Gastric Tolerability and Prolonged Prostaglandin Inhibition in the Brain with a Nitric Oxide-Releasing Flurbiprofen Derivative, NCX-2216 [3-[4-(2-Fluoro-α-methyl-[1,1′-biphenyl]-4-acetyloxy)-3-methoxyphenyl]-2-propenoic acid 4-nitrooxy butyl ester]

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ABSTRACT
NCX-2216 [3-[4-(2-fluoro-α-methyl-[1,1′-biphenyl]-4-acetyloxy)-3-methoxyphenyl]-2-propenoic acid 4-nitrooxy butyl ester] is an NO-releasing flurbiprofen derivative that also contains a ferulic acid (antioxidant) moiety. NCX-2216 has been shown to be effective in reducing β-amyloid deposition in a transgenic mouse model of Alzheimer’s disease. The tolerability of this compound in the stomach and its ability to suppress prostaglandin synthesis in the brain are not known. The purpose of this study was to assess the contribution of nitric oxide (NO) and ferulic acid to the pharmacological properties of NCX-2216 versus flurbiprofen; thus, we compared their gastric tolerability and suppression of prostaglandin synthesis, peripherally and centrally. Oral flurbiprofen produced extensive gastric damage and suppressed gastric prostaglandin synthesis. In contrast, while suppressing prostaglandin production, equimolar doses of NCX-2216 did not cause detectable gastric injury. The NO-releasing moiety of NCX-2216 (but not the ferulic acid moiety) was crucial for the gastric safety of this compound. NCX-2216 substantially inhibited prostanoid synthesis despite not being detectable in plasma and despite producing only low amounts of flurbiprofen in plasma and in the brain. Inhibition of brain prostaglandin synthesis by NCX-2216 (22 mg/kg) persisted for a much longer period of time (up to 48 h) than was seen with flurbiprofen (≤12 h). These results demonstrate that a single administration of NCX-2216 can produce prolonged suppression of brain prostaglandin synthesis without causing gastric injury. It is likely that an active metabolite of NCX-2216 contributes to the suppression of cyclooxygenase activity. NCX-2216 may represent an attractive alternative to conventional nonsteroidal anti-inflammatory drugs for long-term treatment of a variety of inflammatory disorders, especially those occurring in the central nervous system.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for treating pain and inflammation. In recent years, there has been considerable interest in the potential use of this class of drugs for long-term preventative applica-

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ABBREVIATIONS: NSAID, nonsteroidal anti-inflammatory drug; NO, nitric oxide; NCX-2216, 3-[4-(2-fluoro-α-methyl-[1,1′-biphenyl]-4-acetyloxy)-3-methoxyphenyl]-2-propenoic acid 4-nitrooxy butyl ester; ELISA, enzyme-linked immunosorbent assay; COX, cyclooxygenase; NCX-2111, (S)-N-acetyl-S-[α-methyl-4-(2-methylpropyl)benzeneacetyl)cysteine-4-(nitroxy) butyl ester; ES, electrospray; CSF, cerebrospinal fluid; PGE₂, prostaglandin E₂; DPPH, 1,1-diphenyl-2-picrylhydrazyl; PG, prostaglandin; NCX 2057, 3-[4-(hydroxy-3-methoxyphenyl)-2-propenoic acid 4-nitrooxy]butyl ester; NCX 2226, 3-[4-(2-fluoro-α-methyl-[1,1′-biphenyl]-4-acetyloxy)-3-methoxyphenyl]-2-propenoic acid 4-nitrooxy butyl ester.
Jantzen et al. (2002) examined the effects of a nitric oxide-releasing derivative of flurbiprofen (NCX-2216) in a double transgenic (amyloid precursor protein and presenilin-1) mouse model of Alzheimer’s disease. NCX-2216 was found to significantly reduce β-amyloid deposition, whereas the parent drug flurbiprofen elicited severe gastroenteropathy, cachexia, and, in some instances, death. NCX-2216 is somewhat different from the originally described NO-NSAIDs (Wallace et al., 1994a) in that it consists not only of flurbiprofen and an NO-releasing moiety, but also the antioxidant ferulic acid (Fig. 1). The rationale behind the design of this compound is that the antioxidant and anti-inflammatory activities of the ferulic acid and flurbiprofen moieties, respectively, would target critical steps in the pathogenesis of Alzheimer’s disease (McGeer and Rogers, 1992; Behl, 1999), whereas the NO-releasing moiety would protect the stomach from the detrimental effects of flurbiprofen. However, the extent to which the different components of NCX-2216 would contribute to gastric safety and to other biological activities of this compound is not clear. Moreover, little is known of the ability of NCX-2216 to deliver flurbiprofen to the systemic circulation and to the brain or the ability of this compound to inhibit prostaglandin synthesis in the stomach and brain.

