CENTRAL ASIAN JOURNAL of MEDICAL SCIENCES
CAJMS

Original Article

Cent Asian J Med Sci. 2021 March;7(1):3-7 https://doi.org/10.24079/CAJMS.2021.03.002

Dehydrocostus Lactone Inhibits the Expression of Inducible *Slfn-4* in LPS-Activated Microglia

Zhao Fuquan[1,4], Shiirevnyamba Avirmed[2], Bilegtsaikhan Tsolmon[3], Anujin Batbold[2], Seesregdorj Surenjid[1]

[1]Department of Mongolian Medicine Study, International School of Mongolian Medicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia; [2]Division of Graduate Educational Policy and Management, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia; [3]Department of Administration and Human Resources, Second State Central Hospital, Ulaanbaatar, Mongolia; [4]Department of Neuropathology, Hospital of Inner Mongolian University for the Nationalities, Tongliao, China

Submitted: November 18, 2020
Revised: December 5, 2020
Accepted: January 6, 2021

Corresponding Author
Shiirevnyamba Avirmed, MD, PhD
Division of Graduate Educational Policy and Management,
Mongolian National University of Medical Sciences,
Ulaanbaatar 14210, Mongolia
Tel: +976-9400-0269
E-mail: shiirevnyamba@mnums.edu.mn

Objectives: Dehydrocostus lactone (DDL) natural product of Eerdun Wurile that has been reported to have anti-inflammatory, anticancer, and anti-proliferation effect. However, no research has been done regarding mechanism of DDL on microglial cells in the brain. Moreover, *Schlafen (Slfn)* genes are considered to play important regulatory roles for normal cell growth and differentiation of the immune and hematopoietic systems. Among schlafen genes, *Slfn-4* is much modulated during macrophage stimulation and differentiation and implicates macrophage in responses to pathogens. The present study was conducted to investigate effects of DDL on *Slfn-4* gene expression in lipopolysaccharide (LPS)-stimulated microglial cells. **Methods:** The anti-inflammatory effects of DDL were studied using LPS-stimulated murine BV2 microglia. BV2 were cultured in DMEM then 4μM DDL were added. Then BV2 was treated with 1 ng/ml LPS for 24 hours to stimulate. **Results:** LPS alone increased *Slfn-4* mRNA expression in BV2 microglial cells ($p < 0.01$). In contrast, DDL pre-treatment significantly inhibited LPS-induced *Slfn-4* mRNA expression ($p < 0.05$). **Conclusions:** DDL inhibits the expression of *Slfn-4* gene transcripts related with inflammation, activated by LPS in the microglia cells.

Keywords: Microglia, Dehydrocostus Lactone, Lipopolysaccharide, qRT-PCR

Introduction

Neuroinflammation is a common pathological feature and a reaction infection, brain injury and neurodegenerative diseases. Microglia are the resident macrophages in the brain and trigger the primary host defense for detecting foreign pathogens and neuronal injures in the central nervous system [1]. When neuroinflammation is initiated, microglia are activated and alter morphology, migrate and produce cytotoxic and cytotropic immune mediators [2-6]. But prolonged activation of microglia could lead to neuronal injury by releasing variety of cytotoxic molecules such as proinflammatory mediators, reactive oxygen intermediates, nitric oxide, nerve growth factor and chemotactic cytokines [7]. Such studies have been done using the BV2 mice microglial cell line activated by lipopolysaccharides (LPS). They are found in outer cell membrane of Gram-negative bacteria and are potent activators of the inflammatory response [8-9]. LPS causes activation of microglia during infection and this effect is useful study the effects of various treatments on microglial gene expression.

Schlafen (Slfn) genes are considered to play important regulatory roles during normal cell growth and differentiation of the immune, hematopoietic and other systems. The *Slfn* genes are mainly detected in immune system cells and differentially regulated during cells development [10-12]. In addition to regulation in cell development, *Slfn* genes' expression increases after viral and bacterial infections and *Slfn* family members become involved in macrophage stimulation against pathogens [12-15]. Significantly, *Slfn-4* is modulated during macrophage stimulation and differentiation implicating its role in the macrophage response to pathogens [12]. Attenuating this activation seems to have a neuroprotective effect following neuronal injury and therefore is a target for therapeutic intervention.

