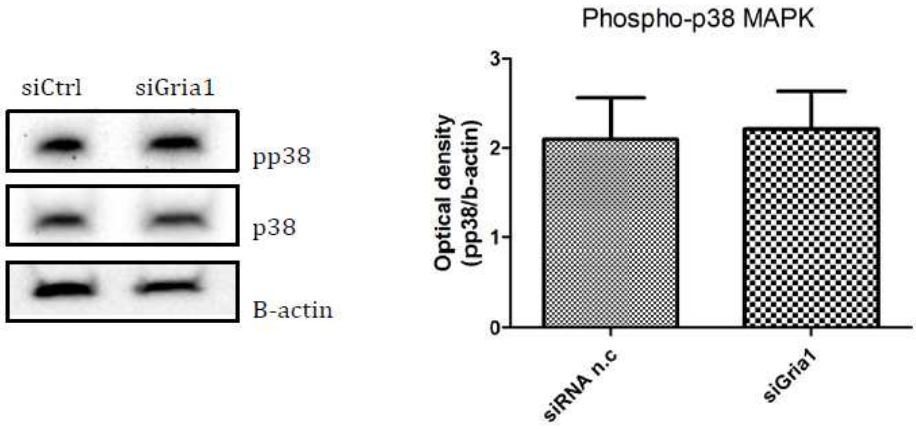


Figure 4. phospho-p38 MAPK did not change in *Gria1* knockdown microglia.

Western blot were performed to detect phospho-p38, p38, and β-actin in *Gria1* knockdown microglia. Protein expression is relative to β-actin ±SEM of three independent experiments.

Figure 5

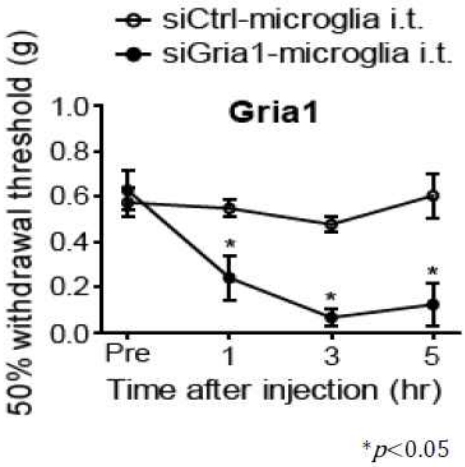


Figure 5. *Gria1* knockdown microglia induce pain hypersensitivity.

Paw withdrawal threshold after intrathecal injection of *Gria1* knockdown microglia (si*Gria1*-microglia) into naive mice (n=6 mice). Negative control siRNA transfected microglia (siCtrl-microglia) was used (n=7 mice). *p<0.05 compared to respective controls. Data are shown as means ± SEM.

DISCUSSION

Previously done study on single cell microarray for microglia after L4 spinal nerve injury, it was found that *Gria1* is decreased. I validated whether low level of *Gria1* expression in microglia contributes to the pain. In vitro, the level of *Gria1* and GluR1 protein of primary cultured microglia was decreased after ATP treatment. Cytokine array was performed in the supernatant of ATP-treated primary microglia and I found that the level of TNF-α increased. The level of TNF-α also increased in the supernatant of *Gria1* knockdown microglia. Phospho-p38 MAPK did not increase in *Gria1* knockdown microglia compared to the control. In vivo, mice which had intrathecal injection of *Gria1* knockdown microglia showed pain hypersensitivity.

There are many evidences support the release of ATP from injured neuron and nerve terminal [28]. It is noted that ATP play an important role to microglia in inflammatory or injury condition in CNS by activating microglia. When microglia is activated with ATP, it evokes the release of substances such as cytokines and chemokines [9, 11, 29]. Even though substance such as LPS can induce a strong immune response and activate microglia [30-32], I used ATP as microglia stimulator to focus on injury condition instead of inflammatory condition. In resting state, macrophage and microglia express P2X receptor which enables ATP to affect resting microglia at the resting state [33]. Concentration of ATP used varies depending on the target cytokine and in between the range of 50μM to 1mM and I decided to treat the 50μM of ATP for 1hr. As, when ATP 50μM is treated on microglia, its *Gria1* is down-regulated while activating the microglia. During the experiment, due to the difficulty in gaining enough amounts

of primary cultured microglia, I used BV-2 microglia cell line since the cell line is more stable than primary cells. Therefore, using the BV-2 microglia cell line would be a better option for the mechanism study. I first validated the result of Fig.1 in BV-2 microglia cell line in identical condition. In contrary to our expectations, ATP concentration, such as 50μM to 200μM, did not significantly change *Gria1* mRNA level of BV-2 microglia cell line. At the end, as I assumed that *Gria1* down-regulation induces release of TNF-α which causes pain, I applied extreme concentration of ATP (1mM, for 3hrs) that gives optimal release of TNF-α on BV-2 microglia cell line. In consequence, *Gria1* mRNA level significantly decreased (n=3). GluR1 protein level was also down-regulated after ATP (1mM) was treated for 3hrs. It was indicative that primary cultured microglia had more sensitivity compared to BV-2 microglia cell line. To identify functional properties of *Gria1* knockdown microglia, I planned to use BV-2 cell line which is easily accessible. However, BV-2 cell line did not transfected with si*Gria1* (10nM to 100nM). Thus, I could not use BV-2 cell line in knockdown experiments.

