ORIGINAL RESEARCH

Guggulipid and Nimesulide Differentially Regulated Inflammatory Genes mRNA Expressions via Inhibition of NF-kB and CHOP Activation in LPS-Stimulated Rat Astrocytoma Cells, C6

Rituraj Niranjan · Chandishwar Nath · Rakesh Shukla

Abstract Neuroinflammation is an integral part of neurodegenerative diseases. Lipo-polysacharide (LPS) induces reactive astrogliosis, the cellular manifestation of neuroinflammation, in various models of neurological diseases, but its mechanism of action is still not properly known. The effect of guggulipid and nimesulide on LPS-induced neuroinflammatory changes is also not properly understood. This work demonstrated the mechanism of actions of guggulipid and nimesulide on inflammatory genes expressions in LPS-stimulated rat astrocytoma cells, C6. We observed that LPS (10 µg/ml) treatment of rat astrocytoma cells, C6, for 24 h significantly increased intracellular Ca^{2+} ion and expression of inducible nitric oxide synthase (iNOS), nuclear factor kappa-B (NF-kB), C/EBP homologous protein 10 (CHOP), c-fos, and c-jun proteins. At transcriptional stage, LPS upregulated mRNA levels of cyclooxygenase-2 and IL-6 with downregulation in IL-1α, IL-1β, and microsomal prostaglandin E synthase-1 (mPGES-1) through activating NF-kB translocation. Treatment with guggulipid reversed these LPS-induced changes in rat astrocytoma cells. Treatment with nimesulide also attenuated LPS-induced Ca^{2+} ion, iNOS, NF-kB, and c-fos expressions, but does not significantly influence CHOP, c-jun protein expressions, and mRNA levels of IL-6, IL-1α, IL-1β, and mPGES-1 genes. In conclusion, our findings elucidated the molecular mechanism of neuroinflammation in response to LPS and its modulation by guggulipid and nimesulide in rat astrocytoma cells (C6), which suggest the use of these drugs in the treatment of neuroinflammation-associated disorders.

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Abbreviations

mPGES-1	Microsomal prostaglandin E synthase-1
LPS	Lipo-polysacharide
TNF-α	Tumor necrosis factor-α
CHOP	C/EBP homologous protein 10
NF-kB	Nuclear factor kappa-B
COX-2	Cyclooxygenase-2
GFAP	Glial fibrillary acidic protein
GL	Guggulipid
iNOS	Inducible nitric oxide synthase
IL-1α	Interleukin-1α
IL-1β	Interleukin-1β
IL-6	Interleukin-6
GAPDH	Glyceraldehydes-phosphate dehydrogenase
Ne	Nimesulide

Introduction

Neuroinflammation is an integral part in the pathophysiology of neurodegenerative diseases and other CNS disorders. In neuroinflammation-activated astroglial glial cells release, an array of inflammatory mediators (proinflammatory cytokines, nitrite, prostaglandins, etc.) that contributes to neuronal demise (Hull et al. 2000; Minagar et al. 2002). The important intracellular events that maybe associated to the glial cells activation are the upregulation of Ca^{++} ions concentration, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expressions, and activation of transcription factor nuclear factor kappa-B (NF-kB). Moreover, the expressions of C/EBP

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homologous protein 10 (CHOP), c-fos, and c-jun proteins may also be upregulated resulting in the modulation of proinflammatory cytokines genes and other inflammatory genes expressions (Butterfield 2003). Therefore, the intracellular molecules of neuroinflammatory cascade may be the potential target(s) to prevent the proinflammatory cytokines- and prostaglandins-mediated neurodegeneration.

Guggulipid is a standardized guggul ethyl acetate extract developed by CDRI as anti-hyperlipidemic agent from the resin of *Commiphora wightii* (Pratap et al. 2005). Importantly, guggulipid has been shown as a potent memory enhancer and antioxidant in the streptozotocin-induced mouse model of dementia (Saxena et al. 2007). Guggulsterones, the active components of guggulipid, were found to inhibit NF-kB activation supporting its anti-inflammatory activity (Shishodia and Aggarwal 2004). Nimesulide, a standardized anti-inflammatory drug, also showed beneficial effect in rat model of ischemia (Candelario-Jalil et al. 2005). Recently, we have also reported that guggulipid and nimesulide have attenuated inflammatory mediator release and glial fibrillary acidic protein (GFAP) expression in lipo-polysacharide (LPS)-stimulated C6 rat astrocytoma cells C6 (Niranjan et al. 2010). Although these studies show the potent inflammatory actions of guggulipid and nimesulide, their effect on LPS-induced intracellular mechanism of actions on astrocytes was not properly understood.

Therefore, this study was undertaken to investigate the effects of guggulipid and nimesulide on neuroinflammatory events associated to the Ca^{++} ion level; mRNA expressions of inflammatory genes (interleukin-1β [IL-1β], interleukin-6 [IL-6], interleukin-1α [IL-1α], COX-2, and microsomal prostaglandin E synthase-1 [mPGES-1]); protein expressions of iNOS, NF-kB, CHOP, c-fos, and c-jun genes; and NF-kB translocation in stimulated rat astrocytoma cell line, C6.

Materials and Methods

Materials

Primary antibodies, rabbit polyclonal anti-iNOS, anti-NF-kB, anti-CHOP, anti-β-actin, anti-c-fos, anti-c-jun secondary goat anti-rabbit HRP-conjugated, and secondary goat anti-rabbit fluorescent-conjugated (Alexa-fluor 546) antibodies were purchased from Santa Cruz Biotechnology (USA). RT–PCR (5-prime two steps) kit was purchased from Eppendorf (India), and primers specific for mPGES-1, COX-2, glyceraldehydes-phosphate dehydrogenase (GAPDH), IL-1α, IL-1β, and IL-6 were purchased from Metabion International AG (Germany). Guggulipid was obtained from Medicinal Chemistry division of Central

Drug Research Institute (CDRI), Lucknow, India. Other chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA).

