Gemfibrozil, a Lipid-lowering Drug, Inhibits the Induction of Nitric-oxide Synthase in Human Astrocytes

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Gemfibrozil, a lipid-lowering drug, inhibited cytokine-induced production of NO and the expression of inducible nitric-oxide synthase (iNOS) in human U373MG astroglial cells and primary astrocytes. Similar to gemfibrozil, clofibrate, another fibrate drug, also inhibited the expression of iNOS. Inhibition of human iNOS promoter-driven luciferase activity by gemfibrozil in cytokine-stimulated U373MG astroglial cells suggests that this compound inhibits the transcription of iNOS. Since gemfibrozil is known to activate peroxisome proliferator-activated receptor-α (PPAR-α), we investigated the role of PPAR-α in gemfibrozil-mediated inhibition of iNOS. Gemfibrozil induced peroxisome proliferator-responsive element (PPRE)-dependent luciferase activity, which was inhibited by the expression of ΔhPPAR-α, the dominant-negative mutant of human PPAR-α. However, ΔhPPAR-α was unable to abrogate gemfibrozil-mediated inhibition of iNOS suggesting that gemfibrozil inhibits iNOS independent of PPAR-α. The human iNOS promoter contains consensus sequences for the binding of transcription factors, including interferon-γ (IFN-γ) regulatory factor-1 (IRF-1) binding to interferon-stimulated responsive element (ISRE), signal transducer and activator of transcription (STAT) binding to γ-activation site (GAS), nuclear factor-κB (NF-κB), activator protein-1 (AP-1), and C/EBPβ-binding site; therefore, we investigated the effect of gemfibrozil on the activation of these transcription factors. The combination of interleukin (IL)-1β and IFN-γ induced the activation of NF-κB, AP-1, C/EBPβ, and GAS but not that of ISRE, suggesting that IRF-1 may not be involved in cytokine-induced expression of iNOS in human astrocytes. Interestingly, gemfibrozil strongly inhibited the activation of NF-κB, AP-1, and C/EBPβ but not that of GAS in cytokine-stimulated astroglial cells. These results suggest that gemfibrozil inhibits the induction of iNOS probably by inhibiting the activation of NF-κB, AP-1, and C/EBPβ and that gemfibrozil, a prescribed drug for humans, may further find its therapeutic use in neuroinflammatory diseases.

It is now increasingly clear that glial cells (astrocytes and microglia) in the central nervous system (CNS) induce the expression of inducible nitric-oxide synthase (iNOS) and the production of NO in response to proinflammatory cytokines, including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) (1–4). Although the NO produced by iNOS has bactericidal and tumoricidal properties, it also plays an important role in pathophysiology of inflammatory neurological diseases including demyelinating disorders (e.g. multiple sclerosis, experimental allergic encephalopathy), neurodegenerative disorder like Alzheimer’s disease, and in ischemic and traumatic brain injuries associated with the activation of glial cells and the production of proinflammatory cytokines (5–8). NO derived from activated glial cells is assumed to contribute to oligodendrocyte degeneration in demyelinating diseases and neuronal death during ischemia and trauma (5, 6). Therefore, characterization of intracellular pathways required to transduce the signal from the cell surface to the nucleus for the induction of iNOS is an active area of investigation, since compounds capable of antagonizing signaling steps for the induction of iNOS may have therapeutic effect in NO-mediated pathophysiological conditions.

Peroxisome proliferator-activated receptors (PPARs), members of the nuclear hormone receptor superfamily, have been implicated in a variety of human diseases (9). Three isotypes have been described to date, PPAR-α, PPAR-β, and PPAR-γ (9). Activation of PPAR-α mainly leads to the induction of a variety of genes such as those coding for the enzymes for β- and α-oxidation of fatty acids (10). Gemfibrozil, an activator of PPAR-α, has been often prescribed in patients to lower the level of triglycerides (11, 12). This drug decreases the risk of coronary heart disease by increasing the level of high density lipoprotein cholesterol and decreasing the level of low density lipoprotein cholesterol (11, 12). Activation of PPAR-α is also capable of modifying the stress response by activation of heat shock factor 1 (HSF-1) and induction of HSP70 (13, 14). Recently it has been shown that activation of HSP70 inhibits the expression of iNOS in astrocytes (15), suggesting that the expression of iNOS may also be regulated by activators of
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RESULTS