I used supernatant on cytokine array after checking the down-regulation of the level of *Gria1* mRNA in ATP-treated primary microglia with real time PCR. I had a doubt on the increase in release of TNF-α to the supernatant of *Gria1* knockdown microglia as *Gria1* down-regulation can decrease the expression of GluR1 protein which might cause stationary intracellular calcium level that affects cytokine release [34]. However, the result indicated that TNF-α release was increased from the *Gria1* down-regulated ATP-treated microglia. I targeted TNF-α because it is generally considered to be cytotoxic and causes inflammatory response for autoimmune disorder. It is also known to be involved in the pathological condition of many neurodegenerative diseases [35].

I also checked the level of another cytokine; macrophage-colony stimulating factor (M-CSF). However, mRNA level of *M-CSF* of *Gria1* knockdown microglia did not change. Since there was a number of possible cytokine targets, confirming multiple cytokines in supernatant of *Gria1* knockdown microglia with cytokine array was thought to be a good option.

As an activation marker, I have confirmed the p38 MAPK phosphorylation by *Gria1* knockdown microglia as in previous researches, phosphorylation of p38 MAPK indicated microglia activation in neuropathic pain [36]. However, pp38 level of *Gria1* knockdown microglia did not increase compared to the control. p38 MAPK is phosphorylated in early time point whereas, I speculate that *Gria1* down-regulation contributes to maintenance of pain in late phase. Therefore, *Gria1* is down-regulated after microglia activation. Furthermore, it is needed to confirm that the *Gria1* knockdown microglia is activated through different activation marker such as CD40 and CD68 which is known to increase after microglial activation [37, 38].

In addition, I have confirmed if *Gria1* knockdown microglia induced neuropathic pain when injected to the spinal cord. As *Gria1* seems to play a role in maintaining pain, further study should be done in behavior test that observe the effect of injected microglia for a longer period of time. And also, need to examine whether *Gria1* down-regulation induced allodynia can be reversed by spinal TNF inhibitor. In addition, injection of *Gria1* overexpressed microglia should be tested to see whether they reverse mechanical hypersensitivity induced by ATP-activated microglia.

For another further study, it should be confirmed that if overexpression of *Gria1* have a preventative effect on microglial

activation induced by ATP stimulation. This can elucidate whether *Gria1* is critical for microglia activation. I will examine TNF-α production of *Gria1* overexpressed microglia following ATP stimulation.

In conclusion, I have found that decreased *Gria1* expression of microglia causes pain. *Gria1* knockdown microglia release TNF-α which may contribute to induce pain hypersensitivity.

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국 문 초 록

신경병증성 통증에서
소교세포 *Gria1* 유전자 감소의 기능

소교세포는 중추신경계에 존재하는 면역 세포이며, 소교세포가 활성화되면 신경병증성 통증을 일으킬 수 있다고 알려져 있다. 이전에 L4 척추신경 손상 모델에서 소교세포에 대한 single cell microarray를 진행했었고, 신경 손상을 준 후 7일째 소교세포의 *Gria1*이 감소된 것을 발견했었다. *Gria1*은 AMPA 리셉터인 glutamate receptor 1으로 번역되는 유전자이다. GluR1을 포함한 GluR이 대뇌 피질의 소교세포에 발현한다고 알려져 있지만, 척수 내 소교세포의 *Gria1*이 신경병증성 통증을 일으키는 데 기여하는지에 대해선 잘 알려져 있지 않다. 그러므로 이 연구의 목적은 소교세포가 활성화되었을 때 *Gria1*이 감소하는지, 그리고 *Gria1*의 발현 감소가 신경병증성 통증을 유발하는데 기여하는지 밝히는 것이다.

신경이 손상된 상황을 In vitro에서 재현하기 위해, 대뇌 피질의 소교세포를 배양하여 ATP를 처리했다. ATP를 처리하여 소교세포를 활성화시켰을 때, 소교세포의 *Gria1*과 GluR1의 발현이 감소하는지 real time PCR, 면역염색법을 사용하여 확인했다. ATP 처리한 소교세포에서 분비되는 다양한 cytokine들을 cytokine array를 통하여 확인했다. *Gria1* knockdown 소교세포에서 TNF-α가 분비되는지 ELISA로 확인했으며, Western blot으로 phospho-p38 MAPK가 증가하는지 확인했다. 그리고 소교세포 내 *Gria1* 발현 감소의 기능적 중요성을 확인하기 위해 in vivo 실험을 시행했다. 정상 쥐의 척수 내 *Gria1*이 감소된 소교세포를 주입하고 Von Frey filament를 시행하여 통증이 생기는지 확인했다.

ATP를 처리한 소교세포에서 *Gria1* mRNA와 GluR1 단백질 발현이 대조

군과 비교하여 유의하게 감소했다. ATP를 처리하여 *Gria1*의 발현이 감소한 소교세포의 supernatant에서 TNF-α가 증가했다. 소교세포 내 *Gria1*을 knockdown시켰을 때도 supernatant에서 TNF-α의 양이 증가함을 확인했다. 그러나 *Gria1* knockdown 소교세포에서 pp38 MAPK의 양은 변하지 않았다. 행동 실험에서 *Gria1* knockdown 소교세포를 정상 쥐에 투여했고, paw withdrawal threshold가 유의하게 감소하는 것을 통해 통증이 생기는 것을 확인했다.

결론적으로, 소교세포가 활성화되었을 때 *Gria1*의 발현이 감소되고 이것은 말초 신경 손상 후 신경병증성 통증을 일으키는데 기여하는 것을 확인했다. 활성화된 소교세포의 *Gria1* 발현 감소는 TNF-α가 분비되게 하여 통증을 일으킬 수 있는 가능성을 제시했다.

주요어 : 소교세포, *Gria1*, glutamate receptor1 (GluR1), TNF-α, 통증
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