LPS was initially dissolved in sterile phosphate-buffered saline (PBS) (stock) and subsequent dilutions were made in the medium. Guggulipid was dissolved (0.5%) in methyl cellulose and subsequent dilutions were made in medium as per the required concentrations. Stock solution of nimesulide (Sigma–Aldrich) was dissolved in the ethanol and subsequent dilutions were made in the medium. Final concentration of ethanol in the medium was always kept less than 0.01% in all the treatments (Decker et al. 2009; Niranjan et al. 2010).

Cell Culture and Treatments

C6 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) nutrient mixture medium supplemented with 10% heat-inactivated fetal bovine serum at 37°C in a humidified atmosphere of 5% CO_2/95% air. Cells were incubated with LPS in the absence or presence of guggulipid (in µg/ml: 0.78, 1.56, 3.12, and 6.25) or nimesulide (in µg/ml: 0.75 and 1.5) for 24 h. Measurement for Ca^{++} ion level; protein expressions of iNOS, c-fos, and c-jun; NF-kB and CHOP; and mRNA levels of IL-1α, IL-1β, IL-6, COX-2, and mPGES-1 were done in respective groups. Selection of the doses on different parameter was based on our previous study (Niranjan et al. 2010) in which guggulipid (in µg/ml: 3.12 and 6.25) or nimesulide (in µg/ml: 0.75 and 1.5) has shown significant effect on the neuroinflammatory parameters except cytokine where only single highest dose was used.

Estimation of Intracellular Calcium (Ca^{++}) Ion by FURA-2AM

Intracellular calcium level was measured by fluorescence probe FURA-2 AM following the method of Grynkiewicz et al. (1985). In brief, C6 cells were seeded at the cell density of 1,00,000 cells/ml in six-well plates. After the treatment period, medium was aspirated and cells were then incubated with the HBSS buffer (137 mM NaCl, 5.4 mM KCl, 0.49 mM $MgCl_2$, 0.44 mM KH_2PO4, 0.64 mM Na_2HPO_4, 3 mM $NaHCO_3$, 5.5 mM glucose, 1.26 mM $CaCl_2$, and 20 mM HEPES) for 1 h at 37°C in incubator containing 5 µM FURA-2AM as a final concentration. Fluorescence was read using a spectrofluorometer (using Cary Eclipse software, VARIAN optical spectroscopy instruments, Australia) at the dual wave length ex = 340/380 nm and em = 510 nm. Calcium level was calculated and calcium influx was expressed as percent increase in basal level.

Immunocytochemistry for Expressional Analysis of c-fos, c-jun, NF-kB (P-65), and CHOP Proteins and NF-kB Translocation

C6 cells (1×10^4/well) were seeded in 96-well plates for expressional studies and treated for 24 h by separate sets of guggulipid and nimesulide in the presence and absence of LPS. For NF-kB translocation, C6 cells were seeded at the cell density of 2×10^5/well in six-well plate containing cover slips at their bottom and treatment was given for 24 h. After treatment period, cells were treated with 0.05% H_2O_2 in methanol for 1 h at room temperature in dark with mild agitation to make them permeable. Cells were treated with blocking buffer (0.02% BSA + 0.002% triton-x 100) for 30 min at room temperature. Cells were then treated with the primary antibody of c-fos, c-jun, NF-kB, and CHOP proteins at 1:100 dilutions in blocking buffer for 1 h with mild agitation. Cells were again treated with secondary antibody HRP-conjugated for expression studies or Alexa-flour-546 antibodies for translocation, at 1:200 dilutions in blocking buffer for 1 h with mild agitation. Cells were washed with PBS at each step. For expression analysis, color was developed by diaminobenzidine (DAB)-enhanced liquid substrate system kit (provided by Sigma) for 20 min. Images were captured by inverted phase contrast and up right fluorescent microscope and expression analysis was done by the Leica Q-win soft ware (Leica Microsystems, Milton Keynes, UK).

Western Blotting for iNOS Protein

Cell lysate of C6 cells after incubation with LPS in the presence or absence of guggulipid or nimesulide were prepared in 200 µl of the lysis buffer, containing 100 mM NaCl, 50 mM Tris–HCL, 1 mM EDTA, 1 µg/ml aprotenin, 100 µg/ml phenylmethylsulfonyle fluoride, and 10 µg/ml pepstatin. Cell lysate was then centrifuged at 10,000 g for 5 min at 4°C. Protein estimation in supernatant was done by Follin Lowry method. Samples were mixed with the 3X loading buffer containing 100 mM Tris–HCl (pH = 6.8), 200 mM dithiothritol, 4% sodiumdodecyl sulfate, 0.2% bromophenol blue, and 20% glycerol and were boiled for 5 min at 100°C. A 100 µg of protein for iNOS was separated on 8–12% SDS–PAGE and transferred to the Polyvinylidene Fluoride (PVDF) membrane. Membrane was blocked by blocking buffer (5% non-fat dry milk, 10 mM Tris pH = 7.5, 100 mM NaCl, and 0.1% tween-20) over night at 4°C. Membrane was treated with primary anti-iNOS antibodies at 1:1,000 dilutions at room temperature for a period of 2 h. A dilution of 1:2,000 was used for anti-beta actin antibodies. After washing, membranes were again treated with HRP-conjugated secondary antibodies in a 1:2,000 dilution for 1 h at room temperature. Blots were then developed by the enhanced chemiluminescence system provided by the Amersham Biosciences. Densitometry analyses of bands were accomplished by the Alpha Image gel documentation system (Alpha Innotech, USA).

