Chinese Journal of Physiology 61(3): 137-143, 2018
DOI: 10.4077/CJP.2018.BAG544

Angiostrongylus Cantonensis-Conditioned Culture Medium Induces Myelin Basic Protein Alterations *via* Erk1/2 and NF-κB Activation in Rat RSC96 Schwann Cells

Jo-Ping Chin[1, #], Chien-Min Chen[2, 3, #], Tsong-Hai Lee[4, 5], Chii-Wen Chou[1], and Cheng-You Lu[5, 6]

[1]Division of Neurosurgery, Tungs' Taichung Metroharbor Hospital, Taichung 43503
[2]Division of Neurosurgery, Department of Surgery, Changhua Christian Hospital, Changhua 50006
[3]School of Medicine, Kaohsiung Medical University, Kaohsiung 80708
[4]College of Medicine, Chang Gung University, Taoyuan 33305
[5]Stroke Center and Department of Neurology, Linkou Chang Gung Memorial Hospital, Taoyuan 33305
and
[6]Department of Nursing, Central Taiwan University of Science and Technology, Taichung 40601, Taiwan, Republic of China

Abstract

Eating of excessive raw or undercooked environmental snails produces angiostrongyliasis demyelination caused by *Angiostrongylus cantonensis (A. cantonensis)*. The aim of this study was to investigate the association between extracellular signal-regulated kinase (Erk)1/2-nuclear factor (NF)-κB pathway and myelin basic protein (MBP) expression in RSC96 Schwann cells treated with *A. cantonensis*-conditioned culture medium, which was prepared by culturing the third-stage (L3) nematode larvae in DMEM for 72 h. The supernatants were collected and filtered before use. Our results showed that MBP was produced in the RSC96 cells at 16 h to 48 h post-stimulation (PS). Phosphorylated (p)-NF-κB levels were significantly increased from 8 h to 48 h PS, as were the p-Erk1/2 levels at the same time points. Additionally, expression of p-NF-κB and MBP was significantly decreased by treatment with QNZ, an NF-κB inhibitor. Treatment with PD98059, an Erk kinase inhibitor, efficiently reduced p-Erk1/2, p-NF-κB and MBP expression in the Schwann cells. These results suggest that *A. cantonensis*-conditioned culture medium induced suppression of the Erk1/2-NF-κB signaling pathway leading to reduced MBP production in RSC96 Schwann cells. Thus, inhibiting this signaling intermediate involved in MBP expression may be a potential method for controlling inflammatory development of *A. cantonensis*-induced MBP changes in preceded demyelination.

Key Words: ***A. cantonensis*, Erk1/2, MBP, NF-κB, Schwann cells**

Corresponding authors: [1] Chii-Wen Chou, MD, Ph.D., Division of Neurosurgery, Tungs' Taichung Metroharbor Hospital, Taichung 43503, Taiwan, Republic of China. E-mail: 108106@ctust.edu.tw; [2] Cheng-You Lu, Ph.D., Stroke Section, Department of Neurology and Stroke Center, Chang Gung Memorial Hospital, Linkou Medical Center, Taoyuan 33305, Taiwan, Republic of China. Tel: +886-3-3281200 ext. 8883, E-mail: ymt150@gmail.com
[#]These authors contributed equally to this work.
Received: July 3, 2017; Revised: February 22, 2018; Accepted: March 26, 2018.
©2018 by The Chinese Physiological Society. ISSN : 0304-4920. http://www.cps.org.tw

Introduction

Angiostrongylus cantonensis (*A. cantonensis*) is a nematode parasite found in the lung of rats. When humans unintentionally ingest third-stage (L3) nematode larvae in food items such as snails, slugs or vegetables that are eaten raw or undercooked, angiostrongyliasis is developed (1). The most prominent features of angiostrongyliasis include severe central nervous system (CNS) inflammation, eosinophilic meningitis (9) or demyelination (18).

The integrity of the myelin wrapping around myelinated axons in the mature mammals is important (28). The myelin originates from and is a part of the Schwann cells in the peripheral nervous system (PNS) and the oligodendroglial cells in the CNS. Myelin is a fatty white substance that surrounds the axon of some nerve cells, forming an electrically insulating layer, and is, therefore, essential for the proper functioning of the nervous system (24, 28). Demyelination is the loss of the myelin sheath, and is the hallmark of some inflammation diseases, including multiple sclerosis (2, 19), demyelination in angiostrongyliasis (3, 9), or acute disseminated encephalomyelitis (14). Myelin basic protein (MBP) is one of the proteins composing the myelin sheath (3), and it maintains the correct structure of the myelin, and interacts with the lipids in the myelin membrane (4). MBP dysregulation leading to myelin damages affect function and existence of the organism (6).

