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Nordihydroguaiaretic acid inhibits IFN-γ-induced STAT tyrosine phosphorylation in rat brain astrocytes

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Abstract

The Janus kinase (JAK) and signal transducers and activators of transcription (STAT) signal cascades are major pathways that mediate the inflammatory functions of interferon-γ (IFN-γ), an important pro-inflammatory cytokine. Therefore, regulation of JAK/STAT signaling should modulate IFN-γ-mediated inflammation. In this study, we found that nordihydroguaiaretic acid (NDGA), a well-known lipoxygenase (LO) inhibitor, suppressed IFN-γ-induced inflammatory responses in brain astrocytes. In the presence of NDGA, interferon regulatory factor-1 expression was significantly reduced. Expression of monocyte chemotactic protein-1 and interferon-gamma inducible protein-10 mRNA in response to IFN-γ was significantly suppressed in the presence of NDGA, as was tyrosine-phosphorylation of JAK and STAT. However, the 5-LO products, leukotriene B_4 (LTB_4) and leukotriene C_4, were not detected in cells treated with IFN-γ, indicating that the effect of NDGA seemed to be independent of 5-LO inhibition. In addition, two other 5-LO inhibitors (Rev5901 and AA861) did not mimic the effect of NDGA, and the 5-LO metabolites, 5-hydroxyeicosatetraenoic acid and LTB_4, were unable to reverse NDGA-driven suppression of STAT activation or affect basal STAT phosphorylation. Taken together, these results suggest that NDGA regulates IFN-γ-mediated inflammation through mechanisms unrelated to the inhibition of 5-LO.

Keywords: Inflammation; Interferon-γ; 5-Lipoxygenase; STAT; NDGA

Interferon-γ (IFN-γ) plays a key regulatory role in inflammation as a pro-inflammatory cytokine. The signal transduction pathways involved in the action of IFN-γ have been well studied, from receptor binding to subsequent activation of gene transcription. IFN-γ binding to the receptor triggers the phosphorylation and activation of a tyrosine kinase, Janus kinase (JAK), which then phosphorylates signal transducer and activator of transcription (STAT). Phosphorylated STAT forms active homodimers that move into the nucleus, bind to IFN-γ-activated sites (GAS) [1], and induce transcription of IFN-γ-responsive genes [2,3] such as interferon regulatory factor-1 (IRF-1), interferon-gamma inducible protein-10 (IP-10), macrophage chemoattractant protein 1 (MCP-1), intracellular adhesion molecule-1 (ICAM-1), inducible nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α), and class II major histocompatibility complex (MHC) molecules [4–8]. Therefore, the JAK/STAT signaling pathway appears to be an ideal target for regulation of IFN-γ-mediated inflammation [9,10].

In the brain, astrocytes and microglia participate in inflammation, where they induce expression of pro-in-

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flammatory mediators following injury. These pro-inflammatory mediators directly damage brain cells and/or recruit immune cells from the surrounding tissues and blood, as shown by observations of microglia migration from surrounding tissues into the damaged area and infiltration of T cells from blood after brain trauma and ischemia [11,12]. Astrocytes and microglia can communicate with T cells through the expression of MHC molecules. The activated T cells release IFN-γ, which may help sustain the activation of astrocytes and microglia [13].

Leukotrienes are emerging as critical mediators of inflammatory responses in allergy, asthma, and arthritis [14]. Recently, it was reported that leukotrienes also have important roles in brain inflammatory reactions and formation of cerebral edema [15]. 5-LO is the key enzyme that metabolizes arachidonic acid and produces leukotrienes [16].

In this study, we revealed that nordihydroguaiaretic acid (NDGA), a well-known inhibitor of 5-LO, reduced IFN-γ-induced inflammatory responses in cultured rat brain astrocytes. NDGA treatment also reduced IFN-γ-induced JAK/STAT phosphorylation and expression of inflammatory mediators such as IRF-1 and IP-10. However, the inhibitory effect of NDGA seems to be independent of 5-LO inhibition. These results suggest that NDGA inhibits inflammation through separately blocking JAK/STAT activation and 5-LO.