In the present study, we have examined the gastric tolerability of NCX-2216 and some structurally related compounds and their ability to suppress gastric prostaglandin synthesis. We have also examined whether or not NCX-2216 administration results in significant systemic and central levels of flurbiprofen and its effects on brain prostanoid synthesis. Our results demonstrate that, despite the suppression of gastric prostaglandin synthesis, NCX-2216 does not induce damage in the stomach, that the absence of gastric damage is attributable to the NO-releasing moiety of the compound, and that this compound produces prolonged suppression of prostaglandin synthesis in the brain.

**Materials and Methods**

**Animals.** Male Wistar rats were obtained from Charles River Canada (Montreal, PQ, Canada) and were housed at the Animal Care Service of the University of Calgary. The rats were deprived of food for 18 to 20 h prior to an experiment.

**Gastric Tolerability and Prostaglandin Synthesis.** Groups of 5 to 6 male Wistar rats were fasted overnight then given NCX-2216 or flurbiprofen by orogastric gavage. The drugs were initially dissolved in dimethyl sulfoxide then diluted in 1% carboxymethylcellulose (the final concentration of dimethyl sulfoxide was 1%). An additional group of five rats received only the vehicle. Flurbiprofen was tested at 1, 3, 10, and 30 mg/kg (4, 12, 40, and 120 μmol/kg, respectively). NCX-2216 was tested at equimolar doses to those of flurbiprofen. Three hours after drug or vehicle administration, the rats were killed, and the stomach was excised for blind assessment of the degree of mucosal damage (Wallace et al., 2000).

We then compared the effects of flurbiprofen at 30 mg/kg to equimolar doses of NCX-2216, ferulic acid, NCX-2057, and NCX-2226 (see Fig. 1). The same vehicle was used as described above. The rats were euthanized 3 h after drug administration; and the extent of gastric damage was blindly evaluated. Samples of the corpus region of the stomach were then excised and processed, as described previously, for the measurement of gastric prostaglandin synthesis (Wallace et al., 2000). Prostaglandin E2 (PGE2) was measured using a specific ELISA.

**Antioxidant Activity.** Because oxygen-derived free radicals have been reported to contribute to the pathogenesis of NSAID-induced gastric damage (Vaananen et al., 1991), it is possible that antioxidant properties of the various test drugs could contribute to any observed gastric tolerability of those drugs. The antioxidant activity of the test drugs was therefore measured using a spectrophotometric assay (Vaananen et al., 1991) modified from that originally described by Smith et al. (1987). The following compounds were dissolved in 95% ethanol: flurbiprofen, NCX-2216, ferulic acid, NCX-2057, NCX-2226, and ascorbic acid. Serial dilutions of each drug

![Fig. 1. Chemical structures of NCX-2216 and other related compounds. The molecular weights of the compounds were as follows: flurbiprofen, 244.3; NCX-2216, 537.5; NCX-2057, 311.3; ferulic acid, 94.2; and NCX-2226, 420.0.](image-url)
were then made in 95% ethanol. An aliquot (20 μl) of each drug, at various dilutions, was added to the wells of a 96-well plate. A stable free radical [1,1-diphenyl-2-picrylhydrazyl (DPPH)], also dissolved in 95% ethanol (180 μl), was then added to each well, and absorbance at 540 nM was monitored over a 10-min period. DPPH is a purple-colored substance, which is converted to a colorless substance in the presence of antioxidants. The concentration of each test compound that reduced absorbance to 50% was then calculated (EC50). Ascorbic acid was included in this analysis as a positive control. All test compounds were assessed at concentrations ranging from 1 to 300 μM.

Plasma Levels of Flurbiprofen and NCX-2216. Groups of 35 male Wistar rats were given flurbiprofen (5 mg/kg) or an equimolar dose of NCX-2216 via orogastric gavage. Subgroups of five rats from each group were anesthetized with Halothane at 30 min, 1, 2, 4, 6, 12, and 24 h after administration of the test drugs. Blood was immediately drawn from the inferior vena cava and centrifuged to permit collection of serum. The serum samples were frozen and stored at −20°C until the time of assay for drug levels (see below).