RT–PCR for Transcriptional Analysis of IL-1β, IL-1α, IL-6, COX-2, and mPGES-1 Genes

RNA was isolated by tri-reagent (Sigma–Aldrich). RNA (2 µg) was quantitated spectrophotometrically and was reverse transcribed using oligo-(dT) primers by kit 5-prime (manual master script kit & manual master script RT–PCR system) according to the manufacturer's protocol (eppendroff). Equal amount of cDNA was subjected to subsequent PCR analysis in a total volume of 50 µl containing 0.5 µM of primers specific for concerned gene. Detail of primers is given in (Table 1). PCR was performed at following conditions (1) 5 min at 94°C; (2) 45 s at 94°C, 45 s at 60°C for GAPDH or 65°C for mPGES-1 or 55°C for

Table 1 Details of PCR conditions used

Probe	Cycles	Orientation	Sequence	Length (bp)	*T*-an °C
GAPDH	30	Forward	5′-TGAAGGTCGGTGTGAACGGATTTGGC-3′	983	68
		Reverse	5′-CATGTAGGCCATGAGGTCCACCAC-3′		
IL-1β	35	Forward	5′-CACCTCTCAAGCAGAGCACAG-3′	79	58
		Reverse	5′-GGGTTCCATGGTGAAGTCAAC-3′		
IL-1α	35	Forward	5′-AAGACAAGCCTGTGTTGCTGAAGG-3′	85	65
		Reverse	5′-TCCCAGAAGAAAATGAGGTCGGTC-3′		
IL-6	35	Forward	5′-TCCTACCCCAACTTCCAATGCTC-3′	79	65
		Reverse	5′- TTGGATGGTCTTGGTCCTTAGCC-3′		
COX-2	35	Forward	5′-TGCGATGCTCTTCCGAGCTGTGCT-3′	479	55
		Reverse	5′-TCAGGAAGTTCCTTATTTCCTTTC-3′		
mPGES-1		Forward	5′-ATGACTTCCCTGGGTTTGGTGATGGAG-3′	459	65
		Reverse	5′-ACAGATGGTGGGCCACTTCCCAGA-3′		

COX-2 or 58°C for IL-1β or 65°C for IL-6 and IL-1α, 45 s at 72°C for 35 cycles to all except GAPDH (30 cycles); and (3) 10 min at 70°C. Controls included RNA subjected to the RT–PCR procedure without addition of reverse transcriptase, and PCR performed in the absence of cDNA that always yielded negative results.

Statistical Analysis

All data are expressed as means ± SEM and are representative of an average of at least three separate experiments. Statistical analysis was done by ANOVA followed by Newman–Keuls test as post hoc test. GraphPad prism

(version 3) was used to perform statistical tests. The value $P < 0.05$ was considered statistically significant.

Results

Effect of Guggulipid and Nimesulide on LPS-Induced Intracellular Ca^{++} Ion Level and iNOS Expression in C6 Cells

C6 cells were treated with LPS (10 µg/ml) alone and in combination with guggulipid or nimesulide for 24 h. LPS significantly increased intracellular Ca^{++} level and iNOS
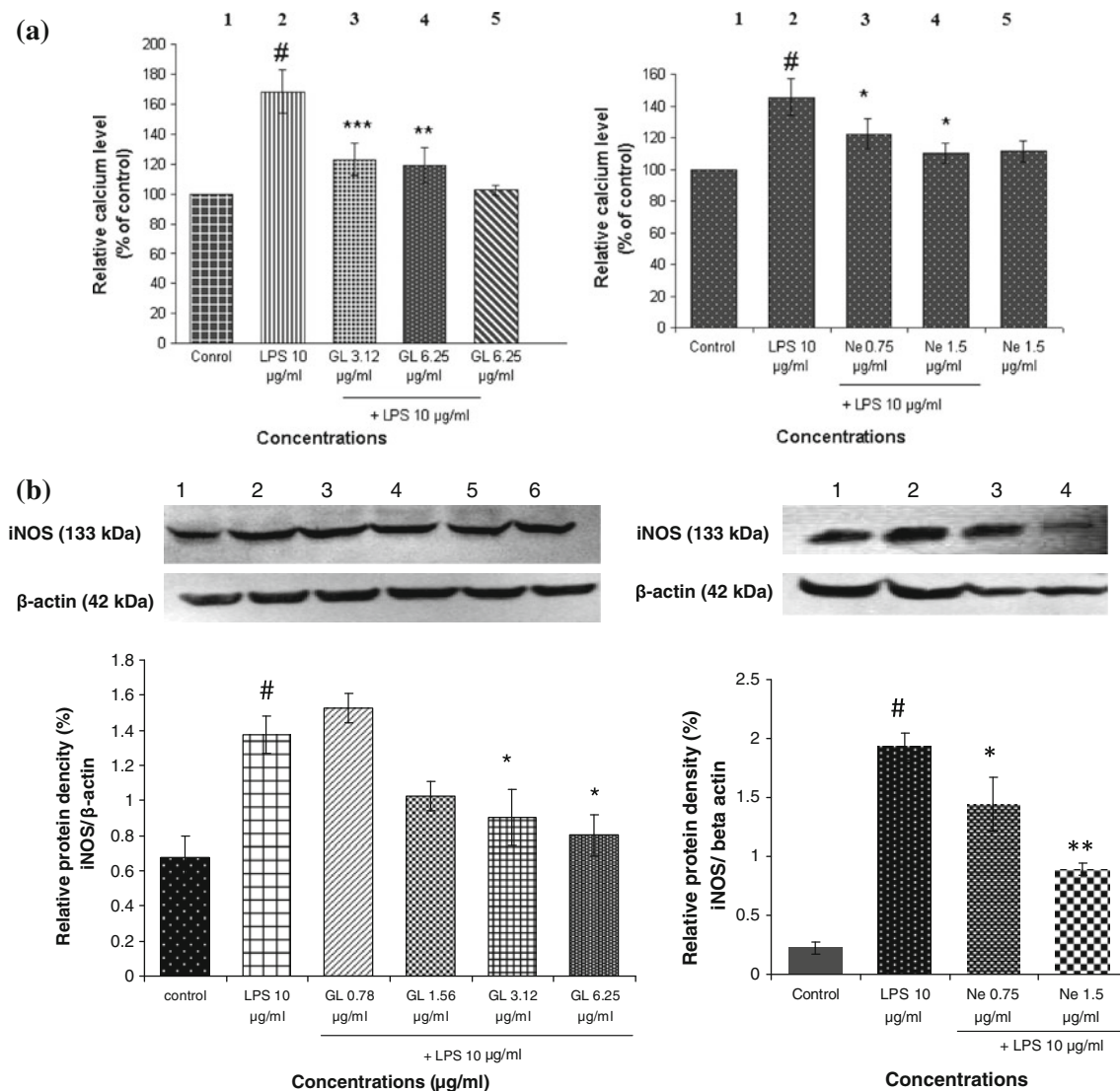

