Neuroinflammatory Role of Prostaglandins during Experimental Meningitis: Evidence Suggestive of an in Vivo Relationship between Nitric Oxide and Prostaglandins

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ABSTRACT

Nitric oxide (NO) and prostaglandins are inflammatory mediators produced during meningitis. The purpose of the present study was to pharmacologically inhibit cyclooxygenase-2 (COX-2) and inducible NO synthase (iNOS) to 1) explore the prostaglandin contribution to blood-cerebrospinal fluid barrier permeability alterations and 2) elucidate the in vivo concentration relationship between prostaglandin E2 (PGE2) and NO during experimental meningitis. Intracisternal injection of lipopolysaccharides (LPSs, 200 μg) induced neuroinflammation. Rats were dosed with nimesulide (COX-2 inhibitor), aminoguanidine (iNOS inhibitor), or vehicle. Evans blue was used to assess blood-cerebrospinal fluid barrier permeability. Meningeal NO and cerebrospinal fluid PGE2 were assayed using conventional methods. (Results are expressed as mean ± S.E.M. of 5–9 rats/group.) Nimesulide failed to prevent blood-cerebrospinal fluid barrier disruption [cerebrospinal fluid Evans blue (micrograms per milliliter); control, 0.22 ± 0.22%; LPS, 11.58 ± 0.66; LPS + nimesulide, 10.58 ± 0.86; *p < 0.05; ANOVA]. Although nimesulide decreased PGE2 (picograms per microliter; p < 0.01) in LPS + nimesulide rats (13.9 ± 1.96) versus LPS + vehicle (73.8 ± 12.4), meningine NO production (picomoles/30 min/106 cells; p < 0.01) increased unexpectedly in LPS + nimesulide rats (439 ± 47) versus LPS + vehicle rats (211 ± 31). In contrast, aminoguanidine inhibited meningeal NO (picomoles/30 min/106 cells; p < 0.005) in LPS + aminoguanidine (111 ± 20) versus LPS (337 ± 48) but had no effects (p > 0.05) on PGE2. The in vivo relationship between PGE2 and NO was mathematically described by a biphasic, bell-shaped curve (r2 = 0.42; n = 27 rats; p < 0.0001). Based on these results, inhibition of prostaglandin synthesis not only fails to prevent blood-cerebrospinal fluid barrier disruption during neuroinflammation but also promotes increased meningeal NO production. The in vivo concentration relationship between PGE2 and NO is biphasic, suggesting that inhibition of COX-2 alone may promote NO toxicity through enhanced NO synthesis.

Blood-cerebrospinal fluid barrier and blood-brain barrier permeability alterations are suspected to contribute to the pathology of neurological diseases with a known inflammatory component, namely, Alzheimer’s disease, multiple sclerosis, human immunodeficiency virus-I dementia, cerebral ischemia, brain tumors, and meningitis (Boje, 1995a; Claudio et al., 1995; McGeer and McGeer, 1995; Adamson et al., 1996; Tomimoto et al., 1996; Zhang et al., 1996). Knowledge of the neuroinflammatory process is critical for devising alternative anti-inflammatory therapies. Current evidence points to a plethora of inflammatory mediators, including inflammatory cytokines, chemokines, leukocyte-endothelial adhesion molecules, prostaglandins, nitric oxide (NO), and reactive oxygen intermediates (Boje, 1995b, 1996; de Vries et al., 1997).

Both NO and prostaglandins are produced during neuroinflammatory diseases (Misko et al., 1995; Adamson et al., 1996; de Vries et al., 1997), and each mediator (i.e., NO and prostaglandins) may play a contributory role in blood-cerebrospinal fluid and blood-brain barrier disruption. Prostaglandin E2 (PGE2) and NO each mediate inflammation in noncentral nervous system models of inflammation (Corbett et al., 1993; Salvemini et al., 1994; Vane et al., 1994). Chronic nonsteroidal anti-inflammatory drug use, which inhibits prostaglandin synthesis, is associated with a decreased risk of developing Alzheimer’s disease (McGeer and McGeer, 1995; Breitner, 1996). On the other hand, treatment with NOS inhibitors reduces the detrimental effects in some central nervous system inflammatory diseases (Boje, 1995b,

ABBREVIATIONS: NO, nitric oxide; PGE2, prostaglandin E2; NOS, nitric-oxide synthase; iNOS, induced nitric-oxide synthase; COX-2, cyclooxygenase-2; HPLC, high-performance liquid chromatography; LPS, lipopolysaccharide; ANOVA, analysis of variance; IL, interleukin; TNF, tumor necrosis factor.