Previous study revealed that *A. cantonensis* infection caused the alterations of myelin proteins, including MBP, in inflammatory demyelination of BALB/c mice (6, 9). However, the mechanism of MBP change in the above process is unclear. Activation-associated secreted protein had been found in many helminthes, which was associated with pathogenesis and stage transition (8). Additionally, Yang *et al*. has mentioned that the activation-associated secreted protein expressed most highly in the brain-stage larvae (L3 stage) of *A. cantonensis* and played a role in the pathogenesis of human angiostrongyliasis (31). Moreover, L3 larvae, maintenance *in vitro*, kept high infective rate to the snail and rat (32). Therefore, we used L3 stage *A. cantonensis*-conditioned culture medium to treat rat RSC96 Schwann cells to investigate the mechanism of MBP alteration *in vitro*. Then, hoping the above finding mechanism will be validated by animal experiments *in vivo*.

Materials and Methods

Chemicals and Reagent

The inhibitor PD98059 (CAS Number: 167869-21-8) and QNZ (CAS Number: 253-82-7) were purchased from Sigma-Aldrich (St. Louis, MO, USA). extracellular signal-regulated kinase (Erk) 1/2 (#9102), phosphorylated (p)-Erk1/2 (#9101), nuclear factor (NF)-κB (p65) (#8242), p-NF-κB (p65) (#3033), MBP (#78896) and β-actin (#4967) were purchased from Cell Signaling Technology (Danvers, MA, USA).

Larval Preparation

The infective larvae (L3) of *A. cantonensis* originally obtained from wild giant African snails (*Achatina fulica*) were purchased from Heping District (Taichung, Taiwan). The larvae within tissues were recovered using a modification of the method of Parsons and Grieve (26). Briefly, the shells of the snails were crushed, the tissues were homogenized and digested in a pepsin–HCl solution (pH 1–2, 500 I.U. pepsin/g tissue), and incubated with agitation in a 37°C water bath for 2 h. Host cellular debris was removed from the digest by centrifugation at 1,400 × *g* for 10 min. The larvae in the sediment were collected by serial washing in double-distilled water and counted under a microscope. The morphological criteria for identification of L3 of *A. cantonensis* was described previously (10). The larvae were then cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS), 4 mM L-glutamate, 1.5 g/l sodium bicarbonate, 4.5 g/l glucose in humidified atmosphere and 1% penicillin/streptomycin in a 5% CO_2 humidified incubator at 37°C. After 72 h of incubation, the supernatant was collected and centrifuged with Amicon Ultra 0.5 ml filters (30K MWCO) (Merck Millipore, Burlington, MA, USA).

Cell Culture

Rat RSC96 Schwann cells were purchased from the Bio-resource Collection and Research Center (BCRC, Hsinchu, Taiwan). Cells were cultured in DMEM supplemented with 10% FBS, 4 mM L-glutamate, 1.5 g/l sodium bicarbonate, 4.5 g/l glucose in humidified atmosphere and 1% penicillin/streptomycin in a 5% CO_2 humidified incubator at 37°C. Cells were cultured in serum-free medium for 4 h and treated with *A. cantonensis*-conditioned culture medium under different conditions.

Treatment of Cells

Based on time-dependent experiments, p-Erk1/2, p-NF-κB and MBP reached relatively high levels in the RSC Schwann cells at 24 h of treatment using the *A. cantonensis*-conditioned medium. Therefore, 24 h

was chosen as a suitable time point to treat the Schwann cells with PD98059 or QNZ. For QNZ treatment, *A. cantonensis*-conditioned medium-treated cells were randomly divided into six groups: control groups, stimulated groups, dimethyl sulfoxide (DMSO) (0.1% DMSO) vehicle control groups, and 5 nM, 10 nM and 20 nM QNZ treatment groups. Additionally, in PD98059 treatment, *A. cantonensis*-conditioned medium-treated cells were randomly divided into six groups: control groups, stimulated groups, vehicle (0.1% DMSO) control groups, 5 μM, 10 μM and 20 μM PD98059 treatment groups. QNZ and PD98059 were added to the cells as DMSO solutions with a final concentration of 0.1% DMSO, as in the DMSO vehicle group samples.