Fig. 1 Effect of guggulipid and nimesulide on the LPS (10 µg/ml) induced Ca^{++} ion level and iNOS expression in C6 cells. All given concentrations are in µg/ml. **a** *Left panel*: Lanes 1 = control, 2 = LPS, 3 = LPS + GL 3.12, 4 = LPS + GL 6.25, and 5 = GL 6.25. *Right panel*: Lanes 1 = control, 2 = LPS, 3 = LPS + Ne 0.75, 4 = LPS + Ne 1.5, and 5 = Ne 1.5. **b** *Left panel*: Lanes 1 = control, 2 = LPS, 3 = LPS + GL 0.78, 4 = LPS + GL 1.5, 5 = LPS + GL 3.12, and 6 = LPS + GL 6.25. *Right panel*: Lanes 1 = control, 2 = LPS, 3 = LPS + Ne 0.75, and 4 = LPS + Ne 1.5. #$P < 0.001$ significant with control group, *$P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ significant with LPS group. β-actin was taken as an internal control

expression when compared with the control group. Guggulipid (in μg/ml: 3.12 and 6.25) and nimesulide significantly decreased the intracellular Ca^{++} ion level (Fig. 1a). However, guggulipid and nimesulide per se did not influence the calcium ion release. LPS significantly increased iNOS expression (Fig. 1b). Guggulipid (6.25 μg/ml) and nimesulide (1.5 μg/ml) significantly decreased iNOS expression (Fig. 1b).

Effect of Guggulipid and Nimesulide on LPS-Induced COX-2, mPGES-1, IL-1β, IL-1α, and IL-6 mRNA Expressions in C6 Cells

Treatment of C6 cells for 24 h with LPS (10 μg/ml) significantly upregulated COX-2 mRNA expression with downregulation in mPGES-1 gene expression. Guggulipid significantly downregulated the LPS increased COX-2 expression with upregulation in mPGES-1 mRNA expression (Fig. 2a). Nimesulide also significantly downregulated the LPS-induced COX-2 mRNA expression with no significant effect on mPGES-1 mRNA expression

(Fig. 2b). Furthermore, LPS (10 μg/ml) increased the expression of IL-6, decreased the expression of IL-1α and IL-1β significantly. Guggulipid reversed all these LPS-induced changes in IL-1α, IL-1β, and IL-6 expression significantly (Fig. 3a). Nimesulide (1.5 μg/ml) did not significantly affect the LPS-induced changes in IL-1α, IL-1β, and IL-6 expressions (Fig. 3b).

The influence of Guggulipid and Nimesulide on LPS-Induced CHOP and NF-kB (p-65) Expression and NF-kB Translocation in C6 Cells

Treatment of C6 cells with LPS (10 μg/ml) caused an increase in NF-kB (p-65) and CHOP expressions, which were significantly inhibited by guggulipid treatment dose dependently (Fig. 4a and b). Nimesulide did not affect CHOP expression but significantly downregulated NF-kB expression. Furthermore, as shown in Fig. 4c, LPS significantly upregulated translocation of NF-kB from the cytosol to the nucleus. Guggulipid (6.25 μg/ml) and nimesulide (1.5 μg/ml) significantly inhibited NF-kB translocation.