Dehydrocostus lactone (DDL) is one of the major components of the dried root of saussurea Lappa Clarke which has been used in Asian traditional medicine. Moreover, DDL has been used as a key component of Eerdun Wurile, a Mongolian medicine which is regularly utilized to treat neurological diseases [16-17]. DDL has been reported to have anticancer, anti-proliferation and anti-inflammatory activity by inducing cells to undergo cell cycle arrest, apoptosis, differentiation and inhibition of overproduction of inflammatory mediators [18-21]. However, the action of the DDL on reducing neural inflammation has not been fully elucidated.

Lee et al. in 1999 studied effect of DDL on TNF-alpha in LPS activated macrophage [22]. Other researchers reported the neuro anti-inflammatory effect of cryptolepine on LPS activated microglia [23]. However, we are not aware of any studies of DDL's effect on *Slfn-4* gene expression in LPS activated microglia. Such research is fundamental to discover Eerdun Wurile's neuroinflammatory effect and perhaps lead to its use as a treatment to prevent neuroinflammatory diseases in the future. The present study was conducted to investigate effects of DDL on *Slfn-4* gene expression in LPS-stimulated mice microglial cells.

Materials and Methods

Study design

This is experimental and *in vitro* study using cell cultures from at least three independent experiments in 3 different study groups. The anti-inflammatory effects of DDL were studied using LPS-stimulated murine BV2 microglia. BV2 were cultured in DMEM then 4μM DDL were added in the medium for 30 minutes. Then BV2 was treated with 1 ng/ml LPS for 24 hours to stimulate. DDL anti-inflammation group (BV2+DDL+LPS), LPS-activated group (BV2+LPS) and control group (only BV2) were analyzed.

Reagents

LPS and DDL were purchased from Sigma-Aldrich Co. LL (Darmstadt, Germany). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco (Thermo Fisher Scientific, USA). RNAiso Plus were obtained from Takara Biomedical Technology Co., Ltd., (Beijing, China) and a QIAGEN RNeasy Mini kit were obtained from QIAGEN (Hilden, Germany). Oligo dT and Super-Script III RT were purchased from Invitrogen (Carlsbad, CA, USA) and SYBR Green from Applied Biosystems (Foster City, CA, USA).

Cell culture and treatment

BV2 microglial cells were obtained from ATCC (Manassas, VA, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified incubator with 5% CO_2. Cells were cultured in 75 cm^2 cell culture flask (Corning, Action, MA, USA) and were split twice a week. For the experiments, cells were plated on 5×10^5 cells/35 mm dishes and cultured for 24 hours before treatments.

The BV2 cells cultured in three different conditions, respectively. 1) BV2 cells were cultured only with medium, and the second, the cells were pretreated with DDL at 4μM for 30 minutes respectively. 2) BV2 cells were treated with 1 ng/ml LPS for 24 hours to activate. 3) BV2 cells were treated only with 1 ng/ml LPS for 24 hours to activate.

RNA isolation and quantification real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted using RNAiso Plus and a QIAGEN RNeasy Mini kit according to the manufacturer's instruction. The expression levels of *Slfn-4* and other mRNA in BV2 cells were assessed by quantitative real-time PCR. In brief, total RNA from each sample was reverse transcribed using oligo dT and Super-Script III RT. qRT-PCR was conducted ABI 7500 sequence detection system (Applied Biosystems, Foster City, CA, USA) using SYBR Green. The sequence-specific primers used to detect expression of the *Schlafen-4* were forward primer 5'-GCCCTCTGTTCAAGTCAAGTGTCC-3', reverse primer 5'-CCAGATGAAATCCTTTCCACGA-3'.

Statistical analysis

The data for each group were expressed as mean ± standard deviation. Because of the small sample sizes, examination for differences among the three groups was conducted using the Kruskal-Wallis test with a critical p-value of $p < 0.05$. Multiple post-hoc comparisons were performed using the Wilcoxon sign rank test. The critical p-value for the post-hoc tests was adjusted using the Bonferroni method. Since there were 3 groups, there were 3 possible pairwise comparisons. Consequently, the critical p-value for the post-hoc tests was $p < 0.05/6 = 0.016$. All analyses were performed using SPSS 25.0.