Western Blotting

RSC96 cells were lysed in radioimmunoprecipitation assay (RIPA) buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 2 mM $MgCl_2$, 0.1% sodium dodecyl sulphate (SDS) and 1% Triton X-10) containing the following protease inhibitors: 0.1 mM phenylmethanesulfonylfluoride or phenylmethylsulfonyl fluorid (PMSF), 10 μM sodium orthovanadate, 1 mM NaF and protease inhibitors tablet (Roche, Branchburg, NJ, USA), and centrifuged at 13,000 rpm for 30 min at 4°C and the supernatants were collected. Protein concentrations were determined using the Bradford Assay (Bio-Rad, Hercules, CA, USA), after which the protein samples were separated by 15% SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The resolved protein bands were then transferred to a polyvinylidene fluoride (PVDF) membrane (Merck Millipore). The membrane was blocked with 5% de-fat milk in phosphate buffered saline (PBS) (pH 7.4) and then exposed to the appropriate antibodies (1:1,000). All bands were visualized using horseradish peroxidase-conjugated secondary antibodies (1:10,000) (Santa Cruz Biotechnology, California, CA, USA) using an enhanced chemiluminescence system (Merck Millipore). Western blotting analysis results reported here are representative of at least three independent experiments.

Statistical Analysis

Results obtained from the different groups were compared by use of the nonparametric Kruskale-Wallis test, followed by post-testing using Dunn's multiple comparisons of means. All results are presented as mean ± standard deviation (SD). A *P*-value of less than 0.05 or 0.01 was considered as significant. Statistical analyses were performed using the SigmaPlot 11.0 edition.

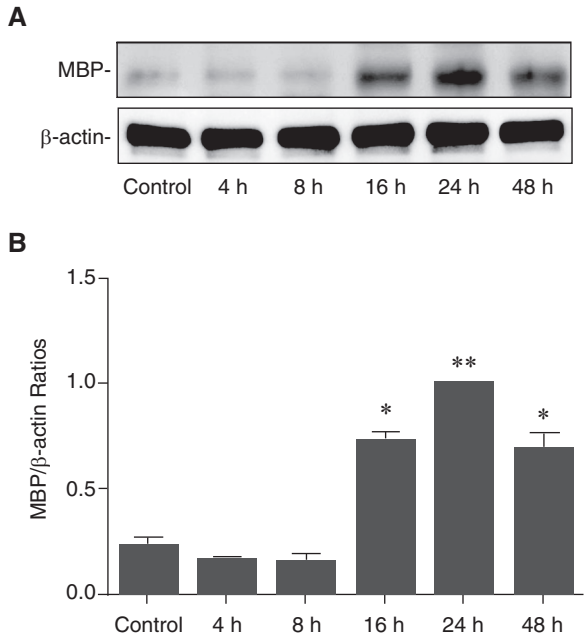

Fig. 1. Protein levels of MBP in RSC96 cells treated with *A. cantonensis*-conditioned medium in western blot analysis. (A) MBP bands were detected at all time points. β-actin was used as a loading control. (B) Quantitative analysis of the MBP bands. The analysis was performed using a computer-assisted imaging densitometer system on cell homogenates. Mean ± SD of three independent experiments in duplicates. **P* < 0.05, ***P* < 0.01 indicate a significant difference as compared with the control groups.

Results

The Levels of MBP on RSC96 Cell Treated with A. Cantonensis-Conditioned Culture Medium

By western blotting analysis, in the cell homogenates, the MBP were detected low levels at 4 h and 8 h as similar to control. Furthermore, the MBP were significantly increased at 16 h, 24 h and 48 h in the cell homogenates. The experiments were repeated three times in different cell homogenates with consistent results (Fig. 1).

Elevated Expression of NF-κB and Erk1/2 in RSC96 Cells Treated with A. Cantonensis-Conditioned Culture Medium

Using western blotting analysis, we performed immunoblots of the RSC96 cell homogenates treated with *A. cantonensis*-conditioned medium. The total NF-κB and Erk1/2 bands were detected in all groups. p-NF-κB and p-Erk1/2 levels significantly increased in the cell homogenates between 8-48 h of treatment compared with the control (Fig. 2).