Materials and methods

Reagents. Rat IFN-γ was purchased from Calbiochem (Los Angeles, CA, USA). NDGA, baicalein, and AA861 were purchased from Biomol (Plymouth Meeting, PA, USA). 5(*S*)-HETE and leukotriene B_4 (LTB_4) were purchased from Cayman Chemical (Ann Arbor, MI, USA), as was the LTB_4 and LTC_4 Assay kit. Dulbecco's modified Eagle's medium (DMEM) and minimal essential medium (MEM) were purchased from Hyclone (Logan, UT, USA) and Sigma (St. Louis, MO, USA), respectively. Antibodies against STAT1, phosphorylated STAT1 and STAT3 were purchased from Cell Signaling Technology (Beverly, MA, USA). The anti-phosphorylated JAK2 antibody was purchased from Upstate Biotechnology (Charlottesville, VA, USA). Peroxidase-conjugated secondary antibodies were purchased from Vector Lab (Burlingame, CA, USA), and the enhanced chemiluminescence (ECL) system was purchased from Sigma. RT-PCR primers were purchased from Bioneer (Seoul, Korea). RNAzol B and Avian Myeloblastosis Virus reverse transcriptase were purchased from TEL-TEST (Friendswood, TX, USA) and Promega (Madison, WI, USA), respectively.

Cell culture. Primary astrocytes and microglia were cultured from the cerebral cortices of 1- to 3-day-old Sprague–Dawley rats as previously described [17,18]. Briefly, the cortices were triturated into single cells in MEM containing 10% FBS and plated into 75 cm^2 T-flasks (0.5 hemisphere/flask) for 2–3 weeks. Microglia were detached from the flasks by mild shaking and filtered through a nylon mesh to remove astrocytes and cell aggregates. Microglia were seeded in dishes, washed 1 h later to remove unattached cells, and attached cells were used in experiments. Astrocytes remaining in the flask were harvested with 0.1% trypsin. Purified astrocytes were plated in dishes and cultured in MEM supplemented with 10% FBS. C6 rat astroglioma cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, CCL-107). Cells were grown in DMEM supplemented with 10% (v/v) FBS. BV2 murine microglial cells were cultured in Dulbecco's modified Eagle's medium containing 5% fetal bovine serum.

Reverse transcription and polymerase chain reaction (RT-PCR). Total RNA was extracted using RNAzol B according to the manufacturer's instructions, and cDNA was prepared with Avian Myeloblastosis Virus reverse transcriptase. PCR was performed with 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 90 s, using the following primers: (R) 5′-ATG CAG GTC TCT GTC ACG ACT-3′, (F) 5′-CTA GTT CTC TGT CAT ACT GG-3′ for MCP-1; (R) 5′-GCT GAA GAG ATT TGT ACC T-3′, (F) 5′-TCC AGA AGC ACC ATG AAC C-3′ for IP-10; and (R) 5′-AGA TCC ACA ACG GAT ACA TT-3′, (F) 5′-TCC CTC AAG ATT CTC AGC AA-3′ for GAPDH.

Western blot analysis. Cells were washed twice with cold phosphate-buffered saline, and then lysed in ice-cold modified RIPA buffer (50 mM Tris–HCl, pH 7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM Na_3VO_4, and 1 mM NaF) containing protease inhibitors (2 mM phenylmethylsulfonyl fluoride, 100 μg/ml leupeptin, 10 μg/ml pepstatin, 1 μg/ml aprotinin, and 2 mM EDTA). The lysate was centrifuged for 10 min at 12,000*g* at 4 °C and the supernatant was collected. Proteins were separated by SDS–PAGE and transferred to a nitrocellulose membrane. The membrane was incubated with primary antibodies, reacted with peroxidase-conjugated secondary antibodies, and then visualized using an enhanced chemiluminescence system.