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Nitrite production (µg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.2 ± 0.8</td>
</tr>
<tr>
<td>IL-1β only</td>
<td>18.6 ± 1.9</td>
</tr>
<tr>
<td>IFN-γ only</td>
<td>15.9 ± 0.9</td>
</tr>
<tr>
<td>IL-1β + IFN-γ</td>
<td>196.3 ± 26.6</td>
</tr>
<tr>
<td>IL-1β + IFN-γ + arginine</td>
<td>21.2 ± 1.8</td>
</tr>
<tr>
<td>IL-1β + IFN-γ + L-NMA</td>
<td>19.1 ± 2.1</td>
</tr>
<tr>
<td>IL-1β + IFN-γ + d-NMA</td>
<td>194.7 ± 21.3</td>
</tr>
<tr>
<td>Gemfibrozil only</td>
<td>5.9 ± 1.1</td>
</tr>
<tr>
<td>IL-1β + IFN-γ + gemfibrozil</td>
<td>20.8 ± 2.8</td>
</tr>
</tbody>
</table>

*p < 0.001 versus IL-1β + IFN-γ.

Co.). The same amount of RNA was hybridized with probe for glyceroldehyde-3-phosphate dehydrogenase (GAPDH). Assay of iNOS Promoter-driven Reporter Activity—Construction of pNF-κB-Luc, the 7.2-kb human iNOS promoter-luciferase construct, has been described previously (19). Cells plated at 50–60% confluence in six-well plates were cotransfected with 1 µg of pNF-κB-Luc and 50 ng of pRl-TK (a plasmid encoding Renilla luciferase, used as transfection efficiency control; Promega) by LipofectAMINE Plus (Invitrogen) following manufacturer's protocol (3, 4). Twenty-four h after transfection, cells were treated with different stimuli for 12 h. Firefly and Renilla luciferase activities were obtained by analyzing total cell extract according to standard instructions provided in the Dual Luciferase Kit (Promega) in a TD-20/20 Luminometer (Turner Designs). Relative luciferase activity of cell extracts was typically represented as the ratio of firefly luciferase values/ml Luciferase activity × 10³.

Assay of Transcriptional Activities of Different Proinflammatory transcription Factors—Cells plated at 50–60% confluence in six-well plates were cotransfected with 1 µg of either pNF-κB-Luc (NF-κB-dependent reporter construct), pC/EBPβ-Luc (C/EBPβ-dependent reporter construct), pGAS-Luc (GAS-dependent reporter construct), or pISRE-Luc (ISRE-dependent reporter construct) and 50 ng of pRl-TK using LipofectAMINE Plus. Construction of pC/EBPβ-Luc has been described earlier (4). This C/EBPβ-sensitive promoter contains four consensus C/EBP binding sites. Other reporter constructs (pNF-κB-Luc, pAP-1-Luc, pGAS-Luc, and pISRE-Luc) were obtained from Stratagene. After 24 h of transfection, cells were treated with different stimuli for 6 h. Firefly and Renilla luciferase activities were obtained as described above.

Statistics—Statistical comparisons were made using one-way analysis of variance followed by Student’s t test.

TABLE I

Induction of NO production in human U373MG astroglial cells

<table>
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<th>Treatments</th>
<th>Nitrite production (µg/mg protein)</th>
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</tr>
<tr>
<td>IL-1β + IFN-γ + gemfibrozil</td>
<td>20.8 ± 2.8</td>
</tr>
</tbody>
</table>

*p < 0.001 versus IL-1β + IFN-γ.
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1. Time course of cytokine-induced NO production and its suppression by gemfibrozil in human U373MG astroglial cells.

Fig. 1. Time course of cytokine-induced NO production and its suppression by gemfibrozil in human U373MG astroglial cells. Cells preincubated with 200 μM gemfibrozil for 2 h in serum-free DMEM/F-12 received the combination of IL-1β (10 ng/ml) and IFN-γ (10 units/ml). At different hours of stimulation, the concentration of nitrite was measured in supernatants using the “Griess reagent” as described under “Materials and Methods.” Data are expressed as the mean of two separate experiments.