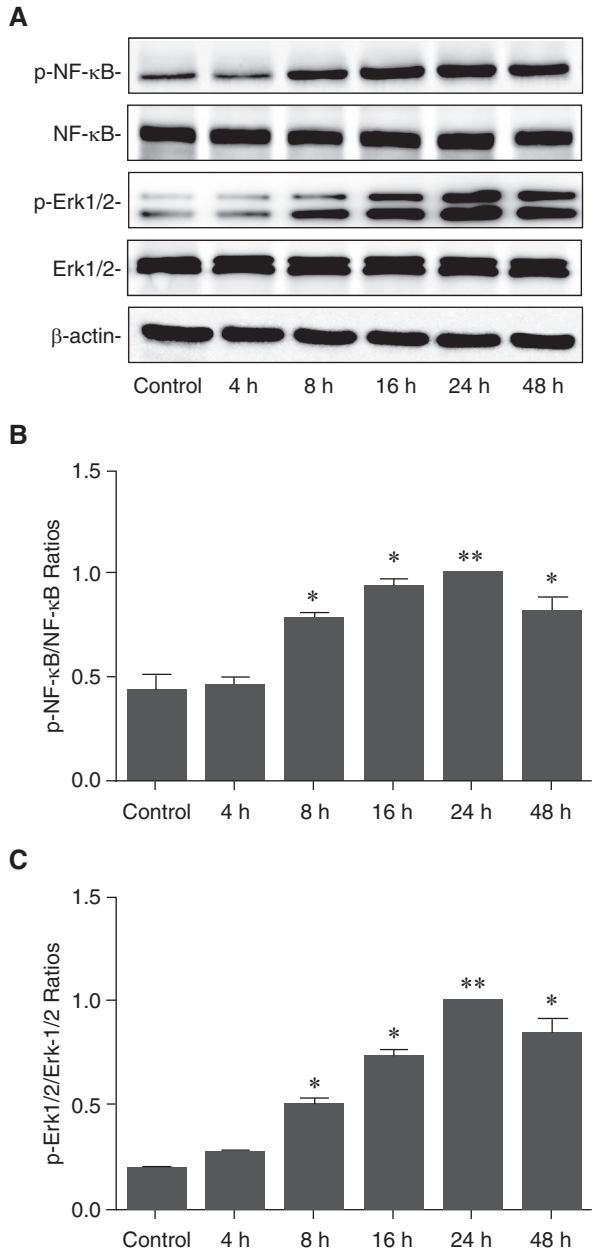

Fig. 2. Protein levels of NF-κB, p-NF-κB, Erk1/2 and p-Erk1/2 in RSC96 cells treated with *A. cantonensis*-cultured medium. (A) p-NF-κB, NF-κB, p-Erk1/2 and Erk1/2 bands were detected at all time points. β-actin was used as a loading control. See legend to Fig. 1 on quantitative analysis and *P* values.

Influence of QNZ Treatment on NF-κB and MBP Levels

NF-κB, p-NF-κB and MBP protein levels in the cell homogenates were also monitored using western blotting analysis. The p-Erk1/2, p-NF-κB and MBP levels reached relatively high levels at 24 h (Figs. 1 & 2). Thus, this time point was used for the following inhibition tests. In the QNZ inhibitory tests, p-NF-κB and MBP levels were significantly increased in the stimulated groups compared to the control groups. p-NF-κB and MBP did not significantly change in the vehicle and the 5 nM QNZ groups. However, marked reductions were observed in the 10 and 20 nM QNZ groups. The total NF-κB bands were detected in all groups. The experiments were repeated three times with similar results (Fig. 3).

Influence of PD98059 Treatment on Erk1/2, NF-κB and MBP Levels

Protein levels of Erk1/2, p-Erk1/2, NF-κB, p-NF-κB and MBP in cell homogenates were assessed using western blotting analysis. In the PD98059 inhibitory tests, p-Erk1/2 and p-NF-κB levels were significantly increased in the stimulated groups compared to the control groups. p-Erk1/2 and p-NF-κB did not significantly change in the vehicle and the 5 μM PD98059 groups. However, marked reductions were observed in the 10 and 20 μM PD98059 groups. The total Erk1/2 and NF-κB bands were detected in all groups. Furthermore, MBP was significantly decreased in the 20 μM PD98059 groups compared to the stimulated groups in three experimental repeats (Fig. 4).

Discussion

Schwann cells are the principal glial cells of the PNS. In peripheral nerves, Schwann cells form myelin, which facilitates rapid conduction of action potentials along axons in the vertebrate nervous system (7). Myelination in the PNS requires close contact between Schwann cells and the axon. In contrast, demyelination impairs nerve conduction, induces inflammatory cascade and causes axonal loss (5, 21). Moreover, demyelination is often accompanied by MBP production: BALB/c mice infected with *A. cantonensis* elicit demyelination, which implicates alterations of myelin proteins, including MBP (17). Interestingly, we likewise found MBP production in RSC96 Schwann cells in response to treatment in *A. cantonensis*-conditioned culture medium in a time-dependent manner.