Fig. 2 Result of RT–PCR of inhibitory effect of guggulipid and nimesulide on LPS (10 μg/ml) induced COX-2 and mPGES-1 transcription. All concentrations are given in μg/ml. **a** COX-2 mRNA expression; *Left panel* = LPS + GL; Lanes 1 = control, 2 = LPS, 3 = LPS + GL 0.78, 4 = LPS + GL 1.5, 5 = LPS + GL 3.12, and 6 = LPS + GL 6.25. *Right panel*: Lanes 1 = control, 2 = LPS, and 3 = LPS + Ne 1.5. **b** mPGES-1 mRNA expression; Lanes 1 = control, 2 = LPS, 3 = LPS + GL 3.12, 4 = LPS + GL 6.25, 5 = LPS + Ne 0.75, and 6 = LPS + Ne 1.5. #$P < 0.001$ significant with control group, **$P < 0.001$ and ***$P < 0.001$ significant with LPS group. GAPDH was taken as an internal control
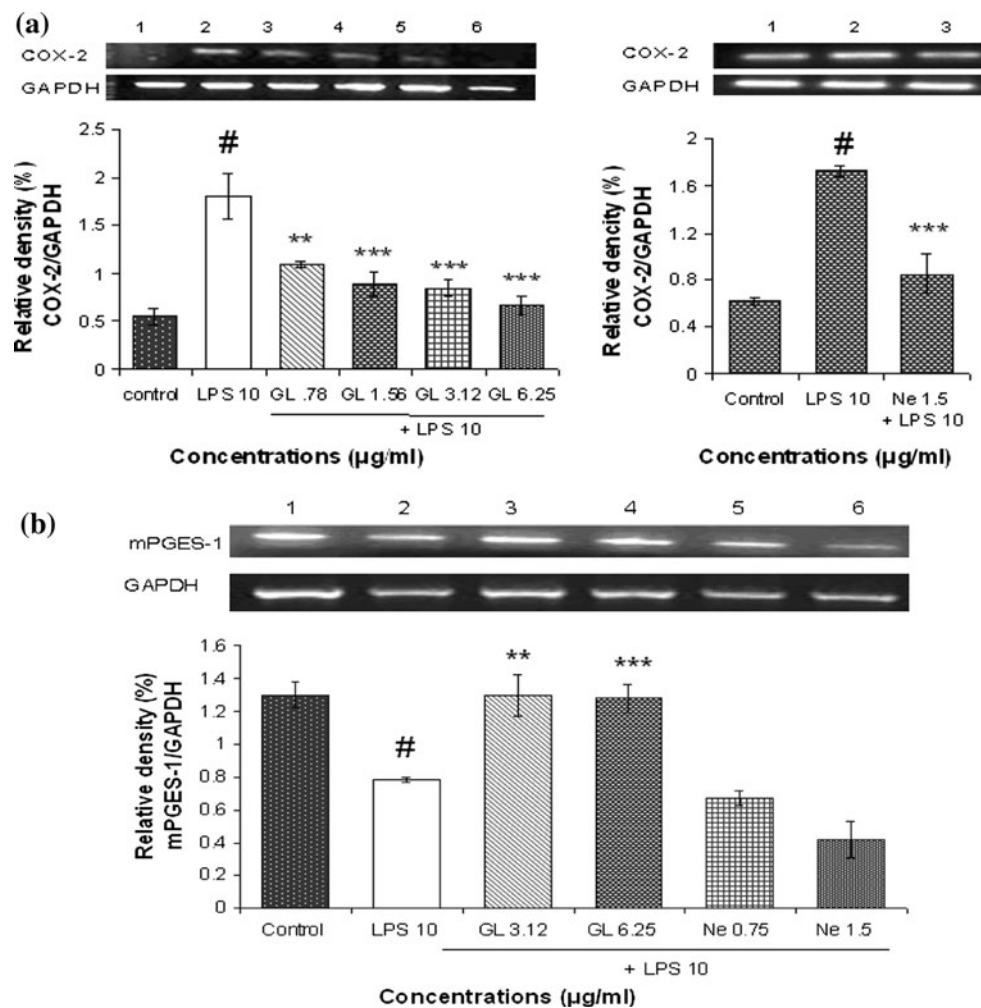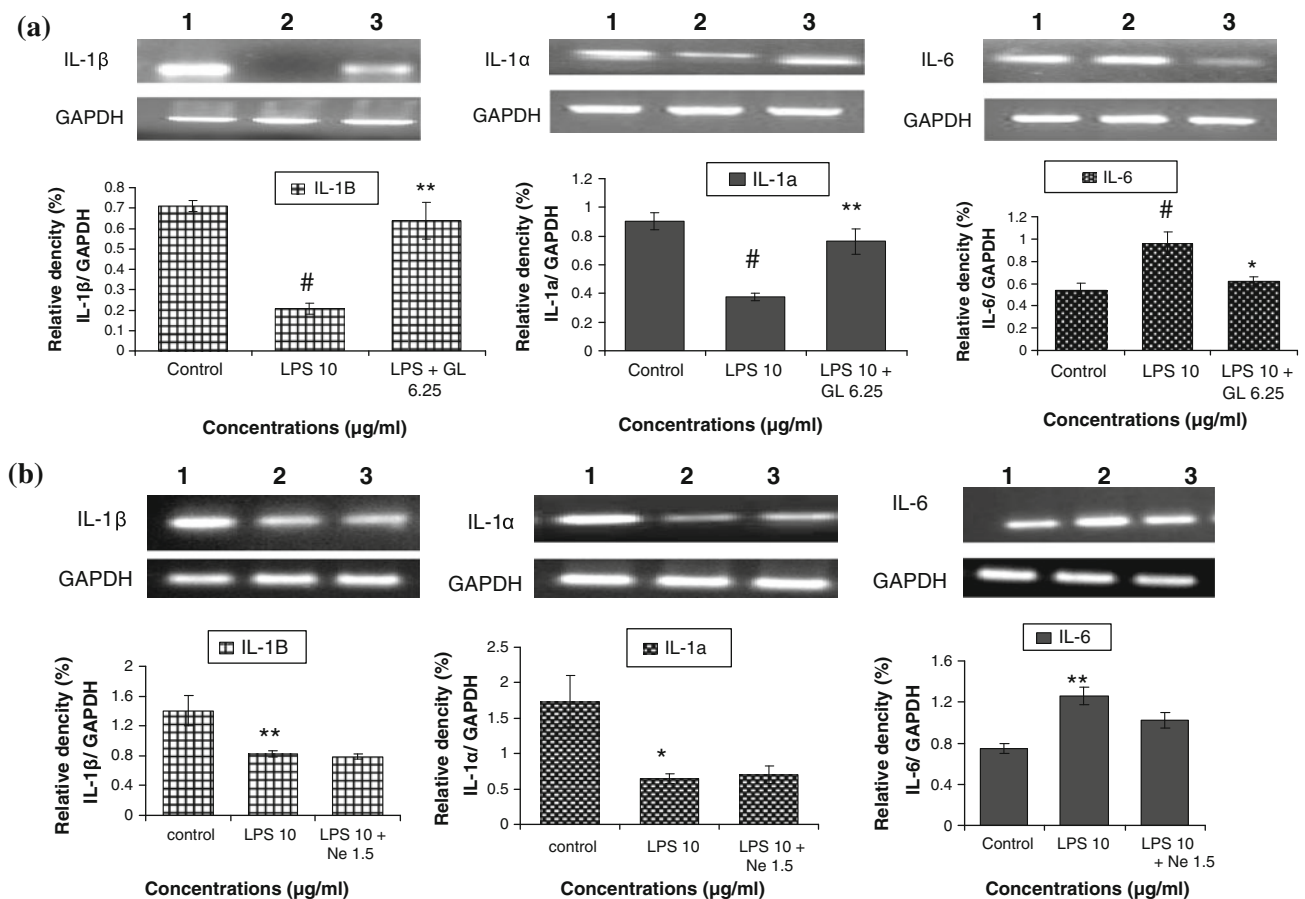
Fig. 3 Result of RT–PCR of modulatory effect of guggulipid and nimesulide on LPS (10 μg/ml) induced IL-1α, IL-1β, and IL-6 expression in C6 cells. All concentrations are given in μg/ml. **a** LPS + GL; Lanes 1 = control, 2 = LPS, and 3 = LPS + GL 6.25. IL-1β, #P < 0.01 significant with control group, **P < 0.01 significant with LPS group, IL-1α, #P < 0.01 significant with control group, **P < 0.01 significant with LPS group, IL-6, #P < 0.05 significant with control group, *P < 0.05 significant with LPS group. GAPDH was taken as internal control. **b** LPS + Ne; Lanes 1 = control, 2 = LPS, and 3 = LPS + Ne 1.5. IL-1β, *P < 0.05 significant with control group, IL-1α, *P < 0.05 significant with control group, IL-6, **P < 0.01 significant with control group