2. Gemfibrozil dose-dependently inhibits the expression of iNOS in cytokine-stimulated human U373MG astroglial cells.

Fig. 2. Gemfibrozil dose-dependently inhibits the expression of iNOS in cytokine-stimulated human U373MG astroglial cells. Cells preincubated with different concentrations of gemfibrozil for 2 h in serum-free DMEM/F-12 received the combination of IL-1β (10 ng/ml) and IFN-γ (10 units/ml). A, after 24 h of stimulation, the concentration of nitrite was measured in the supernatants. Data are mean ± S.D. of three different experiments. a, p < 0.001 versus control; b, p < 0.005 versus IL-1β + IFN-γ; c, p < 0.001 versus IL-1β + IFN-γ. B, cell homogenates were immunoblotted with antibodies against mouse macrophage iNOS as described under “Materials and Methods.” C, after 6 h of stimulation, total RNA was isolated, and Northern blot analysis for iNOS mRNA was carried out as described under “Materials and Methods.”
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Human primary astrocytes preincubated in serum-free DMEM/F-12 for 2 h with 200 μM gemfibrozil, received IL-1β, IFN-γ, and TNF-α alone or in different combinations. After 24 h of incubation, nitrite concentrations in the supernatants. Data are expressed as the mean ± S.D. of three different experiments. The concentrations of different cytokines were as follows: IL-1β, 10 ng/ml; IFN-γ, 10 units/ml; TNF-α, 10 ng/ml.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Nitrite production (μg/mg protein/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.3 ± 0.7</td>
</tr>
<tr>
<td>IL-1β</td>
<td>24.5 ± 2.5</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>6.1 ± 0.7</td>
</tr>
<tr>
<td>TNF-α</td>
<td>5.8 ± 0.8</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>5.1 ± 0.6</td>
</tr>
<tr>
<td>IL-1β + IFN-γ</td>
<td>215.3 ± 22.3</td>
</tr>
<tr>
<td>IL-1β + IFN-γ + TNF-α</td>
<td>219.2 ± 28.5</td>
</tr>
<tr>
<td>IL-1β + IFN-γ + gemfibrozil</td>
<td>24.8 ± 2.8</td>
</tr>
<tr>
<td>IL-1β + IFN-γ + TNF-α + gemfibrozil</td>
<td>25.7 ± 3.1</td>
</tr>
</tbody>
</table>

* p < 0.001 versus IL-1β + IFN-γ.
* b p < 0.001 versus IL-1β + IFN-γ + TNF-α.

phiNOS(7.2)/Luc, a construct containing the human iNOS promoter fused to the luciferase gene (19), and activation of this promoter was measured after stimulating the cells with cytokines in the presence or absence of gemfibrozil. The combination of IL-1β and IFN-γ-induced iNOS promoter-driven luciferase activity by about 3.9-fold (Fig. 5). Consistent with the effect of gemfibrozil on the expression of iNOS, gemfibrozil itself had no effect on iNOS promoter-driven luciferase activity but it dose-dependently inhibited iNOS promoter-driven luciferase activity in cytokine-stimulated cells (Fig. 5), suggesting that gemfibrozil inhibits cytokine-induced production of NO and the expression of iNOS mRNA by inhibiting the activation of iNOS promoter.

Role of PPAR-α in Gemfibrozil-mediated Inhibition of iNOS in Human U373MG Astroglial Cells—Since gemfibrozil is a known activator of PPAR-α (9, 20, 21), a member of the nuclear hormone receptor superfamily, we examined whether gemfibrozil inhibited the induction of iNOS through the activation of PPAR-α. PPARs bind to a consensus sequence known as PPRE (9). Therefore, to study the activation of PPAR, cells were transfected with tk-PPREx3-Luc, a PPRE-dependent luciferase construct, and luciferase activity was measured. As shown in Fig. 6A, gemfibrozil alone was able to induce PPRE-dependent luciferase activity in a dose-dependent manner and the maximum induction (~4-fold) was observed at 100 μM or higher concentration of gemfibrozil. In contrast, the combination of IL-1β and IFN-γ capable of inducing iNOS inhibited PPRE-dependent luciferase activity (Fig. 6A). However, gemfibrozil treatment blocked the inhibitory effect of cytokines on the activation of PPRE and stimulated PPRE-dependent luciferase activity over basal level even in the presence of cytokines (Fig. 6A). To analyze the role of PPAR-α in gemfibrozil-induced activation of PPRE, we used ΔhPPAR-α, the dominant-negative mutant of human PPAR-α (25). Marked abrogation (p < 0.001) of gemfibrozil-induced activation of PPRE (Fig. 6B) by the expression of ΔhPPAR-α but not that of the empty vector suggests that gemfibrozil induced PPRE-dependent reporter activity through the activation of PPAR-α in human astroglial cells.

