Effects of triflusal on oxidative stress, prostaglandin production and nitric oxide pathway in a model of anoxia-reoxygenation in rat brain slices


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Abstract

Acetylsalicylic acid (ASA) is the most widely used drug in the prevention of ischemic vascular accidents, mainly because of its antithrombotic effect. Recently, evidence of a neuroprotective effect has appeared. The aim of this study was to evaluate the neuroprotective effect of triflusal, a fluorinated derivative of ASA, in a model of anoxia-reoxygenation in rat brain slices. Rats ($n = 10$ per group) were treated for 7 days with 1, 10 or 50 mg/kg/day p.o. of triflusal or ASA or solvent (control group), then brain slices were obtained and subjected to a period of anoxia followed by 180 min of reoxygenation. We measured oxidative stress parameters (lipid peroxidation, glutathione system), prostaglandins (PGE$_2$), nitric oxide pathway activity (NO) (nitrites + nitrates, constitutive and inducible NO synthase activity) and cell death (lactate dehydrogenase (LDH) efflux). Triflusal decreased cell death in rat brain slices subjected to reoxygenation after anoxia by 21%, 42% and 47% with 1, 10 and 50 mg/kg/day, respectively. This effect was proportionately greater than the effect of ASA (0%, 25% and 24%). The antioxidant effects of triflusal on the biochemical mechanisms of cell damage studied here were also greater than the effects of ASA: lipid peroxidation was reduced by 29%, 35% and 36% with triflusal, and 0%, 19% and 29% with ASA. Inducible NO synthase activity was reduced by 25%, 27% and 30% with triflusal, and 0%, 25% and 24% with ASA. Triflusal can be considered an alternative to ASA as a neuroprotective agent, at least in the experimental model of anoxia-reoxygenation used in the present study.

Theme: Disorders of the nervous system
Topic: Ischemia

Keywords: Triflusal; Salicylate; Antiplatelet agent; Brain anoxia; Oxidative stress; Nitric oxide

1. Introduction

Aspirin (acetylsalicylic acid, ASA) is the most widely used drug for the secondary prevention of thrombotic phenomena [4]. In the brain, ASA reduces the incidence of thrombotic events in patients who have had a prior stroke. This effect is due mainly to its antiplatelet action [15], which prevents the formation of arterial platelet thrombi, and through a possible neuroprotective effect via a direct action on brain tissue [14,18].

Triflusal is a fluorinated salicylate derivative (2-acetoxy-4-trifluoromethylbenzoic acid) that inhibits platelet function [9], increases leukocyte nitric oxide production [30,31], induces a downregulation of nuclear factor-$\kappa$B and decreases inducible nitric oxide synthase (iNOS) expression in brain during postnatal excitotoxic damage [3]. Moreover, it has been demonstrated that the efficacy of triflusal is not superior to that of aspirin in preventing vascular events in patients with cerebral infarction, although triflusal treatment was associated with a significantly lower rate of hemorrhagic complications (16.7% in the triflusal group versus 25.2% in the aspirin group, $P < 0.001$) [24].

One of the principal mechanisms of brain damage during ischemia is the formation of free radicals, which,
together with the impairment of enzymatic antioxidant mechanisms, give rise to tissue oxidative stress [20]. Oxidative stress has also been directly related to increased nitric oxide production, mainly via the inducible pathway [19], and to the altered intracellular production of prostanoids [17]. In this connection, SA—and ASA, to a much lesser extent—are able to capture hydroxyl ions [27], the main free radicals that act as vehicles for most of the tissue damage that occurs in ischemia.

The aim of the present study was to compare the effects of triflusal and aspirin on cerebral oxidative stress, prostanoid production and the nitric oxide pathway in a model of anoxia-reoxygenation in rat brain slices.

2. Materials and methods

2.1. Materials

Lactate dehydrogenase reagent kits were obtained from Biosystem (Barcelona, Spain). L-[^3H]Arginine and prostaglandin E2 enzyme immunoassay kits were from Amersham International (Little Chalfont, Buckinghamshire, UK). Triflusal and aspirin were supplied by J. Uriach and Cia. (Barcelona, Spain). All other reagents were from Sigma (St. Louis, MO, USA).