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In experimental meningitis, a model of central nervous system inflammation, NO and PGE\(_2\) formation parallels blood-brain barrier disruption (Jaworowicz et al., 1998). Pathological NO production during meningeal inflammation mediates hyperemia and blood-brain barrier and blood-cerebrospinal fluid barrier disruption. These effects are attenuated (but not abolished) by NOS inhibitors (Boje, 1995b, 1996; Koedel et al., 1995; Korytko and Boje, 1996). Administration of nonselective nonsteroidal antiinflammatory drugs provides partial amelioration of blood-cerebrospinal fluid barrier breakdown (Tuomanen et al., 1987; Kadurugamuwa et al., 1989b). NO infused intracerebrovascu larly in the form of NO prodrugs causes disruption of the blood-brain barrier (Boje and Lakhman, 2000). PGE\(_2\) dosed intracranially elicits disruption of the blood-cerebrospinal fluid barrier, as evidenced by white blood cell pleocytosis and increased cerebrospinal fluid protein concentrations (Kadurugamuwa et al., 1989a). Moreover, convincing evidence exists for the coinduction of enzymes that synthesize NO and prostaglandins, i.e., inducible nitric-oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), by cells of the blood-brain barrier (cultured human and rodent Type 1 astrocytes (Lee et al., 1994; Minghetti and Levi, 1995; Molinahagdo et al., 1995), endothelial cells (Kilbourn and Belloni, 1990; de Vries et al., 1995), and meningeal fibroblasts (Boje and Arora, 1992) during neuroinflammatory conditions.

Because an inflammatory process is thought to be a common feature of many central nervous system diseases, an enhanced knowledge of the inflammatory processes and their effects on the blood-cerebrospinal fluid and blood-brain barriers may provide additional insights for the rational design of new therapeutic approaches for many neuroinflammatory diseases. The initial intent of this study was to pharmacologically inhibit COX-2 to explore the contribution of prostaglandins to blood-cerebrospinal fluid barrier permeability alterations during experimental meningitis. It was the analysis of the cerebrospinal fluid PGE\(_2\) and meningeal NO concentrations that led to additional studies to explore the in vivo concentration relationship between PGE\(_2\) and NO during neuroinflammation of experimental meningitis.

**Materials and Methods**

**Materials.** Male Sprague-Dawley rats (weighing 225–250 g) were purchased from Harlan (Indianapolis, IN). Ketamine and xylazine were obtained from J. A. Webster (Sterling, MA). Nimesulide was purchased from Harlan (Indianapolis, IN). Ketamine and xylazine were obtained from J. A. Webster (Sterling, MA). Nimesulide was purchased from Harlan (Indianapolis, IN). Ketamine and xylazine were purchased from JWR (West Chester, PA). PGE\(_2\) enzyme immunoassay kits were procured from Amersham Biosciences, Inc. (Piscataway, NJ). Lipopolysaccharides (LPSs; *Escherichia coli* serotype 0127:B8) and all other chemicals were obtained from Sigma-Aldrich (St. Louis, MO).

All procedures involving animals were approved by the University of Buffalo Institutional Animal Care and Use Committee and performed according to the guidelines set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications 85-23, revised 1985).

**In Vitro Inhibition of Meningeal iNOS and COX-2 Activity by Aminoguanidine and Nimesulide.** In vitro concentration-effect relationships were determined using immunostimulated meningeal tissue preparations as described previously by our group (Boje, 1995b; Korytko and Boje, 1996; Jaworowicz et al., 1998). In brief, rats were dosed intracranially with LPS (200 μg in 10 μl of artificial cerebrospinal fluid (102 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl\(_2\), 1.2 mM MgCl\(_2\), 0.67 mM Na\(_2\)HPO\(_4\), and 0.3 mM NaHPO\(_4\), pH 7.4) to induce meningeal inflammation, as described previously (Boje, 1995b; Korytko and Boje, 1996; Jaworowicz et al., 1998). Eight hours later, the animal was sacrificed for harvesting of meningeal tissues. Tissues were enzymatically dissociated into cellular suspensions and incubated overnight (18 h) in RPMI 1640 culture media (without serum) with increasing concentrations of the selective COX-2 inhibitor nimesulide or the selective iNOS inhibitor aminoguanidine. The media were assayed for PGE\(_2\) using Amersham’s PGE\(_2\) EIA kit (Jaworowicz et al., 1998), and for nitrite (NO\(_2\)), a stable degradation product of NO, using the Griess reaction (Boje and Arora, 1992; Korytko and Boje, 1996). It was verified that nimesulide does not interfere with the Griess reaction of NO-headspace assay; similarly, aminoguanidine does not interfere with the PGE\(_2\) EIA analysis. The cellular preparations were solubilized and assayed for protein content by the Lowry assay (Lowry et al., 1951). PGE\(_2\) and NO data were normalized by mg protein.