LTB_4 and LTC_4 assay. LTB_4 (LTC_4) was measured by enzyme immunoassay (EIA) based on the competition between eicosanoid and an eicosanoid conjugate (tracer) for a limited amount of specific antibody. Briefly, 50 μl of conditioned culture media was mixed with 50 μl of eicosanoid tracer and 50 μl of specific antiserum, in 96-well plates that were coated with mouse monoclonal anti-rabbit antibody for LTB_4 (LTC_4) and pre-washed with specific buffer. After 18 h of incubation at 4 °C, the plates were washed to remove unbound reagents, and the enzyme substrates (Ellman's reagent) were added for 60–90 min at room temperature. The optical density of the samples was determined spectrophotometrically at 405 nm.

Results

Nordihydroguaiaretic acid suppresses IFN-γ-induced inflammatory responses in brain glial cells

Since IFN-γ is a prominent inflammatory cytokine, researchers are currently seeking methods for therapeutically regulating IFN-γ-mediated inflammation. Here, we show that NDGA, a well-known inhibitor of 5-lipoxygenase (LO), significantly suppressed IFN-γ-induced inflammatory responses in brain glial cells. STAT is a major pathway that mediates inflammatory functions of IFN-γ, so we first analyzed the effect of NDGA on IFN-γ-induced STAT activation by Western blotting with phospho-specific antibodies. In the range of 5–20 μM, NDGA dose-dependently suppressed IFN-γ-induced tyrosine phosphorylation of STAT1 and STAT3 in primary cultured astrocytes (Fig. 1A). The suppressive effect of NDGA on STAT1 phosphorylation was detected throughout the tested time period of 5–90 min (Fig. 1B). The inhibitory effect

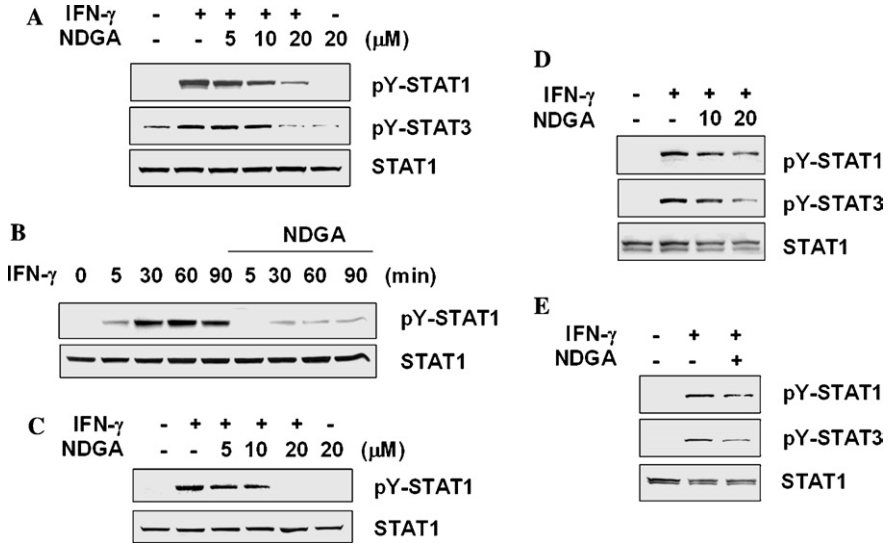

Fig. 1. NDGA reduces IFN-γ-induced phosphorylation of STAT1 and STAT3 in brain glial cells. Cell lysates were separated by SDS-PAGE and Western blot analysis was performed using antibodies against phospho-Tyr-STAT1 (pY-STAT1) and phospho-Tyr-STAT3 (pY-STAT) as described in Materials and methods. The membrane was stripped and analyzed sequentially with anti-STAT1 antibodies. (A) Primary cultured astrocytes were treated with the indicated doses of NDGA for 15 min, and then 10 U/ml IFN-γ was added to cells for 30 min. (B) Primary astrocytes were treated with NDGA for 15 min, and then 10 U/ml IFN-γ was added for the indicated times. (C) C6 astroglioma cells, (D) primary cultured microglia, or (E) BV2 murine microglial cells were treated with NDGA for 15 min, and then 10 U/ml IFN-γ for 30 min.

of NDGA was also observed in C6 rat astroglioma cells (Fig. 1C). In addition, in primary cultured microglia and BV2 murine microglial cells, NDGA produced similar inhibitory effect (Figs. 1D and E). These results suggest that the inhibitory effect of NDGA on IFN-γ-induced STAT phosphorylation could be a general phenomenon rather than being confined to specific cell types.