2.2. Study design

The experimental animals were adult male Wistar rats (body weight 300–350 g). The rats were housed under the customary conditions. All efforts were made to minimize the number of animals used and their suffering. The study protocol was approved by the University of Málaga Ethics Committee for the Use of Animals.

Triflusal or aspirin was given orally for 1 week at doses of 1, 10 or 50 mg/kg/day in a single daily dose between 9.00 and 10.00 am. After this period, animals were anesthetized with ether inhalation, then killed by decapitation. The brain was removed to prepare slices which were exposed to anoxia-reoxygenation in vitro. In all cases, 8 to 10 animals were used for each type of experiment, and a control group (rats treated with the solvent used for salicylate derivatives) was also used for each type of experiment.

2.3. In vitro model of rat brain anoxia

The brain was cut transversally into 0.1-mm slices with a vibrating microtome (Capdem Instruments, San Francisco, CA, USA). The slices were placed in buffer (composition in M: 0.1 NaCl, 5 × 10⁻⁴ KCl, 2.4 × 10⁻² NaHCO₃, 5.5 × 10⁻⁴ KH₂PO₄, 5 × 10⁻⁶ CaCl₂, 2 × 10⁻³ MgSO₄, 9.8 × 10⁻³ glucose, pH 7.4) and perfused with a mixture of 95% O₂ and 5% CO₂. After 30 min to reach equilibrium, the slices were placed in fresh buffer of the same composition except that the concentration of CaCl₂ was 3 × 10⁻³ M, that of MgSO₄ was 1 × 10⁻⁶ M, and no glucose was included. This solution was bubbled with a mixture of 95% N₂ and 5% O₂ for 20 min (hypoxia). Then the slices were placed in fresh buffer containing glucose and the solution was perfused with a mixture of 95% oxygen and 5% CO₂ (reoxygenation).

One brain slice was analyzed for each of the following conditions: (1) after 30 min of incubation and before N₂ perfusion, (2) after 20 min of perfusion with N₂, (3) after 180 min of reoxygenation. For all studies, the tissues were quickly frozen in liquid nitrogen and stored at −80 °C until the day of their analysis, which was done within 7 days of freezing.

2.4. Analytical techniques

2.4.1. Lipid peroxidation

To quantify lipid peroxidation, we measured thiobarbituric acid reactive substances (TBARS) under basal conditions [29]. Cell membrane-enriched fractions of the tissue samples were obtained as described by Bossman and Hems worth [5]. Absorbance was determined spectrophotometrically at 532 nm (Perkin Elmer C-532001 spectrophotometer, USA). The protein concentration was determined with the method of Bradford et al. [6].

2.4.2. Glutathione levels

Total glutathione was measured spectrofluorometrically according to the technique described by Hissin and Hill [17]. Brain tissue was homogenized in 0.1 M sodium phosphate buffer (pH 8.0) with 25% phosphoric acid, then centrifuged at 13 000 × g for 15 min at 4 °C to obtain the supernatant. Duplicate cuvettes were prepared for spectrofluorometry with sodium phosphate buffer, supernatant for each sample, and o-phthalaldehyde.

To determine the proportions of oxidized and reduced glutathione, we incubated supernatant from each sample with 4-vinyl pyridine, then proceeded as for as total glutathione.

2.5. Enzyme activities related to glutathione

Glutathione peroxidase (GSHpx), glutathione reductase (GSSGrd) and glutathione transferase (GSTHt) were determined by spectrophotometric kinetics. Tissue samples were diluted in 0.1 M phosphate-buffered saline (pH 7.0) and 25% phosphoric acid. The mixture was homogenized and centrifuged at 13 000 × g for 15 min at 4 °C. The supernatant was used to determine protein concentration, after neutralization with 0.1 N NaOH, and to determine the follow enzymatic activities:

**Glutathione peroxidase.** GSHpx activity was measured according to the method of Flohé and Gunzler [12]. Briefly, a volume equivalent to 25 μg protein was taken from each supernatant and 0.1 M phosphate-buffered saline was added to a volume of 880 μl, together with 34 mg/ml glutathione reductase, 3 mg/ml GSH, 1.25 mg/ml nicotinamide-adenine...
dinitrophenyl hydrazine (TN). The preparation was until it was absorbed. Every 30 s for 5 min.