The low level of NF-κB activation is helpful for Schwann cell myelination (22, 25, 31). Additionally, manipulating the temporal activation of NF-κB in Schwann cells may offer new therapeutic avenues for PNS and CNS regeneration (23). In contrast, overexpression of NF-κB in CNS or PNS may cause cell damage and inflammation (2, 15, 16). Furthermore, MBP expression could be regulated by NF-κB. (11, 26). In order to determine whether MBP was produced *via* NF-κB in RSC96 Schwann cells treated with *A. cantonensis*-conditioned medium, we analyzed the

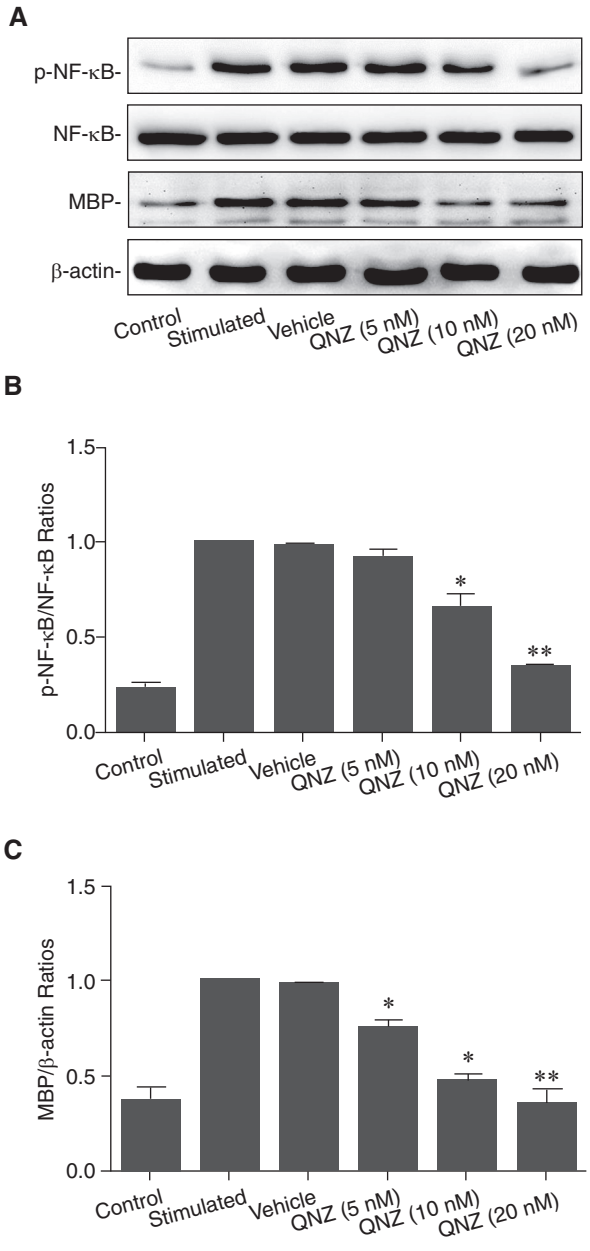

Fig. 3. The effect of QNZ on p-NF-κB, NF-κB and MBP in RSC96 cells treated with *A. cantonensis*-conditioned medium. (A) p-Erk1/2, Erk1/2, p-NF-κB, NF-κB and MBP were detected at all time points. β-actin was used as a loading control. Test groups included untreated cells (Con), RSC96 cells treated with *A. cantonensis*-conditioned medium (stimulated), or with 0.1% DMSO (vehicle), or 5 nM, 10 nM or 20 nM QNZ. See legend to Fig. 1 on quantitative analysis and *P* values.