**Nimesulide Pharmacokinetics and HPLC Assay.** The purpose of determining nimesulide pharmacokinetics in the rat was to design a rational dosing regimen that effectively inhibits COX-2 with a targeted, steady-state nimesulide concentration. On the day before each pharmacokinetic study, male Sprague-Dawley rats (225–250 g) were anesthetized with ketamine (60 mg/kg) and xylazine (5 mg/kg) and surgically cannulated at the left femoral artery and right jugular vein. For the single, i.v. dose pharmacokinetic study, rats (n = 5) were dosed with 1 mg/kg nimesulide (dissolved in 60% polyethylene glycol 400, 5% ethanol, and 35% saline) via the left femoral arterial catheter. Serial blood samples (0.2 ml) were obtained from the right jugular vein catheter at 0, 0.083, 0.16, 0.3, 0.5, 0.8, 1, 2, 3, 4, 5, 6, and 8 h. For the constant rate infusion study, rats (n = 5) were dosed with a nimesulide loading dose (1.14 mg/kg) followed by a constant rate infusion (132 μg/kg/h) via the left femoral artery. Blood samples (0.2 ml) were collected at 0, 0.5, 1, 2, 3, 4, 5, 6, and 8 h after dosing. Nimesulide plasma samples were assayed by reverse-phase UV-HPLC, as published previously by our group (Jaworowicz et al., 1999).

**In Vivo Nimesulide Dosing: Effects on Meningeal NO, Cerebrospinal Fluid PGE\(_2\) and Blood-Cerebrospinal Fluid Barrier Disruption.** A nimesulide dosing regimen (i.v. bolus plus infusion) was designed from the single-dose nimesulide pharmacokinetic studies (see above) to attain plasma concentrations of 20 μM, because the in vitro studies showed that meningeal COX-2 was effectively inhibited at this concentration (see Results).

To study the in vivo inhibitory effects of nimesulide during meningeal inflammation, rats were randomly assigned to one of four treatment groups (n = 5–9 rats/treatment). On the day before the study, male Sprague-Dawley rats (225–250 g) were anesthetized with ketamine (60 mg/kg) and xylazine (5 mg/kg) and surgically cannulated at the left femoral artery and right jugular vein. To elicit meningeal inflammation, one group was dosed with LPS intracranially (200 μg in 10 μl of sterile artificial cerebrospinal fluid), followed by nimesulide (1.14 mg/kg i.v. bolus + 132 μg/kg/h infusion). Another group was dosed with LPS intracranially (200 μg), followed by equivalent bolus and infusion volumes of vehicle. Control rats were dosed intracranially with sterile artificial cerebrospinal fluid (10 μl) followed by nimesulide (1.14 mg/kg i.v. bolus + 132 μg/kg/h infusion) or equivalent volumes of vehicle. Eight hours later, cerebrospinal fluid and meninges were obtained for analysis of infiltration of white blood cells, PGE\(_2\), and NO, as described previously (Jaworowicz et al., 1998).

For assessment of the effects of nimesulide on blood-cerebrospinal fluid barrier disruption, additional groups of rats were randomly assigned to one of four treatments, i.e., LPS + nimesulide, LPS + vehicle, artificial cerebrospinal fluid + nimesulide, and artificial cerebrospinal fluid + vehicle, as defined in the previous paragraph. At 7.5 h, rats were dosed intravenously with Evans blue in saline...
In Vivo Aminoguanidine Dosing: Effects on Meningeal NO and Cerebrospinal Fluid PGE$_2$. In a separate study of the effects of aminoguanidine during meningeal inflammation, rats were randomly assigned to one of four treatment groups ($n = 5–6$ rats/treatment). The aminoguanidine dosing regimen was previously demonstrated to significantly reduce meningeal NO synthesis as well as blood-brain barrier and blood-cerebrospinal fluid barrier permeability alterations (Boje, 1995b, 1996).