Ethical statement

The study was approved by the Research Ethics Committee of Mongolian National University of Medical Sciences in 18th January, 2019 (No.2018/3-01).

Results

First, the effect of DDL on LPS-induced *Slfn-4* mRNA expression was examined by qRT-PCR in BV2 microglial cells (Table 1). BV2 cells were pre-treated with or without DDL at 4 μM for 0.5 hour and then stimulated with LPS at 1 ng/ml for 24 hours. We found that treatment with LPS markedly increased *Slfn-4* mRNA expression (2.078 ± 0.223) in BV2 microglial cells, compared to control conditions ($p < 0.05$) (Table 1). In contrast, pre-treatment of DDL significantly down regulated LPS-induced *Slfn-4* mRNA expression ($p < 0.05$).

Table 1. *Slfn-4* mRNA expression in the microglial cells in three different conditions.

Sample	N	Mean ± SD	95% CI	*p-value
BV2 cell	3	1.0003 ± 0.037	0.909 - 1.091	0.000
BV2 cell + LPS(1ug/ml)	3	2.078 ± 0.223	1.525 - 2.631	
BV2 cell + DDL(4uM) + LPS(1ug/ml)	3	1.284 ± 0.108	1.016 - 1.552	

*Kruskal-Wallis test with $p < 0.05$; [a]pairwise comparison using Wilcoxon test with $p < 0.016$.

As shown in Figure 1, we found that treatment with DDL significantly suppressed the LPS-stimulated *Slfn* mRNA expression level in the BV2 microglial cells ($p < 0.05$) (Figure 1). Those data suggesting that DDL inhibits LPS-induced *Slfn-4* gene expression in in vitro microglia cell line.

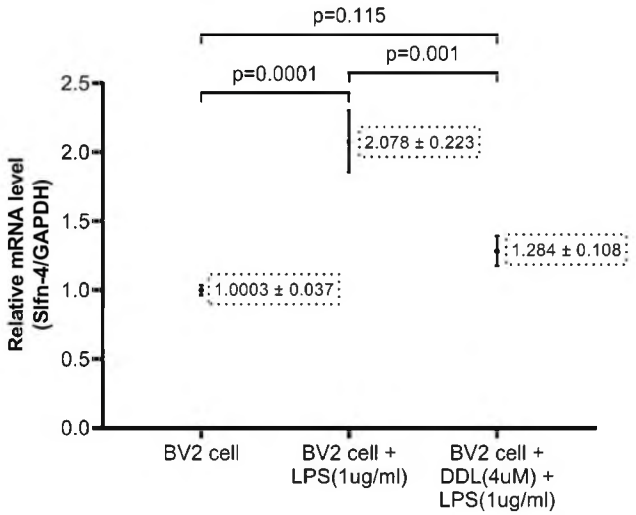

Figure 1. The effect of DDL treatment with LPS-stimulated *Slfn-4* mRNA expression levels in the BV2 microglial cells. The expression of mRNA was determined by RT-PCR. GAPDH gene used to internal control in the assay. Pairwise comparison using Tukey hsd test: p = 0.0001, p = 0.001, and p = 0.115.

Discussion

The *Slfn* genes include mouse and human genome and play important regulatory roles in normal development, regulation of immune response and control of virus replication. The highest induction was observed in the expression *Slfn-4* among *Slfn* family genes [24].

In the present study, we examined the effect of pretreatment with DDL on expression of *Slfn-4* mRNA levels in BV2 microglia that were subsequently activated with LPS. Our study showed mRNA levels of *Slfn-4* were up-regulated during macrophage activation but down-regulated during CSF-1-mediated macrophage differentiation [12]. The present study demonstrates that DDL pretreatment results in a significant decrease of *Slfn-4* mRNA levels in LPS-stimulated BV2 microglia.

Microglia are resident immune cells of the central nervous system (CNS) and play important functional roles in maintaining CNS homeostasis and in recovery from brain injury. Based on their physiological functions, Microglia are classified into pro- (M1) and anti-inflammatory (M2) phenotype [25].