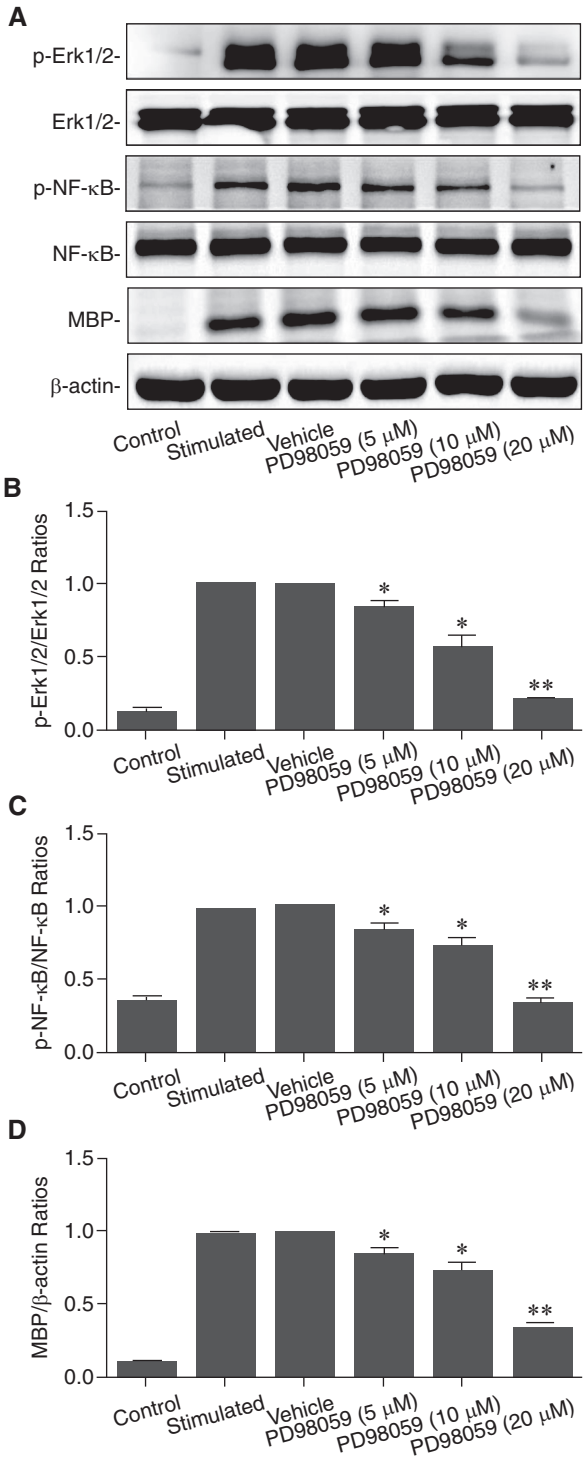

Fig. 4. Effects of PD98059 on Erk1/2, -Erk1/2, NF-κB, p-NF-κB and MBP in RSC96 cells treated with *A. cantonensis*-conditioned medium. (A) p-Erk1/2, Erk1/2, p-NF-κB, NF-κB and MBP were detected at all time points. β-actin was used as a loading control. Test groups included untreated cells (Con), RSC96 cells treated with *A. cantonensis*-conditioned medium (stimulated), or with 0.1% DMSO (vehicle), or 5 μM, 10 μM or 20 μM PD98059. See legend to Fig. 1 on quantitative analysis and *P* values.

NF-κB and MBP expression levels in RSC96 Schwann cells in response to stimulation in *A. cantonensis*-conditioned medium with or without QNZ, an NF-κB inhibitor. Our results showed NF-κB activation in a time-dependent manner, and p-NF-κB and MBP showed decreases in response to QNZ.

Thus, MBP production was regulated by NF-κB in RSC96 Schwann cells treated with *A. cantonensis*-conditioned medium.

Although NF-κB participates in the molecular mechanism of RSC96 Schwann cells in response to stimulation by *A. cantonensis*-conditioned medium, NF-κB may be regulated through different signaling cascades, such as the mitogen-activated protein kinase (MAPK) pathway (12, 13, 20, 30). Human astroglia infected with *Toxoplasma gondii* (20) and murine mast cells in response to *Toxoplasma gondii* infection (29) may activate ERK cascades, leading to downstream increased NF-κB activity. Thus, we analyzed the associations among Erk1/2, NF-κB and MBP in RSC96 Schwann cells in response to *A. cantonensis*-conditioned medium stimulation. Our results showed that Erk1/2 activation and that the Erk1/2, NF-κB and MBP were efficiently reduced by PD98059, an Erk1/2 inhibitor. Thus, we proposed *A. cantonensis*-conditioned medium-induced MBP production might be mediated by the Erk1/2-NF-κB pathways.

We also found that MBP was produced *via* the Erk1/2-NF-κB pathways in Schwann cells treated with *A. cantonensis*-conditioned culture medium. But the nature of the substance of the *A. cantonensis*-conditioned medium that cause this phenomenon was not determined. Further studies are also required to resolve whether MBP production in oligodendroglial cells infected in *A. cantonensis*-conditioned medium is also produced through the Erk1/2-NF-κB pathways. Based on our results presented here and in the previous study of Lin *et al*. (17), we postulate that through blocked Erk1/2-NF-κB activity can improve MBP over-production and RSC96 Schwann cells damage.