Next we examined whether ΔhPPAR-α can block the inhibitory effect of gemfibrozil on the induction of iNOS. Cells were cotransfected with phNOS(7.2)/Luc, ΔhPPAR-α, and pRL-TK (a plasmid encoding Renilla luciferase, used as transfection efficiency control). After 24 h of transfection, cells were incubated with gemfibrozil for 2 h followed by stimulation with cytokines. Consistent, to the inhibitory effect of gemfibrozil on

for 2 h markedly inhibited cytokine-induced production of NO (Table II).

Gemfibrozil Inhibits Human iNOS Promoter-driven Luciferase Activity in Cytokine-stimulated Human U373MG Astroglial Cells—To understand the effect of gemfibrozil on the transcription of iNOS gene, U373MG glial cells were transfected with

![Graph](image-url)
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Fig. 5. Gemfibrozil inhibits human iNOS promoter-derived luciferase activity in cytokine-stimulated human U373MG astroglial cells. Cells plated at 50–60% confluence in six-well plates were cotransfected with 1 μg of phiNOS(7.2)Luc (a construct containing the human iNOS promoter fused to the luciferase gene) and 50 ng of pRL-TK (a plasmid encoding Renilla luciferase, used as a transfection efficiency control) using the LipofectAMINE Plus (Invitrogen). Twenty-four hours after transfection, cells received different concentrations of gemfibrozil. After 2 h of incubation, cells were stimulated with the combination of IL-1β (10 ng/ml) and IFN-γ (10 units/ml) for 12 h under serum-free condition. Firefly (ff-Luc) and Renilla (r-Luc) luciferase activities were obtained by analyzing the total cell extract as described under "Materials and Methods." Data are mean ± S.D. of three different experiments. a, p < 0.001 versus control; b, p < 0.05 versus IL-1β + IFN-γ; c, p < 0.001 versus IL-1β + IFN-γ.

The activation of iNOS promoter (Fig. 5), this drug inhibited iNOS promoter-driven luciferase activity in empty vector-transfected cells (Fig. 7). However, despite the ability of ΔhPPAR-α to block gemfibrozil-mediated activation of PPRE (Fig. 6B), the expression of ΔhPPAR-α did not block the inhibitory effect of gemfibrozil on iNOS promoter-driven luciferase activity in cytokine-stimulated cells (Fig. 7). These results suggest that PPAR-α is not involved in gemfibrozil-mediated inhibition of iNOS in human astroglial cells.

Effect of Gemfibrozil on the Activation of Proinflammatory Transcription Factors in Human U373MG Astroglial Cells—Different proinflammatory transcription factors are known to be involved in the transcription of iNOS (2–4, 26–30). Analysis of human iNOS promoter shows that it has consensus sequences for binding of several transcription factors such as NF-κB, AP-1, C/EBPβ, IRF-1 binding to ISRE, and STAT binding to GAS (29–31). Although several reports have established the involvement of NF-κB, AP-1, and STAT in the induction of iNOS in human cells (29, 32, 33), the role of C/EBPβ in the induction of human iNOS has not been established. Overexpression of dominant-negative molecules provides an effective tool with which to investigate the in vivo functions of different transcription factors and signaling molecules. Therefore, we used the dominant-negative mutant of C/EBPβ (ΔC/EBPβ) (34) to inhibit the activation of C/EBPβ. It is apparent from Fig. 8 that the expression of ΔC/EBPβ but not that of the empty vector inhibited iNOS promoter-driven luciferase activity significantly (p < 0.005) in cytokine-stimulated human U373MG astroglial cells, suggesting the involvement of C/EBPβ in the induction of iNOS in human astroglial cells.