The M1 phenotype is stimulated by LPS or interferon (IFN)-γ and promotes the transcriptional activation of nuclear factor-kB (NF-kB) and produces excessive pro-inflammatory cytokines and oxidative metabolites such as IL-1β, IL-6, TNF-α, chemokines, nitric oxide and reactive oxygen species (ROS) [26-28].

Moreover, increased pro-inflammatory cytokine expressions from activated microglia worsen ischemia-like injury to components of the blood-brain-barrier [29].

M2 phenotype is induced by stimulation of anti-inflammatory cytokines such as IL-4 or IL-1, and it inhibits neuroinflammation and promote tissue regeneration and wound healing [27-31].

We also used this model in this study, and our results show that LPS induced microglial activation (Figure 1). DDL is a natural product and a major sesquiterpene lactone, extracted from the root of Saussurealappa, a well-known Asian traditional Medicine. DDL exhibits various biological activities, including anti-inflammatory, immunomodulatory, anti-ulcer and anti-tumor properties effects in some neurodegenerative disorders and tumors. Moreover, clinically available drugs such as Compound Ancklandia and Berberine tablets which contain DDL, have been used for the treatment of digestive tract diseases with their anti-inflammatory, anti-microbial activities [13, 31].

Previous studies have shown that DDL inhibits cell growth by generating cells to undergo cycle arrest, apoptosis, migration and differentiation [31]. Previous studies have shown that DDL crossed blood-brain barrier and inhibited neoplastic weight and volume through inhibition of the IKKβ/NF-kβ/COX-2 signaling pathway [29, 31].

DDL inhibits activation of NF-kappaB by decreasing TNF-alpha level and suppress the production of nitric oxide by repressing inducible nitric oxide synthase enzyme expression in LPS-activated macrophage cells. Moreover, DDL acts as a promoter of apoptosis by increasing activation of caspase-3 and caspase-8 [17, 31].

In recent years, there is extensive interest by researchers to understand DDL's mechanism of action because of its potential anti-inflammatory and immunomodulatory activities in disorders of CNS. However, the effects of DDL in microglia cells and its mechanisms of action have yet been fully understood.

The applicability of our in vitro finding to animals and humans is unknown, and the DDL's safety profile is unclear. Therefore, further studies are needed. Moreover, other gene expressions will be investigated.

Conclusion

DDL inhibits the expression of *Slfn-4* gene transcripts related with inflammation, activated by LPS in the microglia cells.

References

1. Graeber MB. Changing face of microglia. Science 2010; 330: 783-8.
2. Dheen ST, Kaur C, Ling EA. Microglial activation and its implications in the brain diseases. Curr Med Chem 2007; 14: 1189-97.
3. Tuppo EE, Arias HR. The role of inflammation in Alzheimer's disease. Int J Biochem Cell Biol 2005; 37: 289–305.
4. Banati RB, Gehrmann J, Schubert P, Kreutzberg GW. Cytotoxicity of microglia. Glia 1993; 7: 111–8.
5. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 2005; 308: 1314-8.
6. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 2007; 8: 57–69.
7. Hughes V. Microglia: the constant gardeners. Nature 2012;

485: 570-2.