Effect of Guggulipid and Nimesulide on c-fos and c-jun Expressions in LPS-Stimulated C6 Cells

Treatment of C6 cells with LPS (10 μg/ml) caused an increase in c-fos and c-jun expressions, which were significantly inhibited by guggulipid treatment dose dependently (Fig. 5a and b). Nimesulide also significantly downregulated the LPS-induced c-fos expression, but it did not significantly affect c-jun expression.

Discussion

This study elucidated the molecular mechanism of neuroinflammation associated to the anti-neuroinflammatory effect of guggulipid and nimesulide against LPS-stimulated rat astrocytoma cells C6. Recently, we have reported that guggulipid and nimesulide attenuated inflammatory

mediators and GFAP expression in LPS-stimulated rat astrocytoma cells (Niranjan et al. 2010), but the intracellular mechanism of neuroinflammation was not fully explored.

The major intracellular events that maybe associated to the glial cell activation are the elevated expressions of COX-2, mPGES-1, iNOS, c-fos, c-jun, and CHOP. In addition to these, NF-kB may also be activated, which in turn regulates the expressions of many genes, including proinflammatory cytokines, in particular, IL-1β, IL-1α, and IL-6. These intracellular events of neuroinflammation are still poorly understood in response to guggulipid and nimesulide against LPS stimulation of astrocytes. Therefore, in the present work, we studied the effect of guggulipid and nimesulide on LPS-induced changes in the above-described parameter on rat astrocytoma, C6 cells.

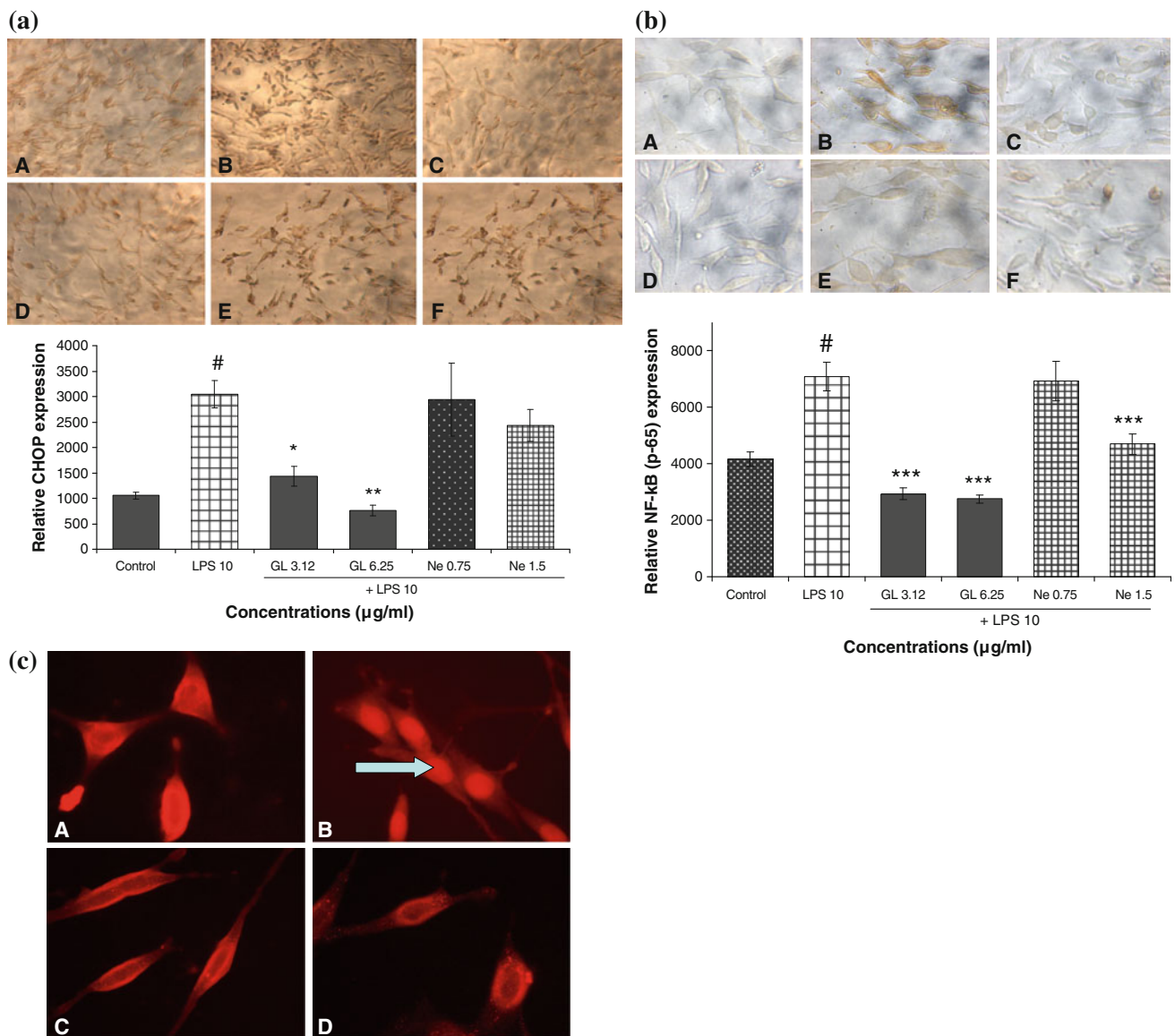The downregulation of LPS-elevated Ca^{++} ion level by guggulipid and nimesulide may point toward

