Acetylsalicylic acid (ASA) reduces the incidence of ischemic stroke mainly through its antithrombotic action; however, it also has a direct neuroprotective effect. The present study was designed to evaluate the effect of ASA on oxidative stress and the activity of nitric oxide synthase (NOS) in an in vitro model of hypoxia in rat brain slices. Rat brain slices were perfused with nitrogen (hypoxia) for a maximum of 120 min, after which we measured lipid peroxidation, glutathione levels, glutathione-related enzyme activities, and constitutive nitric oxide synthase (cNOS) and inducible nitric oxide synthase (iNOS) activities. In brain tissue subjected to hypoxia, ASA reduced oxidative stress and iNOS activity (all increased by hypoxia), but only when used at higher concentrations. The effects of salicylic acid (SA) were similar but more intense than those of ASA. After oral administration, the effect of SA was much greater than that of ASA, and the decrease in cell death with SA was seen much more clearly. In view of the greater effect of SA compared to ASA on changes in oxidative stress parameters in a model of hypoxia, and higher brain concentrations of SA when it is administered alone than when ASA is given (undetectable levels), we conclude that SA plays an important role in the cytoprotective effect in brain tissue after ASA administration.

Key words: acetylsalicylic acid; brain hypoxia; brain oxidative stress; nitric oxide; salicylic acid

Several mechanisms have been invoked to explain the neuroprotective effect of ASA in brain tissue. Inhibition of inducible nitric oxide synthase (iNOS) activity (Moro et al., 2000; Asanuma et al., 2001), inhibition of expression and release of nuclear factors (NFκB) (Grilli et al., 1996), and inhibition of release of amino acids able to excite neurons (Moro et al., 2000; De Cristobal et al., 2002) have all been proposed. When ASA is given orally, however, the main metabolite is salicylic acid (SA) (Levy, 1976), which has not been associated with the neuroprotective effect of ASA.

One principal mechanism 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 (Kontos, 2001). Oxidative stress has also been related directly to increased nitric oxide production, mainly via the inducible pathway (Kim et al., 2002), and to altered intracellular production of prostaglandins (Murdoch and Hall, 1990). In this connection SA, and ASA to a much lesser extent, are able to capture hydroxyl ions (Sagone and Husney, 1987), the main free radicals that act as vehicles for most tissue damage that occurs in ischemia. Moreover, ASA modifies nitric oxide production pathways (López-Farré et al., 1995; De La Cruz et al., 2000a, 2002a) and inhibits cyclooxygenase activity (prostaglandin synthesis) (Roth et al., 1975).

The aim of the present study was to analyze the effects of ASA and SA on cerebral oxidative stress, NOS activities, and cell death in a rat brain slice model of hypoxia.

MATERIALS AND METHODS

Materials

Lactate dehydrogenase (LDH) reagent kits were obtained from Biosystem SA (Barcelona, Spain). L-[3H]Arginine and

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prostaglandin E2 enzyme immunoassay kits were from Amersham (Buckinghamshire, UK). All other reagents including ASA and SA were from Sigma (St. Louis, MO).

**Study Design**

Adult male Wistar rats (body weight 300–350 g) 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.

Two types of experiment were done: in vitro and ex vivo. For in vitro experiments, hypoxia was induced in rat brain slices, and the drugs (ASA and SA) were added to these tissues. For ex vivo, experiments the drugs were given orally (endogastric cannulation) for 1 week at a dose of 1 or 10 mg/kg/day in a single daily dose between 9:00 and 10:00 AM, then the brain was removed to prepare slices that were exposed to hypoxia in vitro. These doses were chosen according to the ability of ASA to selectively inhibit or not inhibit vascular and platelet cyclooxygenase, as an index of a good antithrombotic dose of ASA. In a previous study, we demonstrated that 1 mg/kg/day (oral) did not significantly affect prostacyclin synthesis, whereas 10 mg/kg/day (oral) inhibited synthesis almost completely (De La Cruz et al., 2002b). In all cases, 8–10 animals were used for each type of experiment, rats treated with the solvent used for salicylate derivatives were used as a control group.