As JAK is an upstream signaling molecule of STAT, we next examined the effect of NDGA on JAK activation. In the presence of 20 μM NDGA, IFN-γ induced tyrosine phosphorylation of JAK2 was significantly reduced in both primary cultured astrocytes and C6 cells (Figs. 2A and B). Therefore, the target(s) of NDGA in the IFN-γ signaling pathway appear to be upstream of JAK phosphorylation.

We further examined whether treatment with NDGA could suppress expression of inflammatory mediators in glial cells treated with IFN-γ. When primary astrocytes were treated with IFN-γ, IRF-1 protein expression was detected within 90 min. In the presence of NDGA, IRF-1 protein expression levels were significantly reduced (Fig. 3A left). IP-10 mRNA expression in response to IFN-γ was also significantly suppressed in the presence of NDGA (Fig. 3A, right). Similar results were obtained in C6 cells, where expression of both IRF-1 and IP-10 was reduced in the presence of NDGA (Fig. 3B, left and right). These results suggest that NDGA suppresses IFN-γ-induced inflammatory responses such as JAK/STAT activation and expression of inflammatory mediators.

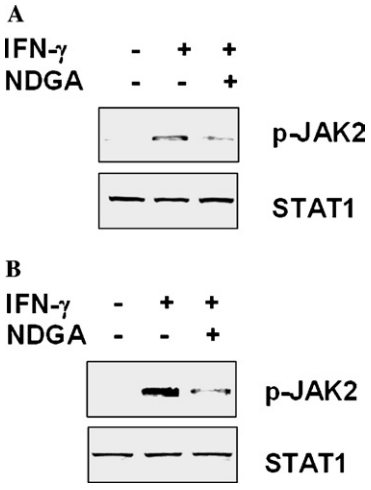

Fig. 2. NDGA reduces IFN-γ-induced activation of JAK2 in brain glial cells. (A) Primary astrocytes and (B) C6 cells were treated with NDGA for 15 min and then with 10 U/ml IFN-γ for 30 min. Western blot analysis was performed using antibodies against phospho-JAK2 (p-JAK2). The membrane was stripped and analyzed sequentially with anti-STAT1 antibodies.

NDGA suppresses IFN-γ-induced STAT phosphorylation independently of lipoxygenase inhibition

We next examined whether IFN-γ activates 5-LO, which catalyzes the oxygenation of arachidonic acid to 5(*S*)-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (5-HpETE) and subsequently to 5(*S*)-hydroxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (5-HETE). The

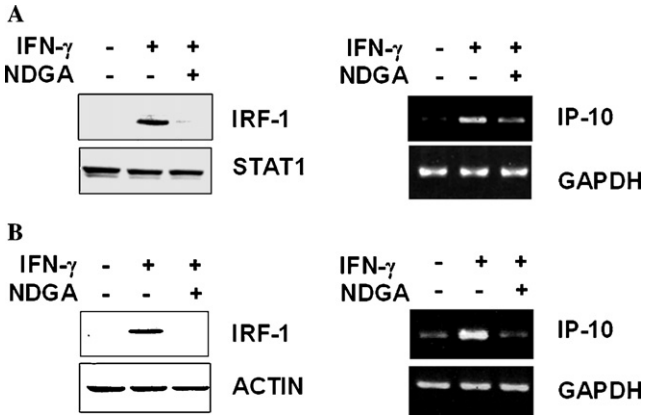

Fig. 3. NDGA reduces IFN-γ-induced IRF-1 and IP-10 expression in brain glial cells. (A) Primary astrocytes and (B) C6 cells were treated with the indicated doses of NDGA for 15 min, and then with 10 U/ml IFN-γ for 90 min and 3 h for Western blot and RT-PCR, respectively. Western blot analysis was performed using anti-IRF-1 antibodies. The membrane was stripped and analyzed sequentially with anti-STAT1 and anti-ACTIN antibodies. IP-10 and GAPDH transcripts were determined by RT-PCR, as described in Materials and methods.