8. Gaowa S, Bao N, Da M, Qiburi Q, Ganbold T, Chen L, et al. Traditional Mongolian medicine eerdunwurile improves stroke recovery through regulation of gene expression in rat brain. J Ethnopharmacol 2018; 222: 249-6.
9. Lee HJ, Kim NY, Jang Mk, Son HJ, Kim KM, Sonh DH, et al. A Sesquiterpens, dehydrocostuslactone, inhibits the expression of inducible nitric oxide synthases and TNF-alpha in LPS-activated macrophages. J Planta Med 1999; 65: 104-8.
10. Duan JA, Hou P, Tang Y, Liu P, Su S. A new sesquiterpene and other constituents from Saussurealappa root. Nat Prod Commun 2010; 5: 1531–4.
11. Sun CM, Syu WJ, Don MJ, Lu JJ, Lee GH. Cytotoxic sesquiterpene lactones from the root of Saussurealappa. J Nat Prod 2003; 66: 1175–80.
12. Zhang S, Won YK, Ong CN, Shen HM. Anti-cancer potential of sesquiterpene lactones: bioactivity and molecular mechanisms. Curr Med Chem Anticancer Agents 2005; 5: 239–49.
13. Cai H, Qin X, Yang C. Dehydrocostus lactone suppresses proliferation of human chronic myeloid leukemia cells through Bcr/Abi-JAK/STAT signaling pathways. J Cell Biochem 2017; 118: 3381-90.
14. Schwarz DA, Katayama CD, Hedrick SM. Schlafen, a new family of growth regulatory genes that affect thymocyte development. Immunity 1998; 9: 657–68.
15. Huang YH, Li D, Winoto A, Robey EA. Distinct transcriptional programs in thymocytes responding to T cell receptor, notch, and positive selection signals. Proc Natl Acad Sci USA 2004; 101: 4936–41.
16. Wendy J. Zuylen V, Garceau V, Idris A. Schroder K, Katharine M, et al. Macrophage activation and differentiation signals regulate schlafen-4 gene expression: evidence for Schlafen-4 as a modulator of myelopoiesis. PLoS ON 2011; 6: e15723. doi:10.1371/journal.pone.0015723.
17. Geserick P, Kaiser F, Klemm U, Kaufmann SHE, Zerrahn J. Modulation of T cell development and activation by novel members of the schlafen (slfn) gene family harbouring an RNA helicase-like motif. Int Immunol 2004; 16: 1535–48.
18. Caron J, Lariviere L, Nacache M, Tam M, Stevenson MM. Influence of Slc11a1 on the outcome of Salmonella enterica serovar enteritidis infection in mice is associated with Th polarization. Infect Immun 2006; 74: 2787–802.
19. Schurr JR, Young E, Byrne P, Steele C, Shellito JE. Central role of toll-like receptor 4 signaling and host defense in experimental pneumonia caused by Gram-negative bacteria. Infect Immun 2005; 73: 532–45.
20. Tang Y, Le M. Differential roles of m1 and m2 microglia in neurodegenerative diseases Mol Neurobiol 2016; 53: 1181-94.
21. Kawabori M, Yenari MA. The role of the microglia in acute cns injury. Metab Brain Dis 2015; 30: 381-92
22. Cherry JD, Olschowka JA, O'Banion MK. Neuroinflammation and m2 microglia: the good, the bad, and the inflamed. J Neuroinflammation 2014; 11: 98-101.
23. Loane DJ, Kumar A. Microglia in the TBI brain: The good, the bad, and the dysregulated. Exp Neurol 2016; 275: 316-27.
24. Yenari MA, Xu L, Tang XN, Qiao Y, Giffard RG. Microglia potentiate damage to blood-brain barrier constituents: improvement by minocycline in vivo and in vitro. Stroke 2006; 37: 1087-93.
25. Nguyen KD, Qiu Y, Cui X, Goh YP. Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. Nature 2011; 480: 104-8.
26. Nair MG, Guild KJ, Artis D. Novel effector molecules in type 2 inflammmation: lessons drawn from helminth infection and allergy. J Immunol 2006; 177: 1393-9.
27. Colton CA. Heterogeneity of microglial activation in the innate immune response in the brain. J Neuroimmune Pharmacol 2009; 4: 399-418.
28. Kim WK, Jang PG, Woo MS. A new anti-inflammatory agent KL-1037 represses pro-inflammatory cytokine and inducible nitric oxide synthase (iNOS) gene expression in activated microglia. Neuropharmacology 2004; 47: 243-52.
29. Wang J, Yu Z, Wang C, Tian X, Huo X, Wang Y, et al. Dehydrocostus lactone, a natural sesquiterpene lactone, suppresses the biological characteristics of glioma, through inhibition of the NF-kappaB/COX-2 signaling pathway by targeting IKKbeta. Am J Cancer Res 2017; 7: 1270-84.
30. Lin X, Peng Z, Su C. Potential anti-cancer activities and mechanisms of costunolide and dehydrocostuslactone. Int J Mol Sci 2015; 16: 10888-906.
31. Kumar M, Hughes L,Nerurkar VR. Expression and regulatory effects of Schlafen genes in flavivirus infection. J Immunol 2016; 196: 217-28.