**Glutathione reductase.** GSSGrd activity was determined according to the technique described by Flohé and Gunzler [12]. The amounts of sample and buffer were the same as in the GSHpx analyses. One hundred microliters of NADPH (0.12 mg/ml) was added, and the microcuvettes were shaken by inversion and incubated as described above for GSHpx. Then 6.1 GSSG was added, the cuvette was agitated by inversion, and the preparation was read at 340 nm. The decrease in absorbance was recorded every 30 s for 5 min.

**Glutathione transferase.** GSHtf activity was determined according to the method of Warlhom et al. [33]. One hundred microliters of GSH (0.3 mg/ml) was added to the volumes of sample and buffer indicated for the other enzyme determinations. The mixture was shaken by inversion and incubated at 37 °C for 3 min. Then 2 mg/ml 1-chloro-2,4-dinitrobenzene was added, the mixture was shaken by inversion, and the preparation was read at 340 nm as for the other enzyme activities.

### 2.6. Lactate dehydrogenase (LDH) assay

Tissue damage was measured by examining the LDH efflux into the incubation solution [22]. Samples of this solution were taken every 30 min and the enzyme activity was measured spectrophotometrically at 340 nm by following oxalate oxidation of NADH (decrease in absorbance) in the presence of pyruvate using an ELX-800 microplate reader (Bio-Tek Instruments, Winooski, VT, USA).

### 2.7. Determination of prostaglandin E₂

Brain tissues were snap-frozen in liquid nitrogen and stored until analysis. The samples were homogenized (1:10 wt/vol) in 15% methanol with 0.1 N phosphate-buffered saline (pH 7.5), then centrifuged at 37,000 × g for 15 min at 4 °C. The supernatant was run through a C18 column (Bio-Rad Laboratories, Hercules, CA, USA) that had been activated with absolute methanol, followed by washing with distilled water. After the sample had been assayed, the column was washed with 15% methanol in distilled water followed by petroleum ether. Prostaglandins were eluted at methylformate. The samples were then dried at room temperature under a nitrogen current and reconstituted with phosphate-buffered saline. The concentration of prostaglandin (PGE₂) was measured with a commercial enzyme immunoassay (Amersham International).

### 2.8. Nitric oxide synthase activity

Samples were homogenized (1:5 wt/vol) in buffer containing 10 mM HEPES, 320 mM sucrose, 1 mM EDTA, 1 mM DL-dithiothreitol (DDT), 10 μg/ml leupeptin and 2 μg/ml aprotonin at 0 °C. The homogenates were centrifuged at 12,000 × g for 20 min at 4 °C, and the supernatant was used to measure NOS synthase. Enzymatic reactions were tested at room temperature for 30 min with a mixture of 40 μl supernatant and 100 μl 40 mM potassium phosphate buffer (pH 7.0) consisting of 4.8 mM DL-valine, 1 mM NADPH, 1 mM MgCl₂, 2 mM CaCl₂, 20 μM l-arginine and 1.25 μM l-[³H]arginine (59 Ci/mol, Amersham Life Science). For each assay, three parallel samples were run: (A) a sample prepared as described above, (B) a sample that included 1 mM GSH, (C) a sample without calcium salts but with 1 mM EDTA and 1 mM EGTA. In all cases, the reaction was stopped by adding cold buffer that consisted of 0.2 mM EDTA. The samples were assayed with 50W-XA Dowex resin columns (Na⁺ form). One hundred microliters of supernatant was added to 5 ml scintillation fluid, and counts per minute (cpm) were recorded with a beta counter. Net NOS activity was calculated as the difference between sample A counts and sample B counts (calcium-