Since gemfibrozil inhibited cytokine-induced activation of human iNOS promoter (Fig. 5), we decided to investigate the effect of gemfibrozil on the activation of these proinflammatory transcription factors in cytokine-stimulated human U373MG astroglial cells. Activation of these transcription factors was monitored by transcriptional activity using the expression of luciferase from respective reporter constructs. It is evident

Fig. 6. The dominant-negative mutant of human PPAR-α (ΔhPPAR-α) inhibits gemfibrozil-induced PPRE-dependent luciferase activity in human U373MG astroglial cells. A, cells plated at 50–60% confluence in six-well plates were cotransfected with 1 μg of tk-PPREx3-Luc, a PPRE-dependent luciferase reporter construct, and 50 ng of pRL-TK using LipofectAMINE Plus. Twenty-four hours after transfection, cells were cotransfected with 0.5 μg of either ΔhPPAR-α or an empty vector and 1 μg of tk-PPREx3-Luc. All transfections also included 50 ng/μl of pRL-TK. Twenty-four hours after transfection, cells were treated with different concentrations of gemfibrozil and/or the combination of IL-1β (10 ng/ml) and IFN-γ (10 units/ml). After 6 h of incubation, firefly (ff-Luc) and Renilla (r-Luc) luciferase activities were assayed. Data are mean ± S.D. of three different experiments. *, p < 0.001 versus 100 μM gemfibrozil; b, p < 0.001 versus 200 μM gemfibrozil.

Fig. 7. ΔhPPAR-α does not block gemfibrozil-mediated inhibition of iNOS promoter activation in cytokine-stimulated human U373MG astroglial cells. Cells were cotransfected with 0.5 μg of either ΔhPPAR-α or an empty vector, 0.5 μg of phiNOS(7.2)Luc, and 50 ng of pRL-TK. Twenty-four hours after transfection, cells were incubated with gemfibrozil for 2 h followed by stimulation with the combination of IL-1β and IFN-γ. After 12 h of incubation, firefly (ff-Luc) and Renilla (r-Luc) luciferase activities were assayed. Data are mean ± S.D. of three different experiments.
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Gemfibrozil Inhibits the Induction of NOS in Human Astrocytes

This manuscript describes the inhibition of nitric oxide synthase (NOS) activity in human astrocytes by gemfibrozil, a fibrate drug. The study was conducted using cytokine-stimulated human U373MG astroglial cells. The key findings include the following:

1. **Activation of NF-κB:** Gemfibrozil inhibited the activation of NF-κB, which is a transcription factor involved in the induction of iNOS.

2. **Activation of C/EBPβ:** The inhibition of C/EBPβ by gemfibrozil further supported the mechanism of iNOS inhibition.

3. **Activation of ISRE:** No activation of ISRE was observed in the presence of gemfibrozil, indicating the selective inhibition of iNOS.

4. **Activation of AP-1:** Gemfibrozil did not affect the activation of AP-1, suggesting a specific inhibition of iNOS.

5. **Activation of GAS:** Similar to AP-1, gemfibrozil did not inhibit the activation of GAS, further validating the selectivity of its action.

6. **Activation of C/EBPβ-Luc:** The inhibitory effect of gemfibrozil on the activation of C/EBPβ-Luc was dose-dependent.

7. **Activation of ISRE-Luc:** Similarly, the inhibition of ISRE-Luc was dose-dependent.

8. **Activation of GAS-Luc:** No significant activation of GAS-Luc was observed in the presence of gemfibrozil.

9. **Activation of NF-κB-Luc:** The inhibitory effect of gemfibrozil on the activation of NF-κB-Luc was dose-dependent.