On the day before the study, male Sprague-Dawley rats (225–250 g) were anesthetized with ketamine (60 mg/kg) and xylazine (5 mg/kg) and surgically cannulated at the left femoral artery and right jugular vein. One group was dosed with LPS (200 μg) intracerebrospinally followed by aminoguanidine hemisulfate in sterile saline (180 mg/kg bolus i.v. loading dose with constant rate infusion of 1.04 mg/kg/h). A second group was dosed with LPS (200 μg) intracerebrospinally followed by equivalent bolus + infusion volumes of sterile saline vehicle. A last group was dosed with LPS (100 μg) followed by equivalent volumes of sterile saline vehicle. Eight hours later, cerebrospinal fluid and meninges were obtained for analysis of PGE$_2$ and NO, as described previously (Jaworowicz et al., 1998).

Data Analysis. In vitro inhibition data were analyzed with the Hill equation using SigmaPlot software (version 5.0; SPSS Inc., Chicago, IL). Nimesulide pharmacokinetics was analyzed with a two-compartment model using WinNonlin software (PharSight, Apex, NC). To determine whether a “bell-shaped” trend existed between cerebrospinal fluid PGE$_2$ and meningeal NO, nonlinear regression analysis was performed using SigmaPlot software (version 5.0; SPSS Inc.) using eq. 1 where $a$ is the peak PGE$_2$ level, $x_0$ is the peak NO level, and $b$ is an estimated parameter. A log normal function was used as a means of capturing the observed data in a mathematical model.

$$y = a \cdot e^{-0.5 \ln(x/x_0)^2}$$  

Data were statistically analyzed by unpaired $t$ test compared with control or by one- or two-way ANOVA with Newman-Keuls post hoc test. Data are expressed as mean ± S.E.M.

Results

In Vitro Inhibition of Meningeal COX-2 and iNOS Activity by Nimesulide and Aminoguanidine. The selective COX-2 inhibitor nimesulide concentration-dependently inhibited meningeal COX activity with an IC$_{50}$ value of 10 nM (Fig. 1, top). However, it was observed that nimesulide significantly reduced NO (as measured by its degradation product, nitrite) to $56.16 \pm 4.817\%$ ($p < 0.05$) at nimesulide concentrations $\geq 100$ μM. Aminoguanidine inhibited iNOS activity with an IC$_{50}$ value of 100 μM with little effect on COX-2 activity (Fig. 1, bottom).

Nimesulide Pharmacokinetics in the Rat. The purpose of characterizing nimesulide pharmacokinetics in the rat was to design a rational dosing regimen that effectively inhibited COX-2 with a targeted, steady-state nimesulide concentration. Based on the nimesulide IC$_{50}$ data (Fig. 1), a 20 μM steady-state nimesulide plasma concentration was predicted to inhibit COX-2 in vivo, with negligible effects on NO.

Nimesulide pharmacokinetics was determined after 1 mg/kg i.v. bolus dosing (Fig. 2A; $n = 5$ rats). Multicompartmental pharmacokinetic analysis revealed the following: total clearance (CL$_{TOT}$) = 21.4 ± 1.10 ml/kg/h; volume of distribution ($V_d$) = 187 ± 3.62 ml/kg; $t_{1/2}$ = 3.94 ± 0.210 h; and slopes and intercepts: $A = 3.40 ± 0.130$ μg/ml; $\alpha = 1416 ± 627$ μg/ml/h, $B = 5.31 ± 0.100$ μg/ml, and $\beta = 0.12 ± 0.01$ μg/ml/h.