Whole Blood Thromboxane Synthesis. Whole blood thromboxane synthesis was measured as an index of systemic cyclooxygenase (COX)-1 activity (Wallace et al., 2000). An aliquot (1 ml) of blood from the samples taken 4 h after drug administration in the studies described above was transferred to a borosilicate tube and allowed to incubate for 45 min at 37°C. The samples were then centrifuged for 10 min (1,500g), and the supernatants were immediately frozen and stored at −20°C until ELISA assays for thromboxane B2 were performed, as described previously (Wallace et al., 2000).

Plasma Sample Preparation. For quantification of plasma NCX-2216 concentrations, the following procedure was followed. Fifty microliters of the internal standard solution (1 μg/ml NCX-2111 in 50% methanol-aqueous solution) was added to a 400-μl aliquot of each plasma sample (or calibration standard). The tubes were briefly vortex-mixed for approximately 10 s then extracted with 4 ml of diethyl ether. The mixture was vortex-mixed for 1 min. The upper organic layer was transferred to another set of clean glass tubes, and the organic solvent was evaporated under N2 at 37°C. The dry residues were reconstituted with 0.2 ml of mobile phase (see below) and transferred to the autoinjector microvials. For quantification of plasma flurbiprofen concentrations, the same procedure described for NCX-2216 was followed, except for the employed internal standard (50 μl of 1 μg/ml ibuprofen in 50% methanol aqueous solution).

Brain Flurbiprofen Levels. Flurbiprofen (10 mg/kg) or NCX-2216 equimolar doses were administered orally to groups of 15 rats each. The dosages of the test drugs were selected because, in the carrageenan-airpouch model (Wallace et al., 1999), we had found them to be equipotent in reducing inflammation (data not shown). At 3, 12, and 24 h thereafter, subgroups of five rats for each treatment group were euthanized, and the left hemisphere of each brain was excised and immediately placed in 3.0 ml of extraction solvent (isopropanol-ethyl acetate-0.1 N HCl; 3:3:1) and 3.0 ml of distilled water. The brain samples were homogenized for 30 s then centrifuged at 1500g for 10 min at 4°C. The organic phase was transferred to a borosilicate tube and evaporated to dryness under a stream of nitrogen at 37°C. The samples were reconstituted in 200 μl of phosphate-buffered saline (pH 7.4) and stored at −70°C until measurement of PGE2 content by ELISA was performed (Wallace et al., 2000). In some experiments, the right hemisphere of the brain from each rat was excised and processed for detection of COX-1 and COX-2 expression by Western blotting, as described in detail previously (Turesin et al., 2003).

Materials

The antibodies for Western blotting and the kits for PGE2 and thromboxane B2 measurement were obtained from Cayman Chemical (Ann Arbor, MI). Ferulic acid, DPPH, and flurbiprofen were obtained from Sigma-Aldrich (St. Louis, MO). NCX-2111, NCX-2226, NCX-2057, and NCX-2226 were synthesized by the NiCoX Research Institute (Bresso, Milan, Italy). All other chemicals and solvents were obtained from Fisher Scientific Co. (Edmonton, AB, Canada).

Results

Gastric Injury and Prostaglandin Synthesis. Oral administration of flurbiprofen to rats resulted in a dose-dependent increase in the severity of hemorrhagic damage in the stomach (Fig. 2). The damage was noted primarily in the corpus region of the stomach, mainly along the crests of rugal folds. Histological examination of the tissues revealed that the damage was confined to the mucosal layer, consisting of extensive epithelial necrosis and hemorrhage. Oral administration of NCX-2216 at doses equimolar to those of flurbiprofen (1–30 mg/kg) did not result in significant gastric damage.
Blind histological tissue evaluation taken from the stomachs of rats treated with NCX-2216 confirmed the absence of damage. To examine the relative contribution of the NO-releasing- and ferulic acid moieties of NCX-2216 to its gastric-sparing properties, we tested the effects of flurbiprofen at 30 mg/kg and equimolar doses of NCX-2216, ferulic acid, NCX-2057 (NO-releasing ferulic acid), and NCX-2226 (flurbiprofen-ferulic acid conjugate) (see Fig. 1).

As shown in Fig. 3 (top panel), only flurbiprofen and those compounds that contained a flurbiprofen moiety (NCX-2216 and NCX-2226) caused a significant reduction of gastric prostaglandin synthesis. NCX-2226 produced gastric damage comparable in severity to that seen with flurbiprofen (Fig. 3, bottom panel). Because NCX-2216 and NCX-2226 differ only by the lack of a NO-releasing group in the latter, these observations suggest that the NO-releasing group is crucial for the gastric tolerability of NCX-2216. Ferulic acid and NCX-2057 did not affect gastric prostaglandin synthesis and did not cause gastric damage.