### Table 1

Mean values of the parameters measured after 30 min oxygenation of brain slices from rats treated orally with solvent (control), triflusol or aspirin (mg/kg/day) for 1 week (n = 10 experiments per group)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Triflusol</th>
<th>Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>TBARS (nmol/mg prot)</td>
<td>3.7 ± 0.3</td>
<td>3.0 ± 0.2</td>
<td>2.4 ± 0.2*</td>
</tr>
<tr>
<td>GSH (μmol/g tissue)</td>
<td>5.0 ± 0.4</td>
<td>5.0 ± 0.2</td>
<td>5.2 ± 0.4</td>
</tr>
<tr>
<td>%GSSG compared to GSH + GSG</td>
<td>6.6 ± 0.7</td>
<td>7.1 ± 0.9</td>
<td>6.9 ± 0.9</td>
</tr>
<tr>
<td>GSHpx (μmol/min)</td>
<td>52.3 ± 5.2</td>
<td>51.9 ± 5.1</td>
<td>51.5 ± 4.9</td>
</tr>
<tr>
<td>GSSGrd (μmol/min)</td>
<td>14.2 ± 1.8</td>
<td>15.7 ± 1.8</td>
<td>14.9 ± 1.4</td>
</tr>
<tr>
<td>GSHtf (μmol/min)</td>
<td>45.0 ± 4.2</td>
<td>43.2 ± 3.8</td>
<td>43.8 ± 4.0</td>
</tr>
<tr>
<td>PGE₂ (pg/mg tissue)</td>
<td>17.9 ± 1.9</td>
<td>15.2 ± 1.3</td>
<td>13.3 ± 1.1*</td>
</tr>
<tr>
<td>NO₂ + NO₃ (nmol/mg prot)</td>
<td>3.2 ± 0.2</td>
<td>3.4 ± 0.3</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>CNOS (nmol/30 min/mg prot)</td>
<td>11.7 ± 1.2</td>
<td>11.5 ± 1.2</td>
<td>12.3 ± 1.1</td>
</tr>
<tr>
<td>iNOS (nmol/30 min/mg prot)</td>
<td>6.9 ± 0.5</td>
<td>6.6 ± 0.6</td>
<td>6.6 ± 0.5</td>
</tr>
<tr>
<td>LDH (UI/mg/min)</td>
<td>0.4 ± 0.03</td>
<td>0.4 ± 0.03</td>
<td>0.4 ± 0.03</td>
</tr>
</tbody>
</table>


*P < 0.05 in comparison to control. TBARS: thiobarbituric acid reactive substances.
dependent activity, equivalent to constitutive NOS, cNOS), or the difference between sample A counts and sample C counts (calcium-independent activity, equivalent to inducible NOS, iNOS). The results were expressed as the production of \[^{[3}H\]citrulline per minute and per milligram of protein.

2.9. Statistical methods

The data in the text, tables and figures are expressed as the mean ± standard error of the mean from 10 experiments in samples from different animals. All statistical analyses were done with the Statistical Program for Social Sciences (SPSS, Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by Bonferroni transformation was used, and differences were considered significant when \(P<0.05\).

3. Results

3.1. Effect of oral administration of triflusal or aspirin in normal (oxygenated) brain tissue

The oral administration of triflusal or aspirin inhibited prostaglandin E\(_2\) production. Triflusal inhibited brain lipid peroxidation; aspirin (10 and 50 mg/kg/day) increased nitric oxide production. None of the other parameters were modified after 30 min of oxygenation (pre-anoxia period) (Table 1).