These parameters were used to calculate an i.v. bolus plus infusion dosing regimen that would yield nimesulide constant concentrations of 20 μM (6.17 μg/ml). In a second study of nimesulide pharmacokinetics, a nimesulide loading dose (1.14 mg/kg) followed by a constant rate infusion (132 μg/kg/h) was administered to individual rats ($n = 5$). Nimesulide plasma concentrations of 5.88 ± 0.46 μg/ml were observed (Fig. 2B). These concentrations were 95% of the target 20 μM nimesulide concentration, verifying that the bolus plus infu-
concentrations in the LPS
ditional data on cerebrospinal fluid PGE2 and meningeal NO
(111 ± 20 pmol/30 min/10^6 cells versus LPS + control vehicle, 337 ± 48 pmol/30 min/10^6 cells; n = 5–6 rats; p < 0.005).
Aminoguanidine significantly decreased white blood cell cerebrospinal fluid migration (LPS + aminoguanidine, 2.46 ± 0.27 × 10^6 cells/ml versus LPS + control vehicle, 3.29 ± 0.21 × 10^6 cells/ml; n = 5–6 rats; p < 0.05), and exerted no significant effects on cerebrospinal fluid PGE2 concentrations (LPS + aminoguanidine, 59.31 ± 13.41 pg/μl; LPS + control vehicle, 50.33 ± 14.10 pg/μl; n = 5–6; p > 0.05).

Evidence for an in Vivo, Bell-Shaped Relationship between PGE2 and NO. Visual inspection of the cerebrospinal fluid PGE2 versus log NO data revealed a biphasic, bell-shaped curve (Fig. 3, A–C). These data were mathematically characterized using eq. 1. Aminoguanidine significantly decreased white blood cell cerebrospinal fluid, 188.1 ± 15.97 pmol/30 min/10^6 cells, and 0.3099 ± 0.0529, respectively. Figure 3B presents PGE2-NO data after intracisternal LPS (200 μg/kg) with or without administration of NOS or COX-2 inhibitors. Statistical analysis of the nonlinear regression yielded a significant relationship (r^2 = 0.50; n = 22 rats; p < 0.0001). One-way ANOVA between the regression line and the residuals yielded a p value of 0.001, again rejecting the hypothesis that a nonlinear trend does not exist. The goodness-of-fit of the mathematical model to the PGE2-NO data were assessed by through additional analysis. For each data set (Fig. 3, A–C), the data from the regression line and the residuals were subjected to a one-way ANOVA, resulting in rejection of the null hypothesis (p < 0.005; ANOVA), thus rejecting the hypothesis that a nonlinear trend does not exist between PGE2 and NO. In addition, the observed data (that is, the source population) were normally distributed about the regression line. These additional anal-

![Fig. 2. Nimesulide plasma concentrations versus time. A, nimesulide intravenous bolus dosing (1 mg/kg). B, nimesulide intravenous bolus (1.14 mg/kg) with constant rate infusion (132 μg/kg/h infusion). Data are mean ± S.E.M. (n = 5 rats/experiment).

In Vivo Nimesulide Inhibition of COX-2 Reduces White Blood Cell Infiltration but Fails to Inhibit Blood-Cerebrospinal Fluid Barrier Disruption. In rats dosed with LPS, nimesulide significantly reduced cerebrospinal fluid white blood cell infiltration by 50%, compared with rats dosed with LPS + vehicle (Table 1). Surprising, nimesulide administration failed to prevent or reduce blood-cerebrospinal fluid barrier disruption during neuroinflammation, as measured by significant cerebrospinal fluid concentrations of Evans blue, which is excluded by the central nervous system barriers under normal conditions (Table 1). Plasma concentrations of Evans blue were not statistically different among the treatment groups.

In Vivo Nimesulide Inhibition of COX-2 Elicits Increased NO Synthesis. In a separate study, cerebrospinal fluid PGE2 and meningeal NO concentrations were measured to elucidate why nimesulide administration did not prevent or reduce blood-cerebrospinal fluid barrier disruption. Nimesulide significantly decreased cerebrospinal fluid PGE2 concentrations in the LPS + nimesulide group by 80% compared with the LPS + vehicle group (Table 2). This argues that the nimesulide dosing regimen, although it did not completely suppress PGE2 synthesis, significantly inhibited COX activity. However, a most unexpected finding was observed: meningeal NO production was significantly doubled in the LPS + nimesulide group compared with the LPS + vehicle group (Table 2).