latter compound is converted into leukotriene A_4, which is the precursor of the biologically active LTB_4 and the cysteinyl leukotrienes (LTC_4, LTD_4, and LTE_4) [14]. Therefore, we assayed the levels of LTB_4 and LTC_4 released into the culture media after IFN-γ treatment, as a measure of 5-LO activity. Unexpectedly, we found that IFN-γ treatment did not affect the production of either LTB_4 or LTC_4, whereas A23187 (calcium ionophore) induced production of LTB_4 (Figs. 4A and B).

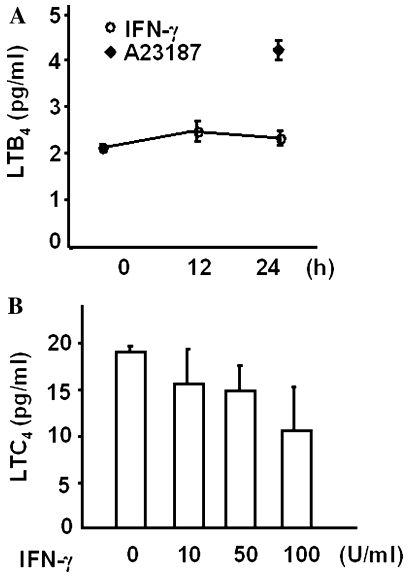

Fig. 4. IFN-γ treatment does not produce LTB_4 or LTC_4. (A) C6 cells were treated with 10 U/ml IFN-γ for the indicated times. A23187 (1 μM) was used as the positive control. LTB_4 was measured in cell-free supernatants by EIA. (B) C6 cells were treated with indicated amount of IFN-γ for 24 h. LTC_4 was measured in cell-free supernatants by EIA. Each value represents the means ± SEM of three samples.

Next, we examined two other 5-LO inhibitors, Rev5901 and AA861 [19,20], suppressed IFN-γ-induced STAT phosphorylation. Unlike our results with NDGA, we found that neither Rev5901 (IC_{50} for 5-LO = 6 μM) nor AA861 (IC_{50} for 5-LO = 0.8 μM) suppressed IFN-γ-induced STAT1 and STAT3 phosphorylation across concentration ranges of 30–100 μM and 5–20 μM, respectively (Figs. 5A and B). Since NDGA also inhibits 12-LO, we also tested the effect of a 12-LO inhibitor, baicalein [21], but found that 2–10 μM baicalein (IC_{50} for 12-LO = 0.12 μM) had no effect on IFN-γ-induced STAT phosphorylation (Fig. 5C). In addition, we found that treatment of 5-LO metabolites such as 5-HETE and LTB_4 [22,23] did not reverse the inhibitory effect of NDGA on STAT phosphorylation nor did it alter basal or IFN-γ-induced STAT1 phosphorylation (Figs. 6A and B). Taken together, these results suggest that NDGA suppresses IFN-γ-induced STAT phosphorylation independent of its ability to inhibit 5-LO enzyme activity.