### Table 2

Mean values of the parameters measured before and after 30 min anoxia, and 180 min after reoxygenation in brain slices from rats treated orally with solvent (control) for 1 week (\(n=10\) experiments per group)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Preamoxia</th>
<th>Postanoxia</th>
<th>Reoxygenation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol/mg prot)</td>
<td>3.7 ± 0.3</td>
<td>3.1 ± 0.3</td>
<td>4.6 ± 0.5*</td>
</tr>
<tr>
<td>GSH (µmol/g tissue)</td>
<td>5.0 ± 0.4</td>
<td>4.8 ± 0.4</td>
<td>3.3 ± 0.2*</td>
</tr>
<tr>
<td>%GSSG compared to GSH + GSSG</td>
<td>6.6 ± 0.7</td>
<td>6.9 ± 0.6</td>
<td>9.6 ± 0.7*</td>
</tr>
<tr>
<td>GSHpx (µmol/min)</td>
<td>52.3 ± 5.2</td>
<td>55.8 ± 5.2</td>
<td>68.6 ± 5.4*</td>
</tr>
<tr>
<td>GSSGr (µmol/min)</td>
<td>14.2 ± 1.8</td>
<td>15.0 ± 1.3</td>
<td>13.2 ± 1.4</td>
</tr>
<tr>
<td>GSHtf (µmol/min)</td>
<td>45.0 ± 4.2</td>
<td>43.2 ± 4.1</td>
<td>34.2 ± 2.9*</td>
</tr>
<tr>
<td>PGE(_2) (pg/mg tissue)</td>
<td>17.9 ± 1.9</td>
<td>19.0 ± 2.0</td>
<td>22.5 ± 2.2*</td>
</tr>
<tr>
<td>NO(_3) + NO(_2) (nmol/mg prot)</td>
<td>3.2 ± 0.2</td>
<td>3.9 ± 0.2</td>
<td>10.6 ± 1.0*</td>
</tr>
<tr>
<td>cNOS (nmol/30 min/mg prot)</td>
<td>11.7 ± 1.2</td>
<td>8.9 ± 0.7*</td>
<td>5.6 ± 0.5*</td>
</tr>
<tr>
<td>iNOS (nmol/30 min/mg prot)</td>
<td>6.9 ± 0.5</td>
<td>7.4 ± 0.6</td>
<td>9.7 ± 0.6*</td>
</tr>
<tr>
<td>LDH (UI/min/mg tissue)</td>
<td>0.4 ± 0.03</td>
<td>0.7 ± 0.07</td>
<td>1.9 ± 0.08*</td>
</tr>
</tbody>
</table>

TBARS: thiobarbituric acid reactive substances. GSH: reduced glutathione. GSSG: oxidized glutathione. GSHpx: glutathione peroxidase activity. GSSGr: glutathione reductase activity. GSHtf: glutathione transferase activity. PGE\(_2\): prostaglandin E\(_2\). cNOS: constitutive nitric oxide synthase. iNOS: inducible nitric oxide synthase. LDH: lactate dehydrogenase activity. *\(P<0.05\) in comparison to preanoxic value.

Fig. 1. Thiobarbituric acid reactive substances (TBARS) in rat brain slices subjected to anoxia followed 180 min of reoxygenation. *\(P<0.05\) in comparison to the control group (\(n=10\) rats per group).

Fig. 2. Reduced glutathione (GSH) and percentage of oxidized glutathione (GSSG) in comparison to total glutathione (GSH + GSSG) in rat brain slices subjected to anoxia followed 180 min of reoxygenation. *\(P<0.05\), **\(P<0.001\) in comparison to the control group (\(n=10\) rats per group).
3.2. Effects of the model of anoxia-reoxygenation

In brain slices subjected to anoxia-reoxygenation, there was an increase in TBARS production, which had risen by 24.3% after 180 min of reoxygenation in comparison to the pre-anoxia value. Reduced glutathione levels decreased 34%. The percentage of oxidized glutathione increased by 45.4% and GSHpx activity by 31.6%, whereas GSHtf activity decreased by 24%.

Brain PGE$_2$ production had increased significantly by 25.7%. In brain slices, nitrite + nitrate concentration increased by 231%, cNOS activity decreased by 52.1% and iNOS activity increased by 40.6%. Efflux of LDH into the incubation medium increased by 375% after 180 min of reoxygenation (Table 2).