Conflict of Interest

The authors have nothing to declare.

References

1. Alto, W. Human infections with Angiostrongylus cantonensis. *Pac. Health Dialog*. 8: 176-182, 2001.
2. Asadikaram, G., Noroozi, S., Ebrahimi Meimand, H.A., Sanjari, M., Zainodini, N., Khoramdelazad, H., Shahrokhi, N. and Kazemi Arababadi, M. Interferon-Ĭbeta 1a modulates expression of RAGE but Not S100A12 and nuclear factor-kappaB in multiple sclerosis patients. *Neuroimmunomodulation* 23: 345-351, 2016.
3. Asipu, A. and Blair, G.E. Regulation of myelin basic protein-encoding gene ranscription in rat oligodendrocytes. *Gene* 150: 227-234, 1994.
4. Deber, C.M. and Reynolds, S.J. Central nervous system myelin: structure, function, and pathology. *Clin. Biochem*. 24: 113-134, 1991.
5. Dutta, R. and Trapp, B.D. Pathogenesis of axonal and neuronal damage in multiple sclerosis. *Neurology* 68 (Suppl 3): S22-S31, 2007.
6. Gendelman, H.E., Pezeshkpour, G.H., Pressman, N.J., Wolinsky, J.S., Quarles, R.H., Dobersen, M.J., Trapp, B.D., Kitt, C.A., Aksamit, A. and Johnson, R.T. A quantitation of myelin-associated glycoprotein and myelin basic protein loss in different demyelinating diseases. *Ann. Neurol*. 18: 324-328, 1985.
7. Glenn, T.D. and Talbot, W.S. Signals regulating myelination in peripheral nerves and the Schwann cell response to injury. *Curr. Opin. Neurobiol*. 23: 1041-1048, 2013.
8. Hawdon, J.M., Jones, B.F., Hoffman, D.R. and Hotez, P.J. Cloning and characterization of Ancylostoma-secreted protein a novel protein associated with the transition to parasitism by infective hookworm larvae. *J. Biol. Chem*. 271: 6672-6678, 1996.
9. Hsu, W.Y., Chen, J.Y., Chien, C.T., Chi, C.S. and Han, N.T. Eosinophilic meningitis caused by Angiostrongylus cantonensis. *Pediatr. Infect. Dis. J*. 9: 443-445, 1990.
10. Hou, R.F., Tu, W.C., Lee, H.H., Chen, K.M., Chou, H.L. and Lai, S.C. Elevation of plasminogen activators in cerebrospinal fluid of mice with eosinophilic meningitis caused by Angiostrongylus cantonensis. *Int. J. Parasitol*. 34: 1355-1364, 2004.
11. Huang, C.J., Nazarian, R., Lee, J., Zhao, P.M., Espinosa-Jeffrey, A. and de Vellis, J. Tumor necrosis factor modulates transcription of myelin basic protein gene through nuclear factor kappa B in a human oligodendroglioma cell line. *Int. J. Dev. Neurosci*. 20: 289-296, 2002.
12. Ju, C.H., Chockalingam, A. and Leifer, C.A. Early response of mucosal epithelial cells during *Toxoplasma gondii* infection. *J. Immunol*. 183: 7420-7427, 2009.
13. Ju, S.M., Song, H.Y., Lee, J.A., Lee, S.J., Choi, S.Y. and Park, J. Extracellular HIV-1 Tat up-regulates expression of matrix metalloproteinase-9 *via* a MAPK-NF-kappaB dependent pathway in human astrocytes. *Exp. Mol. Med*. 41: 86-93, 2009.
14. Kepes, J.J. Large focal tumor-like demyelinating lesions of the brain: intermediate entity between multiple sclerosis and acute disseminated encephalomyelitis? A study of 31 patients. *Ann. Neurol*. 33: 18-27, 1993.
15. Kirkley, K.S., Popichak, K.A., Afzali, M.F., Legare, M.E. and Tjalkens, R.B. Microglia amplify inflammatory activation of astrocytes in manganese neurotoxicity. *J. Neuroinflammation* 14: 99, 2017.
16. Leonoudakis, D., Rane, A., Angeli, S., Lithgow, G.J., Andersen, J.K. and Chinta, S.J. Anti-Inflammatory and neuroprotective role of natural product securinine in activated glial cells: implications for Parkinson's disease. *Mediators Inflamm*. 2017: 8302636, 2017.
17. Lin, K.Y., Chen, K.M., Lan, K.P., Lee, H.H. and Lai, S.C. Alterations of myelin proteins in inflammatory demyelination of BALB/c mice caused by Angiostrongylus cantonensis. *Vet. Parasitol*. 171: 74-80, 2010.
18. Lin, K.Y. and Lai, S.C. Induction of 2',3'-cyclic nucleotide 3'-phosphodiesterase in demyelination of BALB/c mice caused by Angiostrongylus cantonensis. *J. Comp. Pathol*. 141: 248-253, 2009.
19. Lubetzki, C. and Stankoff, B. Demyelination in multiple sclerosis. *Handb. Clin. Neurol*. 122: 89-99, 2014.
20. Lu, C.Y. and Lai, S.C. Induction of matrix metalloproteinase-2 and -9 *via* Erk1/2-NF-kappaB pathway in human astroglia infected with *Toxoplasma gondii*. *Acta Trop*. 127: 14-20, 2013.
21. Matthieu, J.M. An introduction to the molecular basis of inherited myelin diseases. *J. Inherit. Metab. Dis*. 16: 724-732, 1993.
22. Morton, P.D., Dellarole, A., Theus, M.H., Walters, W.M., Berge, S.S. and Bethea, J.R. Activation of NF-kappaB in Schwann cells is dispensable for myelination *in vivo*. *J. Neurosci*. 33: 9932-9936, 2013.
23. Morton, P.D., Johnstone, J.T., Ramos, A.Y., Liebl, D.J., Bunge, M.B. and Bethea, J.R. Nuclear factor-kappaB activation in Schwann cells regulates regeneration and remyelination. *Glia* 60: 639-650, 2012.
24. Mikoshiba, K., Okano, H., Tamura, T. and Ikenaka, K. Structure and function of myelin protein genes. *Annu. Rev. Neurosci*. 14: 201-217, 1991.