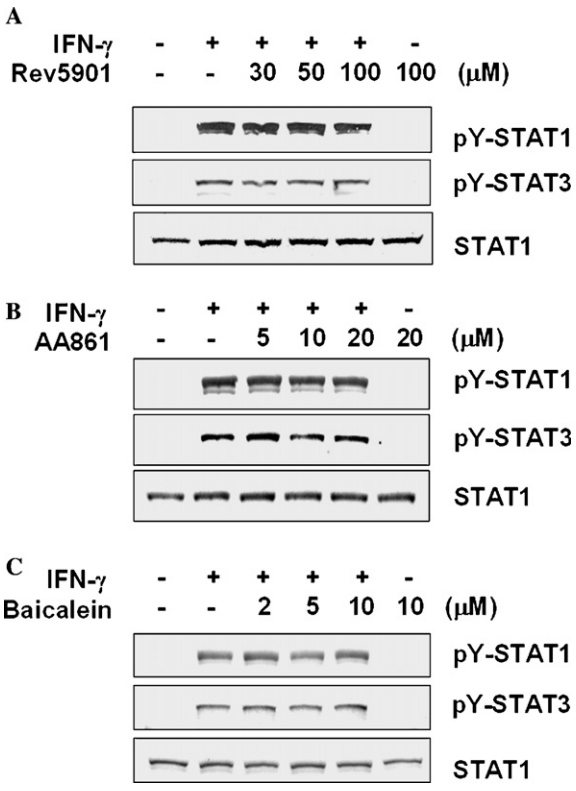

Fig. 5. Rev5901, AA861, and baicalein have no effect on IFN-γ-induced STAT phosphorylation. C6 cells were treated with the indicated doses of Rev5901 (A), AA861 (B), or baicalein (C) for 15 min followed by treatment with 10 U/ml IFN-γ for 30 min. Western blot analyses were then performed using antibodies against pY-STAT1, pY-STAT3, and STAT1.

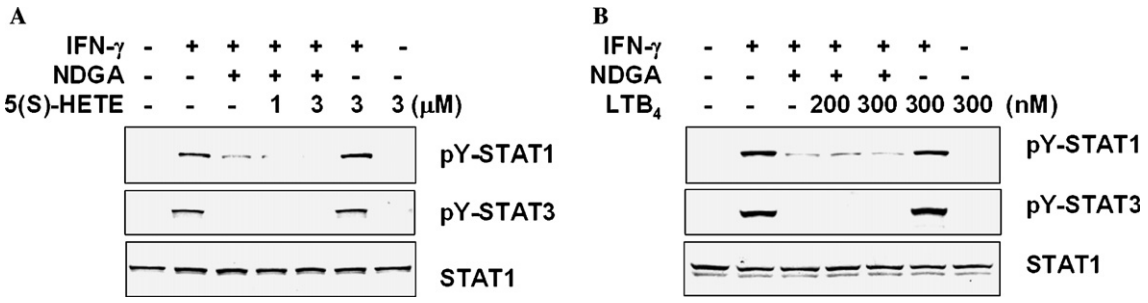

Fig. 6. Treatment with the 5-LO metabolites, 5(*S*)-HETE, and LTB_4, does not reverse the inhibitory effect of NDGA. C6 cells were treated with NDGA (20 μM) for 15 min, and then treated with 5(*S*)-HETE (A), LTB_4 (B), and IFN-γ (10 U/ml) as indicated. After 30 min, cell lysates were separated by SDS–PAGE, and Western blot analysis was performed using pY-STAT1 and pY-STAT3 antibodies. The membrane was stripped and analyzed sequentially with anti-STAT1 antibody.

Discussion

Leukotrienes such as LTB_4 and the cysteinyl leukotrienes are important mediators of inflammation in allergy, asthma, arthritis, and even in injured brain [14]. 5-LO is a key enzyme that catalyzes the production of leukotrienes from arachidonic acid [16]. Although IFN-γ is a major pro-inflammatory cytokine, little information is available as to whether 5-LO could be involved in the pro-inflammatory function of IFN-γ, particularly in brain glial cells. Activation of 5-LO depends on Ca^{2+}, p38 MAPK, and/or rac [24–27]. It has been reported that IFN-γ increases intracellular calcium [28] and activates p38 MAPK [29], and we recently found that IFN-γ activates rac in astrocytes [30]. The lines of evidence led us to examine the possibility of crosstalk between IFN-γ and 5-LO.