3.3. Effect of the oral administration of triflusal or aspirin in rat brain slices subjected to anoxia-reoxygenation

The oral administration of triflusal reduced TBARS production after 180 min of reoxygenation in brain slices. Acetylsalicylic acid reduced TBARS production significantly only at a dose of 50 mg/kg/day (Fig. 1). The decrease in glutathione levels after 180 min of reoxygenation in the control group was smaller in samples treated with oral triflusal or ASA. However, the final levels of reduced glutathione were higher with triflusal than with ASA (Fig. 2). The fraction of glutathione in the form of GSSG was only partially modified by triflusal or ASA (Fig. 2).

In the control group, reoxygenation increased GSHpx activity after 180 min; this was significantly reduced only with ASA (Fig. 3). GSSGrd activity was reduced with triflusal or ASA (Fig. 3). The degree of inhibition of GSHtf activity in the treated groups was similar to that seen in the control group (Fig. 3).

The increase in brain levels of PGE$_2$ caused by reoxygenation was curtailed by both triflusal (65% inhibition with 10 mg/kg/day, 68% inhibition with 50 mg/kg/day) and ASA (34% inhibition with 1 mg/kg/day, 56% inhibition with 10 mg/kg/day and 69% with 50 mg/kg/day) (Fig. 4).

The increase of nitrite + nitrate levels after reoxygenation was reduced after treatment with 10 or 50 mg/kg/day...
triflusal or ASA. cNOS activity was increased by the oral administration of either triflusal or ASA. Inducible NOS activity was increased by reoxygenation in the control group but was less markedly stimulated by triflusal (25% inhibition with 1 mg/kg/day, 27% inhibition with 10 mg/kg/day, 30% inhibition with 50 mg/kg/day) or ASA (0% inhibition with 1 mg/kg/day, 25% inhibition with 10 mg/kg/day, 24% inhibition with 50 mg/kg/day) (Fig. 5).

The increased LDH efflux was significantly lower after 180 min of reoxygenation in brain slices from rats treated with triflusal (21%, 42% and 47% inhibition with 1, 10 and 50 mg/kg/day, respectively) or with ASA (0%, 12% and 26% with 1, 10 and 50 mg/kg/day, respectively) (Fig. 6).

4. Discussion

We show here that in rat brain slices subjected to reoxygenation after anoxia, the oral administration of triflusal decreases cell death, to an extent proportionately greater than the effect of ASA. In experiments designed to elucidate the biochemical mechanisms of tissue damage, triflusal showed an antioxidant effect that was also greater than that of ASA.

To elucidate the possible neuroprotective effect of ASA, we investigated some biochemical mechanisms that others have previously investigated. We searched for evidence of antithrombotic and anti-inflammatory effects, inhibition of the release of excitatory amino acids, modulation of the nitric oxide pathway, and antioxidant effects [4,14,25–27]. However, these diverse pharmacological effects are seen with very different concentrations and doses; this means that the mechanisms that may be involved in ischemic processes in the brain have yet to be entirely explained. We have confirmed that the prevention of thrombotic events plays an important role in the prevention of brain damage in ischemic cerebrovascular accidents.

Triflusal is a fluorinated derivative of ASA that differs from the latter in certain pharmacodynamic and pharmacokinetic characteristics. At antithrombotic doses (10 mg/kg/day p.o.), it has been shown not to reduce vascular prostacyclin synthesis [7] and to have a major effect on
neutrophil nitric oxide production [30,31]. At doses above those used for antithrombotic treatment (30 mg/kg/day), it decreased the expression of iNOS and other antiinflammatory molecules in an excitotoxic lesion model in the postnatal brain, and downregulated the constitutive transcription of NF-κB in the postnatal rat brain [1–3]. However, its antioxidant capacity and its effects on brain prostaglandin production or the nitric oxide pathway have not previously been investigated in an experimental model of cerebral ischemia-reoxygenation.

The absence of an antioxidant effect in oxygenated brain slices is consistent with the effect of other antioxidants, which have shown no clear effect on normal tissues, but have shown such an effect in oxidative tissue stress [13]. Under these conditions, triflusal shows an anti-peroxidative effect in brains subjected to anoxia-reoxygenation, and this effect is significantly greater than that of ASA. In addition, triflusal increased brain concentrations of glutathione to a greater degree than did ASA.