Antioxidant Activity. Assessment of antioxidant activity in vitro revealed that ferulic acid (ED$_{50}$ of $\sim 80 \mu$M) was approximately half as potent as ascorbic acid (ED$_{50}$ $\sim 40 \mu$M) in terms of free radical scavenging (Table 1). NCX-2057 exhibited antioxidant activity comparable to that of ferulic acid. None of the other compounds exhibited antioxidant activity at concentrations as great as 300 $\mu$M.

Plasma Drug Levels and Cyclooxygenase Suppression. Figure 4A shows the plasma levels of flurbiprofen after oral administration of flurbiprofen (10 mg/kg) or an equimolar dose of NCX-2216. NCX-2216 itself was not detected in any plasma sample. Plasma levels of flurbiprofen following administration of NCX-2216 were relatively low. Interestingly, the levels increased 12 to 24 h after oral administration. Despite the low plasma levels of flurbiprofen following administration of NCX-2216, significant inhibition of whole blood thromboxane synthesis was observed. Figure 4B shows the suppression of whole blood thromboxane synthesis by orally administered NCX-2216 and flurbiprofen. At all doses (1–30 mg/kg), both flurbiprofen and NCX-2216 (equimolar doses) produced profound suppression of whole blood thromboxane synthesis, which is an index of COX-1 activity (Wallace et al., 2000). Flurbiprofen produced a more pronounced suppression of thromboxane synthesis than NCX-2216 across the range of doses, but with both compounds, the degree of suppression relative to the vehicle-treated controls was greater than 90%.

Brain Flurbiprofen Levels and Cyclooxygenase Inhibition. NCX-2216 was not detected in brain tissue at any time after its administration. Figure 5 illustrates the brain levels of flurbiprofen following oral administration of equimolar doses of NCX-2216 or flurbiprofen. As with plasma, the levels of flurbiprofen in the brain at 3 h were much less than were seen after the administration of flurbiprofen.
profen itself. However, at 12 and 24 h postadministration, the two drugs delivered similar amounts of flurbiprofen to brain tissue. At 24 h, the brain tissue levels of flurbiprofen were significantly greater in the NCX-2216-treated rats than in flurbiprofen-treated rats.

Of the various compounds tested, only flurbiprofen and the compounds that contained a flurbiprofen moiety (NCX-2216 and NCX-2226) significantly suppressed brain and CSF prostaglandin levels (Fig. 6); however, there were distinct differences among these compounds in terms of the duration of inhibition of PG synthesis after their administration. Although the inhibitory effects of flurbiprofen were only observed for the first 3 to 12 h after administration, marked suppression of brain PG synthesis by NCX-2216 was apparent even 48 h after its administration. Inhibition of brain PG synthesis by NCX-2216 was not apparent 72 h after administration (data not shown). NCX-2226 (flurbiprofen-ferulic acid) also produced more prolonged suppression of prostaglandin levels in the brain and CSF than was seen with flurbiprofen.

### TABLE 1

Antioxidant activity of flurbiprofen and derivatives

The ability of the test compounds at concentrations of 1 to 300 μM to scavenge a stable free radical (DPPH) was assessed in vitro. The EC₅₀ represents the concentration of the test compound that scavenged half of the free radical.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Antioxidant Activity (EC₅₀)</th>
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<tbody>
<tr>
<td>Ascorbic acid</td>
<td>40.1</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>&gt;300</td>
</tr>
<tr>
<td>NCX-2216</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>83.2</td>
</tr>
<tr>
<td>NCX-2057</td>
<td>103.4</td>
</tr>
<tr>
<td>NCX-2226</td>
<td>&gt;300</td>
</tr>
</tbody>
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Ascorbic acid 40.1 ▪ Flurbiprofen ▪ NCX-2216 ▪ NCX-2226

![Fig. 4. A, plasma levels of flurbiprofen after oral administration of flurbiprofen (10 mg/kg) or an equimolar dose of NCX-2216. NCX-2216 itself was not detectable in plasma at any time. B, whole blood thromboxane synthesis 4 h after oral administration of flurbiprofen or equimolar doses of NCX-2216. At all doses tested, both drugs significantly suppressed thromboxane production, which is an index of cyclooxygenase-1 activity. Each group consisted of five rats.](image1)