We found that the well-known 5-LO inhibitor, NDGA, suppressed IFN-γ-induced inflammatory responses, JAK/STAT activation, and inflammatory mediators. However, two other specific 5-LO inhibitors, AA-861 and Rev5901, did not mimic the effect of NDGA in glial cells. Although NDGA inhibits 12-LO (with a slightly lower affinity than that for 5-LO) [31], the 12-LO inhibitor baicalein had no effect on IFN-γ-induced STAT activation. These results suggest that NDGA suppresses IFN-γ-induced inflammatory responses independent of its effects on 5-LO and/or 12-LO. The 5-LO-independent effect of NDGA was further supported by our observation that levels of LTB_4 and LTC_4, two final products of 5-LO, were not induced by IFN-γ treatment of glial cells. In addition, treatment with the 5-LO metabolites 5-HETE and LTB_4 had no effect on STAT activation in the presence or absence of IFN-γ and/or NDGA. As it was recently reported that NDGA inhibits nitric oxide production from TNF-α-treated microglia through 5-LO-independent mechanisms [32], we also examined the effects of inhibitors of phospholipase A_2 (mepacrine) and cyclooxygenase (indomethacin and SC560), which are involved in arachidonic acid metabolism along with lipoxygenase. Unlike the case of NDGA, none of these enzyme inhibitors suppressed IFN-γ-induced STAT1 or -3 phosphorylation (data not shown). These results suggest that the inhibitory effect of NDGA on IFN-γ-signaling could be unrelated to arachidonic acid metabolism. Although further work will be required to fully examine how NDGA suppresses IFN-γ-induced inflammatory responses, our work and previous studies may suggest some possibilities. One possibility is that NDGA might inhibit tyrosine kinase activity, as suggested by West et al. [32]. IFN-γ binding to the receptor triggers the tyrosine phosphorylation and activation of JAK (a tyrosine kinase), which in turn induces tyrosine phosphorylation of STAT. Therefore, NDGA could suppress tyrosine phosphorylation of JAK and STAT through the inhibition of tyrosine kinase activity. Another possibility is that NDGA could suppress IFN-γ-induced inflammatory responses through its antioxidant effects, as NDGA has antioxidant effects and antioxidants have been shown to inhibit IFN-γ function [33,34]. Therefore, the exact target(s) of NDGA in IFN-γ signaling should be further studied in the future.

In sum, 5-LO has emerged as a new therapeutic target of inflammatory diseases such as asthma, inflammatory bowl disease, rheumatoid arthritis, and even neurodegenerative diseases. The results in this study showed that the 5-LO inhibitor, NDGA, suppresses the inflammatory responses of IFN-γ in astrocytes. Therefore, NDGA or NDGA-like drugs could be potent and effective candidate therapeutics for novel treatments of inflammatory or neurodegenerative diseases.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2005.01.025.