**DISCUSSION**

The signaling events transduced by proinflammatory cytokines for the induction of iNOS are poorly understood. A complete understanding of the cellular signaling mechanisms involved in the induction of iNOS should identify novel targets for therapeutic intervention in NO-mediated neuroinflammatory diseases. The studies reported in this manuscript clearly demonstrate that gemfibrozil, an activator of PPAR-α, reduces the induction of iNOS in human astrocytes. Since astrocytes express PPAR-α (35), and NO produced from iNOS has been implicated in the pathogenesis of demyelinating and neurodegenerative diseases (5–7), our results provide a potentially important mechanism whereby activators of PPAR-α may ameliorate neural injury. However, gemfibrozil inhibits the induction of iNOS in human astroglial cells independent of PPAR-α. This conclusion is based on the following observations. First, gemfibrozil inhibited PPRE-dependent luciferase activity, suggesting that gemfibrozil can activate PPAR in human astroglial cells. Second, gemfibrozil inhibited cytokine-induced activation of iNOS promoter suggesting that gemfibrozil inhibits the transcription of iNOS. Third, the expression of ΔhPPAR-α, a dominant-negative mutant of human PPAR-α, blocked gemfibrozil-mediated activation of PPRE suggesting...
that gemfibrozil activates PPRE through PPAR-α. Fourth, ΔHPPAR-α was unable to block the inhibitory effect of gemfibrozil on the induction of iNOS promoter activation, suggesting that gemfibrozil does not require PPAR-α to inhibit the induction of iNOS in human astroglial cells.

In contrast to the marked induction of iNOS mRNA by the cytokine combination (Fig. 2), there was only a 3.9-fold induction of human iNOS promoter in human astroglial cells (Fig. 5), suggesting that in addition to transcriptional mechanisms posttranscriptional events could also play a significant role in regulating the expression of iNOS gene. Several authors have performed nuclear run-on assays to analyze the induction rate of the human iNOS promoter in different human cell lines (30, 36–38). These authors have shown that the endogenous iNOS promoter displays a significant basal activity and that the induction rate of only 2–10-fold by cytokines is much lower as seen for the induction of the iNOS mRNA expression. Taken together, these results suggest that human iNOS gene is regulated at the level of transcription as well as posttranscription.

However, in our experiment, gemfibrozil does not alter the relative rate of degradation of human iNOS mRNA in astroglial cells (Fig. 3), suggesting that gemfibrozil may not couple to posttranscriptional pathways required for the regulation of iNOS expression and that gemfibrozil inhibits the expression of iNOS mRNA mainly at the level of transcription.

Proinflammatory cytokines (TNF-α, IL-1β, or IFN-γ) bind to their respective receptors and induce iNOS expression via activation of NF-κB (2–4, 22, 26–28). The presence of multiple consensus sequences in the promoter region of iNOS for the binding of NF-κB and the inhibition of iNOS expression with the inhibition of NF-κB activation (2–4, 22, 26–28) establishes an essential role of NF-κB activation in the induction of iNOS. Although TNF-α or IL-1β alone is capable of inducing the activation of NF-κB, these cytokines alone were not sufficient to induce the expression of iNOS in human cell lines (39). The fact that a combination of cytokines is required to induce the expression of iNOS suggests that activation of additional transcription factors is also necessary for the expression of iNOS.

Consistently, apart from the consensus sequence for binding of NF-κB, the human iNOS promoter contains consensus sequences for the binding of transcription factors including AP-1, C/EBPβ, IRF-1 binding to ISRE, and STAT binding to GAS (29–31). The bulk of the work regarding the involvement of these transcription factors in the transcriptional regulation of the iNOS gene involved the murine system.

Although the role of these transcription factors in the transcription of human iNOS has not been well established, several evidences point to their possible involvement in the induction of iNOS in human cells. Kleinert et al. (32) have shown that the cytokine mixture induces the tyrosine phosphorylation of JAK-2 in human DLD-1 cells. This activated JAK-2 further induces the DNA binding activity of STAT1α (32). Tyrophostin B42, a specific inhibitor of JAK-2 (33), inhibits the phosphorylation of JAK-2, the activation of STAT1α, and the induction of iNOS (32), suggesting that the JAK-2/STAT1α pathway is an important activator of iNOS transcription. Moss and colleagues (29) have recently shown that activation of both NF-κB and AP-1 is an important step for the transcription of iNOS in human cells. Mutation in NF-κB- as well as AP-1-binding site of the iNOS promoter reduces the transcriptional activity of iNOS promoter (29). Furthermore, they (29) have shown that MAP kinase pathways (ERK and p38) regulate the expression of iNOS in human lung epithelial (A549) cells through the modulation of NF-κB and AP-1. Recently, we have found that activation of C/EBPβ is also necessary for the induction of iNOS (4). Overexpression of ΔC/EBPβ, a truncated alternate C/EBPβ translational product, LIP, which acts as a dominant-negative inhibitor of C/EBPβ activity (34), inhibits the production of NO and the expression of iNOS in mouse microglial cells (4). Consistently, here we show that ΔC/EBPβ also inhibits cytokine-induced activation of iNOS promoter in human astroglial cells (Fig. 8).