![Fig. 5. Brain tissue levels of flurbiprofen 3, 12, and 24 h after oral administration of flurbiprofen (10 mg/kg) or an equimolar dose of NCX-2216. NCX-2216 itself was not detected in any brain tissue sample. *, p < 0.05 versus the corresponding flurbiprofen-treated group. Each group consisted of five rats.](image2)

![Fig. 6. Brain tissue (top panel) and cerebrospinal fluid (bottom panel) PGE₂ synthesis at various times after oral administration of flurbiprofen (10 mg/kg) or equimolar doses of NCX-2216 or NCX-2226. The other compounds shown in Fig. 1 were also tested, but they did not significantly affect prostaglandin synthesis at any time studied. Flurbiprofen and the two flurbiprofen-containing derivatives (NCX-2216 and NCX-2226) significantly inhibited brain and CSF prostaglandin synthesis (*, p < 0.05). The duration of suppression of brain prostaglandin synthesis by NCX-2216 exceeded that of the other compounds. Each group consisted of 5 or 6 rats.](image3)
The prolonged suppression of brain PG levels with NCX-2216 may have been due in part to the down-regulation of COX-2 expression, as other NO-releasing compounds have been reported to down-regulate this enzyme (Fiorucci et al., 2002; Turesin et al., 2003). We therefore measured by Western blotting COX-1 and COX-2 expression in the brain 24 h after oral administration of flurbiprofen or NCX-2216 (Fig. 6). Neither drug affected the expression of COX-2; however, flurbiprofen significantly increased (by ~40%) and NCX-2216 significantly decreased (by ~30%) brain expression of COX-1.

**Discussion**

Nitric oxide-releasing derivatives of aspirin were developed in an attempt to provide similar efficacy to aspirin in various scenarios while greatly reducing toxicity in the stomach (Wallace et al., 1994a; Davies et al., 1997; Wallace and Del Soldato, 2003). In particular, these compounds may be attractive for long-term use aimed at reducing the risk of colorectal cancer or cardiovascular disease. More recently, several epidemiological studies have shown that NSAIDs can delay the progression of Alzheimer’s disease (Etminan et al., 2003). It has also emerged that only a few NSAIDs, among the number currently used in therapy, have the property of reducing β-amyloid secretion in a variety of models (Eriksen et al., 2003; Gasparini et al., 2004). Flurbiprofen appears to be among the most effective β-amyloid-lowering drugs (Gasparini et al., 2004). Indeed, NCX-2216 has been shown to reduce brain deposition of β-amyloid in a double transgenic mouse model of Alzheimer’s disease when administered in the diet over a 5-month period (Jantzen et al., 2002). This compound consists of an NSAID moiety (flurbiprofen), an antioxidant moiety (ferulic acid), and a nitric oxide-releasing moiety. The results of the present study demonstrate that NCX-2216 does not produce damage to the stomach despite being able to suppress gastric PG synthesis as effectively as the parent drug. We further demonstrate that the NO-releasing moiety of NCX-2216 is essential for these gastric-sparing properties, although the ferulic acid moiety does not appear to contribute to this feature of the compound. Moreover, oral NCX-2216 administration resulted in a long-lasting (~48-h) suppression of PG synthesis in the brain.

Like many drugs in the NSAID class, flurbiprofen is a weak acid. It has been suggested for many years that the acidity of many NSAIDs is an important contributing factor to their ability to induce gastrointestinal ulceration, in that the compounds can cause epithelial damage through direct contact and uncouple oxidative phosphorylation (Whitehouse and Rainsford, 1980; Mahmud et al., 1996). Indeed, there have been reports that the gastric tolerability of NSAIDs could be markedly improved simply by “masking” the carboxylic acid residue on acidic NSAIDs, such as flurbiprofen; however, in the present study, we demonstrated that the coupling of ferulic acid to flurbiprofen through an ester bond to the carboxylic acid residue of the latter was not sufficient to reduce the gastric toxicity. The same experiments demonstrated that the antioxidant activity of ferulic acid, which was confirmed in an in vitro assay, did not seem to contribute to the gastric tolerability of NCX-2216. We initially postulated that the ferulic acid moiety could convey some protective effects in the stomach, because free radical scavengers can reduce the severity of experimental NSAID-gastropathy (Del Soldato et al., 1986; Vaananen et al., 1991). Nevertheless, we cannot exclude the possibility that the free radical scavenging moiety and the NO-releasing moiety interacted positively to protect the stomach.