Fig. 4 Effect of guggulipid and nimesulide on LPS (10 µg/ml) induced (a) CHOP and (b) NF-kB expressions and (c) NF-kB translocation in C6 cells. All concentrations are given in µg/ml. In (a) and (b) A control, B LPS, C LPS + GL 3.12, D LPS + GL 6.25, E LPS + Ne 0.75, and F LPS + Ne 1.5. a #$P < 0.05$ significant with control, *$P < 0.05$ and **$P < 0.01$ significant with LPS group. b #$P < 0.001$ significant with control, ***$P < 0.001$ significant with LPS group. (c) NF-kB nuclear translocation: Arrow shows NF-kB (p-65) localization in cytosol and in nucleus. A control, B LPS, C LPS + GL-6.2, and D LPS + Ne 1.5

neuroprotective action of these drugs because intracellular Ca^{++} ion in astrocytes is an important event and involved in the regulation of number of mechanisms, including release of S100B and other neurotoxins (Bezprozvanny 2009; Iuvone et al. 2007; Marambaud et al. 2009). Upregulated expression of iNOS (calcium independent) in response to stimuli or in a pathological state plays the major role in nitric oxide (NO)-mediated neurotoxicity (Ayasolla et al. 2004). We have also recently reported that guggulipid and nimesulide have significantly attenuated the LPS-induced nitrite release (Niranjan et al. 2010). The present study demonstrated that guggulipid and nimesulide

significantly downregulated the LPS-induced iNOS expression revealing their mechanism of action to inhibit nitrite release and thus suggesting wide therapeutic potential of these drugs in NO-mediated disorders.

COX-2 enzyme forms excessive prostanoids and free radicals that mediate the neurotoxicity (Kaufmann et al.1997). Nimesulide is an inhibitor of cyclooxygenase enzymatic activity. The role of nimesulide at transcriptional level of COX-2 gene was not demonstrated. In present study, guggulipid and nimesulide have significantly downregulated LPS-induced COX-2 expression depicting their actions at transcriptional level and thus subsequent
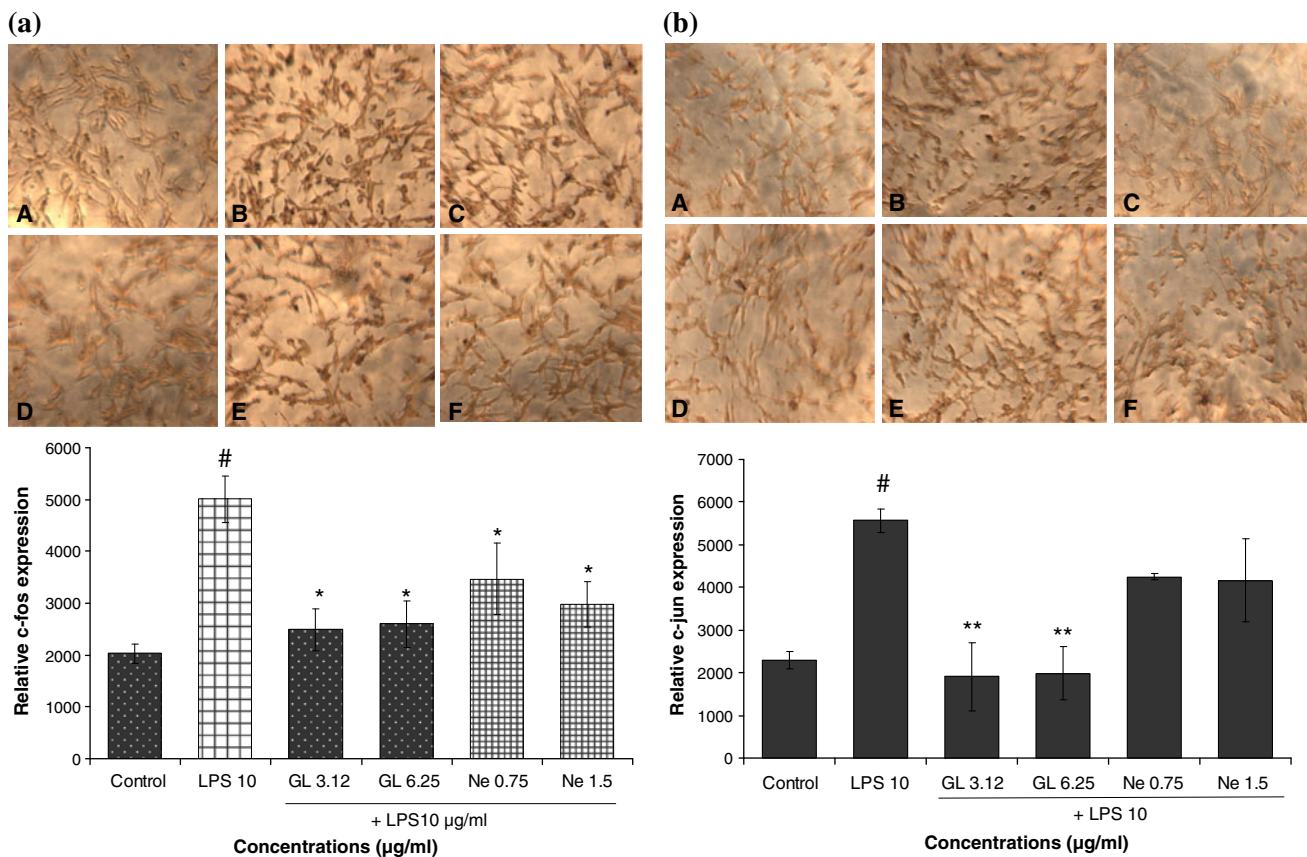
(a)