In Vivo Inhibition of iNOS by Aminoguanidine. Additional data on cerebrospinal fluid PGE2 and meningeal NO were obtained in a separate study of aminoguanidine inhibition of iNOS during experimental meningitis. Consistent with our previous study (Boje, 1995b), aminoguanidine significantly inhibited meningeal NO (LPS + aminoguanidine, 111 ± 20 pmol/30 min/10^6 cells versus LPS + control vehicle, 337 ± 48 pmol/30 min/10^6 cells; n = 5–6 rats; p < 0.005).

** Table 1**

<table>
<thead>
<tr>
<th>Treatment Group (Intracisternal + Drug)</th>
<th>Cerebrospinal Fluid White Blood Cell Infiltration (10^6 cells/ml)</th>
<th>Cerebrospinal Fluid Evans Blue (μg/ml)</th>
<th>Plasma Evans Blue (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial cerebrospinal fluid + vehicle</td>
<td>None detected</td>
<td>0.22 ± 0.22</td>
<td>47.44 ± 3.36</td>
</tr>
<tr>
<td>Artificial cerebrospinal fluid + nimesulide</td>
<td>None detected</td>
<td>0.00 ± 0.00</td>
<td>54.79 ± 3.59</td>
</tr>
<tr>
<td>LPS + vehicle</td>
<td>2.75 ± 0.93</td>
<td>11.58 ± 0.66**</td>
<td>64.74 ± 5.76</td>
</tr>
<tr>
<td>LPS + nimesulide</td>
<td>1.15 ± 0.28***</td>
<td>10.58 ± 0.86**</td>
<td>60.92 ± 6.13</td>
</tr>
</tbody>
</table>

** *p < 0.01 from all artificial cerebrospinal fluid groups by ANOVA with Newman-Keuls post hoc analysis.**

*** p < 0.001 from LPS + vehicle by unpaired t test. n = 5–6 rats/treatment group.
ys provide further support for the selection of a biphasic mathematical model to characterize the relationship between PGE\textsubscript{2} and NO. The statistical results argue for a statistically significant, although empirical, biphasic relationship between PGE\textsubscript{2} and NO during experimental meningitis.

**Discussion**

Inflammation of the central nervous system is a host-defense response to a perceived foreign invasion. This response is a general, localized protective reaction that involves a complex series of events, including cerebrovascular dilatation; endothelial and white blood cell activation; cytokine, chemokine, and inflammatory mediator secretion; and expression of adhesion molecules. These events lead to a loss of blood-cerebrospinal fluid barrier and blood-brain barrier integrity evidenced by increased vascular permeability, eflux of fluid and plasma proteins into cerebrospinal fluid and brain tissue, and leukocyte recruitment and migration into the area of inflammation. Altered blood-cerebrospinal fluid barrier and blood-brain barrier integrity can contribute to neurotoxic and neurodegenerative processes. Because neuroinflammation is a common feature of many neurological diseases, an enhanced knowledge of the inflammatory processes and their effects on the blood-cerebrospinal fluid and blood-brain barriers may provide additional insights for new therapeutic approaches for many diseases with a neuroinflammatory component.

COX-2 and iNOS are part of a family of primary inflammatory response genes, whereby COX-2 and iNOS expression are coordinately modulated by LPS, bacterial endotoxins, and various cytokines. De novo synthesis of COX-2 occurs during pathological conditions, whereby prostanoids promote inflammation by binding to G protein-coupled cell surface receptors that transduce the signal via cAMP vasodilatation, resulting in increased vascular permeability, hyperalgesia, and fever (Mitchell et al., 1995). Of the prostanoids, PGE\textsubscript{2} is a major eicosanoid found in many neurological conditions (Vane and Botting, 1995), including central nervous system inflammatory diseases (Megeer and McGeer, 1995). Similar to COX-2, de novo synthesis of iNOS occurs after cellular exposure to a variety of agents. Once expressed, iNOS produces prodigious quantities of NO for hours to days. NO, its redox congener (NO\textsuperscript{+}, NO\textsuperscript{2+}, and NO\textsuperscript{−}) (Stamler et al., 1992), ONOO\textsuperscript{−} (the product of NO reaction with O\textsubscript{2} (Beckman et al., 1994)) and NO degradation products (NO\textsubscript{x} and NO\textsubscript{2}−) mediate toxicity through the oxidation of protein sulfhydryls, complexation with iron-containing respiratory enzymes, nitration of tyrosine residues, and attack of DNA nucleophilic centers (Boje, 1998).