The experiments with the various compounds with structural similarities to NCX-2216 support the hypothesis that it is the NO-releasing moiety of this compound that accounts for its gastric safety profile. NO donors have been shown to reduce the severity of experimental gastric injury (MacNaughton et al., 1989; Wallace et al., 1994b), and the use of NO donors in a clinical setting has been shown to result in a significant decrease in the risk of NSAID-associated gastrointestinal bleeding (Lanas et al., 2000). The gastric tolerability of NO-releasing derivatives of naproxen and aspirin was reversed in rats pretreated with 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (Brzozowski et al., 2000), a soluble guanylate cyclase inhibitor, further supporting the notion that NO released from NO-NSAIDs is responsible for the markedly reduced gastric toxicity of these compounds through a cGMP-dependent pathway.

NCX-2216 was not detected in any plasma or tissue sample after its oral administration, and only a small amount of flurbiprofen could be detected. Nevertheless, NCX-2216 produced substantial inhibition of whole blood thromboxane synthesis. These observations suggest that a metabolite of...
NCX-2216 is a compound that has COX-1-inhibiting activity. NCX-2216, or a metabolite of this compound, can also suppress COX-2 activity. We have also observed that NCX-2216 exhibited comparable activity to flurbiprofen in suppressing prostaglandin generation in the carrageenan-airpouch model in rats (unpublished observations), a model in which the vast majority of prostaglandin generation occurs via COX-2 (Wallace et al., 1999).

Beneficial effects of NSAIDs in terms of retarding the neurodegeneration associated with Alzheimer’s disease has been attributed to the anti-inflammatory effects of these drugs (McGeer and Rogers, 1992), which in turn are attributable to the suppression of PG synthesis. More recently, it has been found that flurbiprofen and a few other NSAIDs affect β-amyloid metabolism through mechanisms independent of cyclooxygenase metabolism (Eriksen et al., 2003; Gasparini et al., 2004). It was therefore important to establish whether or not NCX-2216 was able to suppress PG synthesis in the brain, and for how long such suppression persisted. Although inhibition of brain PG levels by flurbiprofen persisted for only 3 to 12 h after administration, substantial suppression (>70%) was still evident 48 h after the administration of NCX-2216. There are several possible reasons for this long-lasting effect of NCX-2216. First, it is possible that there is a COX-inhibiting metabolite of NCX-2216 that has a long half-life. Second, it is possible that flurbiprofen is generated from NCX-2216 but in such a manner that its delivery to the brain is longer lasting than after the administration of flurbiprofen itself. Although plasma levels of flurbiprofen were very low following oral administration of NCX-2216, we were able to detect the parent compound in brain tissue for at least 24 h after oral administration to the rat. Initially, the levels of flurbiprofen were markedly lower than could be detected after oral administration of an equimolar dose of flurbiprofen itself; however, at the 24-h timepoint, brain levels of flurbiprofen remained higher in the NCX-2216-treated rats than in the flurbiprofen-treated rats. A third possible explanation for the long-lasting suppression of central prostaglandin synthesis by NCX-2216 is that this drug may down-regulate the expression of COX in the brain. We investigated this possibility by performing Western blots of COX-1 and COX-2 in brain tissue 24 h after oral administration of NCX-2216 or flurbiprofen. Somewhat surprisingly, neither drug affected COX-2 expression, but both drugs slightly altered COX-1 expression. The underlying reasons for the alterations in COX-1 expression are not clear. Although statistically significant, the changes in COX-1 expression were small (30–40%) relative to the profound reduction of PG synthesis. It remains possible, however, that the decreased COX-1 expression in the NCX-2216-treated rats and the increased COX-1 expression in the flurbiprofen-treated rats contributed to the observed differences in brain prostaglandin synthesis. It is interesting that NCX-2226, the flurbiprofen-ferulic acid conjugate, also produced longer suppression of brain PG synthesis than did flurbiprofen.

In recent years, substantial evidence has accumulated that supports the notion that regular ingestion of aspirin and some other NSAIDs can reduce the incidence-certain cardiovascular diseases and colorectal cancer (Wallace et al., 2002; Ricchi et al., 2003). Moreover, regular NSAID use seems to retard the progression of neuroinflammation associated with Alzheimer’s disease (Etmian et al., 2003). Our findings of excellent gastric tolerability and prolonged suppression of brain PG synthesis with NCX-2216, if extendable from our animal studies to humans, suggest that this compound may be an attractive alternative to conventional NSAIDs for such applications.

References
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