(b)

Fig. 5 Effect of guggulipid and nimesulide on LPS (10 μg/ml) induced c-fos and c-jun expressions in C6 cells. All concentrations are given in μg/ml. **A** control, **B** LPS, **C** LPS + GL 3.12, **D** LPS + GL 6.25, **E** LPS + Ne 0.75, and **F** LPS + Ne 1.5. **a** c-fos expression: #$P < 0.01$ significant with control, *$P < 0.05$ significant with LPS group. **b** c-jun: #$P < 0.05$ significant with control, **$P < 0.05$ significant with LPS group, **$P < 0.01$ significant with LPS group

neuroprotection. Other studies have also shown neuroprotection by nimesulide supporting the present findings (Candelario-Jalil et al. 2000, 2005; Cullen et al. 1998). In addition to COX-2, mPGES-1 is also involved in the prostaglandin synthesis which is an important event for maintaining normal brain function in physiological states (Samuelsson et al. 2007). In our study, LPS-downregulated mPGES-1 expression that was normalized by guggulipid but not by the nimesulide.

Role of proinflammatory cytokine by the astrocytes is one of the major immunoregulatory event by which they communicate their immunological instructions. One immune function of astrocytes is IL-6 production (Norris et al. 1994). IL-6 through its IL-6 receptor significantly regulates VCAM-1 gene expression which is understood as a resultant of glial cell activation (Oh et al. 1998). Guggulipid significantly attenuated LPS-induced IL-6 expression suggesting its potential role in the inactivation of astroglial. Another study showed that IL-1α plays a crucial role in modulating glia cells proliferation and thereby guidance and trophic factors for new fibers, in response to brain injury (Parish et al. 2002). A more recent study

showed that sustained IL-1β overexpression mediate chronic neuroinflammation and ameliorates Alzheimer's plaque pathology describing that IL-1β overexpression is a beneficial event in Alzheimer's disease (Shaftel et al. 2007). Guggulipid significantly increased LPS-induced decrease in IL-1α and IL-1β expression in these astrocytoma cells C6 suggesting its beneficial role in regulating these cytokines in the states of infection or brain injury. However, nimesulide failed to reverse the LPS-induced changes in IL 6, IL-1β, and in IL-1α expressions. No significant effect of nimesulide on IL 6, IL-1β, and in IL-1α expressions pointed toward the different signaling mechanisms that may exist in the regulation of COX-2 expression and cytokine genes expressions. In a similar study, it has been shown that diclofenac has downregulated the prostaglandin E 2 (PGE-2) production without affecting the IL-1β, IL-1α, and tumor necrosis factor-α levels (Lavigne et al. 2002), which also provide support to the present data.

Active components of guggulipid (guggulsterones) have shown its inhibitory effect on NF-kB signaling by inhibiting IK-B kinase (Cheon et al. 2006). Anti-inflammatory and antioxidant properties of guggulipid on LPS-induced

C6 glioma cells were also demonstrated by the attenuation of NF-kB (p65) activation. NF-kB is a redox-regulated transcription factor and importantly involved in regulation of the expression of inflammatory genes, early response genes (Zhang and Stanimirovic 2002; Zhou et al. 2008). Guggulipid and nimesulide significantly inhibited LPS-induced NF-kB (p65) translocation in these C6 cells suggesting their effects at transcription factor level. Furthermore, antioxidant effect of guggulipid and nimesulide was also showed on the activation of CHOP. CHOP is also a redox-regulated transcription factor and also involved in the regulation of inflammatory genes (COX-2 and iNOS) (Zhang and Stanimirovic 2002; Zhou et al. 2008). Guggulipid significantly inhibited LPS-induced CHOP activation at low doses but nimesulide failed to attenuate CHOP expression, which may suggest wider anti-inflammatory actions of guggulipid than nimesulide.

The product of c-fos and c-jun also constitutes to the AP-1 transcription factor that regulate number of inflammatory genes (Gadea et al. 2008; Rubio 1997). Guggulipid significantly decreased LPS-induced c-fos and c-jun expressions in these LPS-stimulated C6 cells. Increased c-fos and c-jun expression triggers normal cells toward activated cells. LPS increased c-fos and c-jun genes expression, indicating that it could enhance the inflammatory genes expressions. Thus, regulation of AP-1 transcription factor by guggulipid may be a self regulatory mechanism in astrocytes in neuroinflammatory conditions. Nimesulide failed to attenuate LPS-induced c-jun expression significantly that indicates its limited influence on AP-1 transcription factor.

In conclusion, LPS activates astroglial cells and produces sustained NF-kB and CHOP activation. LPS also regulated differential inflammatory genes expression. The observed anti-inflammatory functions of guggulipid and nimesulide point to the multiple regulatory and therapeutic potentials of these drugs for neuroinflammatory disorders. Guggulipid and nimesulide differentially regulated mRNA expressions of inflammatory genes through inhibition of NF-kB activation in LPS-stimulated rat glioma, cell line (C6), that might reveal the neuroprotective effects of guggulipid and nimesulide. Guggulipid covered wider targets than nimesulide. This study supports the use of guggulipid and nimesulide as a therapeutic agent to reduce astroglial activation following different types of brain insult.

Conflict of interest All authors declare that they have no conflicts of interest.

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