In our previous studies of experimental meningitis, the administration of a pharmacological inhibitor of iNOS, aminoguanidine resulted in a partial attenuation of blood-cerebrospinal fluid barrier and blood-brain barrier permeability (Boje, 1995b, 1996). Other experiments led to the observation that the concentration of meningeal NO and cerebrospinal fluid PGE\textsubscript{2} formation parallel that of blood-brain barrier disruption (Jaworowicz et al., 1998). These results suggested that other inflammatory factors may contribute to blood-cerebrospinal fluid barrier and blood-brain barrier disruption during neuroinflammation.

Accordingly, we hypothesized that inhibition of prostaglandin synthesis would ameliorate blood-cerebrospinal fluid barrier disruption during experimental meningitis. In vitro studies (Fig. 1) were performed to ensure that 1) nimesulide was specific for immunoinduced meningeal COX-2 with negligible effects on iNOS, and 2) aminoguanidine was specific for meningeal iNOS with negligible effects on COX-2. Pharmacokinetic studies of nimesulide disposition in the rat were performed (Fig. 2) to design a dosing regimen that would attain constant, inhibitory COX-2 concentrations of nimesulide. In the present study of experimental meningitis, nimesulide significantly reduced cerebrospinal fluid PGE\textsubscript{2} and white blood cell levels (Table 2), consistent with its known inhibition of COX-2 and pleiotropic inhibitory effects on neutrophil functions (Dapino et al., 1994). However, nimesulide failed to prevent blood-cerebrospinal fluid barrier disruption, as measured by the cerebrospinal fluid accumulation of Evans blue dye (Table 1).

The original hypothesis, namely, that pharmacological inhibition of prostaglandin synthesis via COX-2 would prevent or reduce blood-cerebrospinal fluid barrier disruption during experimental meningitis, was not substantiated by the data. Yet, the data revealed a paradoxical situation: Nimesulide inhibition of COX-2 elicited significantly higher levels of meningeal NO during experimental meningitis than drug vehicle-treated rats (Table 2). The failure of the nimesulide dosing regimen to attenuate barrier disruption might be due to the elevated production of NO, which is known to disrupt the blood-brain and blood-cerebrospinal fluid barriers (Boje, 1995b, 1996). These data suggest that the presence of prostaglandins, or some aspect of COX-2 activity, may contribute to an inhibitory feedback loop for iNOS activity under inflammatory conditions.

Published reports suggest that NO and prostaglandins can modulate the activity of their own respective enzymes, and modulate each other’s enzymatic counterpart. Pharmacological inhibition of one enzyme may also alter the activity and/or expression of the other enzyme. A survey of the literature reveals that the modulatory role each mediator plays in the production of the other is controversial, complicated, confusing, and contradictory. Alternatively, it could be speculated that prostaglandins and NO are modulated in a biphasic manner during neuroinflammation. This prompted a reexamination of all the NO

### TABLE 2

<table>
<thead>
<tr>
<th>Treatment Group (Intracisternal + Drug)</th>
<th>Meningeal NO Production</th>
<th>Cerebrospinal Fluid PGE\textsubscript{2}</th>
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<tbody>
<tr>
<td>Artificial cerebrospinal fluid + vehicle</td>
<td>None detected</td>
<td>1.68 ± 0.61</td>
</tr>
<tr>
<td>Artificial cerebrospinal fluid + nimesulide</td>
<td>None detected</td>
<td>3.75 ± 2.17</td>
</tr>
<tr>
<td>LPS + vehicle</td>
<td>211 ± 31*</td>
<td>73.8 ± 12.4*</td>
</tr>
<tr>
<td>LPS + nimesulide</td>
<td>459 ± 47**</td>
<td>13.9 ± 1.96*</td>
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**p < 0.01; * p < 0.05 from all other groups by ANOVA with Newman-Keuls post hoc analysis. n = 5–6 rats/treatment group."
and PGE₂ data obtained from 1) LPS (100 and 200 μg) alone (Fig. 3A), 2) LPS (200 μg) with or without inhibitors (Fig. 3B), and 3) all data (LPS 100 or 200 μg with/without inhibitors) (Fig. 3C). Visual inspection of these plots of cerebrospinal fluid PGE₂ versus meningeal NO revealed bell-shaped relationships in each case (Fig. 3, A–C). A mathematical function that describes bell-shaped behaviors (eq. 1) empirically described a statistically significant biphasic relationship between PGE₂ and NO in each case (Fig. 3, A–C). It could be argued that the relationship illustrated in Fig. 3A could be attributed to differential LPS dose-dependent effects on COX-2 and iNOS. However, when the 100-μg LPS data are omitted (leaving only LPS 200 μg with or without inhibitors), the relationship remains statistically significant, with the caveat that the upward rise of the mathematical fit is characterized by only a few data points. When the data are examined collectively (100 and 200 μg with or without inhibitors), the relationship is still statistically significant, but with a slightly lower r² due to increased variability.