25. Nickols, J.C., Valentine, W., Kanwal, S. and Carter, B.D. Activation of the transcription factor NF-kappaB in Schwann cells is required for peripheral myelin formation. *Nat. Neurosci*. 6: 161-167, 2003.
26. Paez, P.M., Garcia, C.I. and Pasquini, J.M. Expression of myelin basic protein in two oligodendroglial cell lines is modulated by apotransferrin through different transcription factors. *J. Neurosci. Res*. 83: 606-618, 2006.
27. Parsons, J.C. and Grieve, R.B. Effect of egg dosage and host genotype on liver trapping in murine larval toxocariasis. *J. Parasitol*. 76: 53-58, 1990.
28. Raine, C.S. Morphology of myelin and myelination. In P. Morell (ed.), Myelin, 2nd ed. New York: Plenum. pp. 1-41, 1984.
29. Wang, M.F., Lu, C.Y. and Lai, S.C. Up-regulation of matrix metalloproteinases-2 and -9 *via* an Erk1/2/NF-kappaB pathway in murine mast cells infected with *Toxoplasma gondii*. *J. Comp. Pathol*. 149: 146-155, 2013.
30. Wu, C.Y., Hsieh, H.L., Jou, M.J. and Yang, C.M. Involvement of p42/p44 MAPK, p38 MAPK, JNK and nuclear factor-kappa B in interleukin-1beta-induced matrix metalloproteinase-9 expression in rat brain astrocytes. *J. Neurochem*. 90: 1477-1488, 2004.
31. Xie, H., Yuan, D., Luo, S., Zeng, X., Zeng, X., He, P., Lv, Z. and Wu, Z. Angiostrongylus cantonensis: an optimized cultivation of this parasitic nematode under laboratory conditions. *Parasitol. Res*. 16: 2231-2237, 2017.
32. Yang, X., Li, Z., He, H., Cheng, M., Liu, Q., Zhang, D., Chen, J., Wu, X., He, A., Zheng, X., Wu, Y., Wu, Z. and Zhan, X. Molecular cloning, expression, and characterization of a putative activation-associated secreted protein from *Angiostrongylus cantonensis*. *Parasitol. Res*. 112: 781-788, 2013.
33. Yoon, C., Korade, Z. and Carter, B.D. Protein kinase A-induced phosphorylation of the p65 subunit of nuclear factor-kappaB promotes Schwann cell differentiation into a myelinating phenotype. *J. Neurosci*. 28: 3738-3746, 2008.