Salicylate derivatives are reportedly able to trap hydroxyl free radicals [27]: they may therefore be able to impair the process of lipid peroxidation. Because only small amounts of hydroxyl anions are produced in the normal brain but production is greater under conditions of anoxia-reoxygenation, these compounds probably have a greater antioxidant effect under these conditions but not in situations of normal oxygenation in brain slices. In this connection, several studies have shown the order of potency of salicylate-containing compounds in hydroxyl anion uptake to be: SA>ASA>benzoic acid ([16,21,27,28], among others). Therefore, the increase in reduced glutathione in our experiments may have been the result of a lower need by the brain tissue to protect itself from free radical damage.

Because the structure of triflusal is similar to that of ASA, carboxyl groups may facilitate the uptake of hydroxyl anions at two positions on the ASA or triflusal molecule. However, the trifluoromethane group in triflusal (but absent ASA) provides a third position for hydroxyl anion capture on the surface of the molecule [32]. This may give triflusal its greater capacity for hydroxyl anion uptake and hence its greater ability to inhibit lipid peroxidation.

Acetylsalicylic acid is better able to inhibit, in absolute values, the accumulation of prostaglandin E2 in brain tissue after anoxia-reoxygenation. This finding was logical in view of its mechanism of action. It should be noted that in platelets and in human arterial tissue, the ability of triflusal to inhibit cyclooxygenase activity was 50- to 60-fold weaker that that of ASA [9]. However, in the experimental model we used, the effect of ASA and triflusal was similar in quantitative terms. In models of brain ischemia, inducible cyclooxygenase expression (COX-2) was increased, and prostaglandin production was greater than production from the constitutive isof orm (COX-1) [23]. Triflusal was found to inhibit COX-2 expression to a much greater degree than ASA. This may account for the ability of triflusal to inhibit prostaglandin accumulation in brain tissue in our experimental model.

Both compounds curtail the increase in global nitric oxide production, increase cNOS activity and inhibit iNOS activity. These effects were reported for both compounds in different tissues such as human neutrophils [10,11,23] and rat brain tissue subjected to ischemia [25]. In human neutrophils in vitro, triflusal increased cNOS activity to a greater degree than did ASA, but in a millimolar range [30,31]. The administration of 30 mg/kg/day of triflusal inhibited NFκB expression and activation in rat nerve cells [1,2], and led to a discreet inhibition of the inflammatory factors TNFα and interleukin 1β [8]. These effects may account for the ability of triflusal to inhibit iNOS activity.

We felt it would be worthwhile to compare the results obtained with doses of both compounds that approximated those recommended for antithrombotic treatment and ischemic cerebrovascular accidents in humans. The equivalent doses were 1 mg/kg/day p.o. (approximately 75–80 mg/day in humans) for ASA and 10 mg/kg/day (approximately 600–700 mg/day in humans) for triflusal. Although caution is in order because of the uncertainties of extrapolating results obtained in our rat model to humans, triflusal clearly showed a greater ability to reduce lipid peroxidation, increase concentrations of reduced glutathione, reduce the accumulation of prostaglandins in the brain after anoxia-reoxygenation, increase cNOS activity and reduce iNOS activity. In addition, cell death in brain slices 180 min after reoxygenation was 39.69% lower in animals treated with triflusal at 10 mg/kg/day p.o., whereas no such effect was seen in animals treated with 1 mg/kg/day p.o.

In conclusion, clinical trials with triflusal have shown that it is able to secondarily prevent cerebral thrombotic events to a degree similar to ASA, but that the risk of bleeding with triflusal is lower [24]. The neuroprotective effect of triflusal in tissues subjected to ischemia-reoxygenation is another feature that makes this compound a potentially useful alternative to ASA. Further studies are needed to show whether the effects observed in experimental situations are reflected in parameters of cerebral functioning in humans after a cerebrovascular accident.

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