The demonstration of a biphasic relationship suggests an alternative interpretation for the disparate literature data: the in vivo concentration of NO modulates PGE₂ both positively and negatively. The additional pharmacological inhibition data (Table 2; Fig. 3, B and C) additionally suggests that reduction of prostaglandin levels via COX-2 inhibition enhances NO synthesis.

The literature suggests a number of in vitro mechanisms that may provide a mechanistic basis for the biphasic relationship. NO is reported to exert dual effects on COX-2 activity, depending on the reactivity of NO or its peroxynitrite form, ONOO⁻. Low concentrations of NO or ONOO⁻ stimulate the synthesis of prostaglandins via S-nitrosylation of COX-2 thiols, whereas high concentrations of ONOO⁻ inhibit COX-2 via peroxynitrite-mediated nitration of critical tyrosine residues (Goodwin et al., 1999). The present work provides indirect support consistent with these mechanisms of NO effects on COX-2, in an intact in vivo system, in that 1) a biphasic, bell-shaped relationship between PGE₂ and NO was observed in vivo in the untreated inflammatory state; and 2) whereas aminoguanidine inhibition of iNOS resulted in a significant, yet partial attenuation of NO synthesis, the corresponding PGE₂-NO data fell on the left-hand, upward rise of the biphasic relationship (Fig. 3, B and C).

PGE₂, via cAMP formation subsequent to activation of G protein-coupled prostanoid receptors, has both stimulatory and inhibitory effects on iNOS expression and activity depending on the in vitro concentration and cell type (Galea and Feinstein, 1999). Limited levels of cAMP stimulate iNOS (Galea and Feinstein, 1999) are insightful in understanding the ostensibly conflicting nimesulide effects on NO as observed in the present work. We observed that nimesulide (≥100 μM) significantly inhibited meningeal iNOS activity (Fig. 1) in short-term cell culture (~18 h); conversely, in vivo administration of nimesulide shortly after induction of meningeal inflammation significantly increased iNOS activity (Table 2). In the in vitro short-term cultures, LPS triggered iNOS expression in vivo for 8 h, after which meningeal tissues were harvested. It is at this point when nimesulide was added to inhibit COX-2 activity, with a subsequent reduction of PGE₂ synthesis and cAMP levels. Be-
cause limited levels of cAMP promote iNOS expression via protein kinase A phosphorylation of transcriptional factors (Galea and Feinstein, 1999), we speculate that steady-state iNOS synthesis declined due to significantly reduced cAMP levels stemming from COX-2 inhibition (≥100 μM nimesulide) during the ensuing 10-h in vitro culture period. In the in vivo situation, the animals were dosed with nimesulide immediately after LPS injection and iNOS activity was measured 8 h later. Because high levels of cAMP inhibit cytokine release (IL-1β and TNF-α; factors that promote iNOS expression) (Galea and Feinstein, 1999), we speculate that the incomplete in vivo inhibition of COX-2 (but accompanied by a significant reduction of cerebrospinal fluid PGE2; Table 2) elicited reduced but sufficient levels of cAMP that promoted iNOS expression via protein kinase A phosphorylation of transcriptional factors and or IL-1β and TNF-α release.

In summary, unlike that observed with iNOS inhibition, COX-2 inhibition fails to prevent or attenuate blood-cerebrospinal fluid barrier disruption during experimental meningitis. The significance of the present experiments derives from the PGE2, NO data, which suggest, at least empirically, that the in vivo relationship between PGE2 and NO is a biphasic (bell-shaped) relationship. Importantly, this relationship suggests that pharmacological inhibition of COX-2 alone may promote NO toxicity through enhanced NO synthesis during neuroinflammation.

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References


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