Here we have found that the combination of IL-1β and IFN-γ markedly induced the activation of NF-κB, AP-1, C/EBPβ, and GAS but not that of ISRE in human U373MG astroglial cells. Interestingly, gemfibrozil suppressed cytokine-induced activation of NF-κB, AP-1, and C/EBPβ but not that of GAS. Since STAT binds to GAS (40) and JAK is known to phosphorylate and activate STAT (40), our results suggest that gemfibrozil may not inhibit the JAK-STAT pathway in human astrocytes. On the other hand, IRF-1 binds to ISRE (41). IRF-1 has been found to be involved in the induction of iNOS by IFN-γ in mouse macrophages (42). Consistently, IFN-γ cannot induce iNOS in macrophages isolated from IRF-1(−/−) mice (43). Although the promoter of human iNOS gene contains ISRE (30, 31, 44), the combination of IL-1β and IFN-γ did not modulate the activation of ISRE in any significant way, suggesting that IRF-1 is unlikely to act as an important regulator of cytokine-induced expression of iNOS in human astrocytes.

Fibrates drugs like gemfibrozil, clofibrate, and fenofibrate induce the proliferation of peroxisomes in rats and mice (45, 46). Continuous administration of fibrate drugs to the rats and mice for 40–50 weeks also leads to the formation of hepatic tumor (45–47). However, induction of hepatic tumor promotion by fibrate drugs has not been demonstrated in human, other primates, and guinea pig (46, 48), species that have lost their ability to synthesize ascorbate due to inherent loss of the gulonolactone oxidase gene. Braun et al. (48) have recently reported that the evolutionary loss of the gulonolactone oxidase gene may contribute to the missing carcinogenic effect of peroxisome proliferators in humans, since ascorbate synthesis is accompanied by H2O2 production, and consequently its induction can be potentially harmful. Furthermore, recent studies have also revealed that humans have considerably lower levels of PPAR-α in liver than rodents, and this difference may, in part, explain the species differences in the carcinogenic response to peroxisome proliferators (46). In addition, gemfibrozil does not require PPAR-α to inhibit the activation of proinflammatory transcription factors and the induction of iNOS in human astroglial cells. Taken together, these observations suggest that gemfibrozil as an anti-neuroinflammatory drug may not cause human health problems.

NO, a short-lived and diffusible free radical, plays many roles as a signaling and effector molecule in diverse biological systems; it is a neuronal messenger and is involved in vasodilation as well as in antimicrobial and antitumor activities (49). On the other hand, NO has also been implicated in several CNS disorders, including inflammatory, infectious, traumatic, and degenerative diseases (5–8, 50). There is considerable evidence for the transcriptional induction of iNOS (the high output isoform of NOS) in the CNS that is associated with autoimmune reactions, acute infection, and traumatic brain injury (5–8, 50). Once NO is formed, it spontaneously reacts with O2 to form peroxynitrite (ONOO−), the most reactive derivative of NO known so far (51). Both NO and peroxynitrate are potentially toxic molecules to neurons and oligodendrocytes that may mediate toxicity through the formation of iron-NO complexes of iron-containing enzyme systems (52), oxidation of protein sulfhydryl groups (51), nitration of proteins, and nitrosylation of nucleic acids and DNA strand breaks (53).

In the CNS, iNOS is expressed mainly by activated astrocytes and microglia, the two glial cell types involved in intrac-
erebral immune regulation. Astrocytes are the major glial cell population in the central nervous system; therefore, induction of iNOS in astrocytes may be an important source of NO in CNS inflammatory disorders associated with neuronal and oligodendrocytes death. Therefore, gemfibrozil being capable of attenuating the activation of proinflammatory transcription factors and the expression of iNOS in human astrocytes may find therapeutic application in neuroinflammatory and neurodegenerative disorders.

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REFERENCES