References

[1] T. Decker, P. Kovarik, A. Meinke, J. Interferon Cytokine Res. 17 (1997) 121–134.
[2] T.H. Wu, C.N. Pabin, Z. Qin, T. Blankenstein, M. Philip, J. Dignam, K. Schreiber, H. Schreiber, J. Immunol. 172 (2004) 3243–3251.
[3] B. Raju, Y. Hoshino, K. Kuwabara, I. Belitskaya, S. Prabhakar, A. Canova, J.A. Gold, R. Condos, R.I. Pine, S. Brown, W.N. Rom, M.D. Weiden, Infect. Immun. 72 (2004) 1275–1283.
[4] P. Vanguri, J. Neuroimmunol. 56 (1995) 35–43.
[5] E.M. Frohman, T.C. Frohman, M.L. Dustin, B. Vayuvegula, B. Choi, A. Gupta, S. van den Noort, S. Gupta, J. Neuroimmunol. 23 (1989) 117–124.
[6] S. Majumder, L.Z. Zhou, P. Chaturvedi, G. Babcock, S. Aras, R.M. Ransohoff, J. Neurosci. Res. 54 (1998) 169–180.
[7] S. Fujigaki, M. Takemura, H. Hamakawa, M. Seishima, K. Saito, Adv. Exp. Med. Biol. 527 (2003) 97–103.
[8] Y. Xu, L. Wang, G. Buttice, P.K. Sengupta, B.D. Smith, J. Biol. Chem. 279 (2004) 41319–41332.
[9] X. Hu, C. Herrero, W.P. Li, T.T. Antoniv, E. Falck-Pedersen, A.E. Koch, J.M. Woods, G.K. Haines, L.B. Ivashkiv, Nat. Immunol. 9 (2002) 859–866.
[10] P. Dell'Albani, R. Santangelo, L. Torrisi, V.G. Nicoletti, J. de Vellis, A.M. Giuffrida Stella, J. Neurosci. Res. 65 (2001) 417–424.
[11] F.C. Barone, D.B. Schmidt, L.M. Hillegass, W.J. Price, R.F. White, G.Z. Feuerstein, R.K. Clark, E.V. Lee, D.E. Griswold, H.M. Sarau, Stroke 23 (1992) 1337–1347.
[12] S. Jander, M. Kraemer, M. Schroeter, O.W. Witte, G. Stoll, J. Cereb. Blood Flow Metab. 15 (1995) 42–52.
[13] D. Mason, F. Powrie, Curr. Opin. Immunol. 10 (1998) 649–655.
[14] B. Samuelsson, Science 220 (1983) 568–575.
[15] A. Di Gennaro, C. Carnini, C. Buccellati, R. Ballerio, S. Zarini, F. Fumagalli, S. Viappiani, L. Librizzi, A. Hernandez, R.C. Murphy, G. Constantin, M. De Curtis, G. Folco, A. Sala, FASEB J. 7 (2004) 842–844.
[16] M. Luo, S.M. Jones, M. Peters-Golden, T.G. Brock, Proc. Natl. Acad. Sci. USA 100 (2003) 12165–12170.
[17] D. Giulian, T.J. Baker, L.C. Shih, L.B. Lachman, J. Exp. Med. 164 (1986) 594–604.
[18] H. Pyo, I. Jou, S. Jung, S. Hong, E.H. Joe, Neuroreport 9 (1998) 871–874.
[19] E.J. Kusner, R.L. Marks, D. Aharony, R.D. Krell, Biochem. Pharmacol. 38 (1989) 4183–4189.
[20] Y. Ashida, T. Saijo, H. Kuriki, H. Makino, S. Terao, Y. Maki, Prostaglandins 26 (1983) 955–972.
[21] A. Lebeau, F. Esclaire, W. Rostene, D. Pelaprat, Neuroreport 12 (2001) 2199–2202.
[22] S. Zarini, R.C. Murphy, J. Biol. Chem. 278 (2003) 11190–11196.
[23] A.W. Ford-Hutchinson, Cancer Metastasis Rev. 13 (1994) 257–267.
[24] O. Werz, Curr. Drug Targets Inflamm. Allergy 1 (2002) 23–44.
[25] W.C. Chang, A.B. Parekh, J. Biol. Chem. 279 (2004) 29994–29999.
[26] O. Werz, J. Klemm, O. Radmark, B. Samuelsson, J. Leukoc. Biol. 70 (2001) 830–838.
[27] Y.W. Eom, S.H. Cho, J.S. Hwang, S.B. Yoon, D.S. Na, I.J. Kang, S.S. Kang, W.K. Song, J.H. Kim, Biochem. Biophys. Res. Commun. 284 (2001) 126–132.
[28] S. Franciosi, H.B. Choi, S.U. Kim, J.G. McLarnon, J. Neurosci. Res. 1 (2002) 607–613.
[29] J. Lee, J.S. Shin, J.Y. Park, D. Kwon, S.J. Choi, S.J. Kim, I.H. Choi, J. Neurosci. Res. 74 (2003) 884–890.
[30] E.J. Park, K.A. Ji, S.B. Jeon, W.H. Choi, I.O. Han, H.J. You, J.H. Kim, I. Jou, E.H. Joe, J. Immunol. 173 (2004) 5697–5703.
[31] L. Levine, Biochem. Pharmacol. 32 (1983) 3023–3026.
[32] M. West, M. Mhatre, A. Ceballos, R.A. Floyd, P. Grammas, S.P. Gabbita, L. Hamdheydari, T. Mai, S. Mou, Q.N. Pye, C. Stewart, S. West, K.S. Williamson, F. Zemlan, K. Hensley, J. Neurochem. 91 (2004) 133–143.
[33] M. Anjaneyulu, K. Chopra, Pharmacology 72 (2004) 42–50.
[34] Y.X. Xu, K.R. Pindolia, N. Janakiraman, C.J. Noth, R.A. Chapman, S.C. Gautam, Exp. Hematol. 25 (1997) 413–422.