**In Vitro Model of Rat Brain Hypoxia**

The rats were killed by decapitation and the whole brain was removed immediately. The cerebellum and brainstem were discarded and the remaining tissue was cut transversally into 1-mm slices with a vibratome (Capdem Instruments, San Francisco, CA). Slices were placed in buffer (composition in M: 0.1 NaCl, 5 × 10⁻⁵ KCl, 2.4 × 10⁻⁴ NaHCO₃, 5.5 × 10⁻⁴ KH₂PO₄, 5 × 10⁻⁶ CaCl₂, 2 × 10⁻³ MgSO₄, 9.8 × 10⁻⁵ glucose, pH 7.4) and perfused with a mixture of 95% O₂ and 5% CO₂. After 30 min to reach equilibrium, 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. The solution was perfused with a mixture of 95% N₂ and 5% O₂ for 20 min (hypoxia).

One brain slice was analyzed for each of the following conditions: (1) after 30 min of incubation and before N₂ perfusion; (2) after 30 min of perfusion with N₂; (3) after 60 min of perfusion with N₂; (4) after 90 min of perfusion with N₂; and (5) after 120 min of perfusion with N₂. For all studies, tissues were frozen quickly in liquid nitrogen and stored at −80°C until used (within 7 days of freezing).

![Fig. 1. Thiobarbituric acid reactive substances (TBARS) in brain slices.](image-url)
Lipid Peroxidation Analysis

To quantify lipid peroxidation we measured thiobarbituric acid reactive substances (TBARS) under basal conditions (De La Cruz et al., 1992). Cell membrane-enriched fractions of tissue samples were obtained as described previously by Bossman and Hemsworth (1969). Absorbance was determined spectrophotometrically at 532 nm (Perkin-Elmer C-532001 spectrophotometer; Perkin-Elmer, Cyprus, CA). Blank samples were prepared in an identical manner but incubated at 4°C to avoid TBARS production. The results were expressed as μmol TBARS/mg protein; protein determination was carried out using the method of Bradford (1976).

Analysis of Glutathione Levels

Total glutathione (GSH) was measured spectrofluorometrically according to the technique described previously by Hissin and Hill (1976). Brain tissue was homogenized in 0.1 M sodium phosphate buffer (pH 8.0) with 25% phosphoric acid at a proportion of 1:20, then centrifuged at 13,000 g for 15 min at 4°C to obtain the supernatant. Duplicate cuvettes were prepared for spectrophotometry with sodium phosphate buffer, the supernatant for each sample, and o-phthaldehyde.

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

Analysis of Enzyme Activities Related to Glutathione

Glutathione peroxidase (GSHpx) and glutathione transferase (GSHtf) were determined by spectrophotometric kinetics. Tissue samples were diluted in 0.1 M phosphate-buffered saline (PBS; 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 enzyme activities described below.

**Glutathione peroxidase.** GSHpx activity was measured according to the method of Flohé and Gunzler (1985). Briefly, a volume equivalent to 25 μg protein was taken from each supernatant and 0.1 M PBS was added to a volume of 880 μl, together with 53 μl glutathione reductase, 133 μl GSH, 100 μl nicotinamide-adenine dinucleotide phosphate (NADPH) and 100 μl tertbutyl-hydroperoxide. The absorbance of the preparation was read at 340 nm and the decrease in absorbance was recorded every 30 sec for 5 min.

**Glutathione transferase.** GSHtf activity was determined according to the method of Warholm et al. (1985). GSH (100 μl) was added to the sample and buffer volumes indicated above.

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Fig. 2. Total glutathione (GSH+GSSG) in brain slices. **A:** Time-course of GSH+GSSG in oxygenated (O₂) and hypoxic (N₂) slices. **B:** Percentage of change with respect to pre-hypoxic value after 120 min of hypoxia, in slices incubated with ASA or SA. **C:** Percentage of change with respect to pre-hypoxic value, after 120 min of hypoxia, in hypoxic slices after 1 week of oral treatment with ASA or SA. *P < 0.05, **P < 0.001, with respect to oxygenated samples (A), saline-incubated (B), or saline-treated (C) animals.
Lactate Dehydrogenase Assay

Tissue damage was measured by examining the LDH efflux to the incubation solution (Lobner, 2000). Samples of this solution were obtained every 30 min and the enzyme activity was measured spectrophotometrically at 340 nm by following oxidation of NADH (decrease in absorbance) in the presence of pyruvate using an ELX-800 microplate reader (Bio-Tek Instruments, Winooski, VT).

Nitric Oxide Synthase Activity

Brain tissue was snap-frozen in liquid nitrogen until the assay for NOS activity. Samples were homogenized (1:5 wt/vol) in buffer containing 10 mM HEPES, 320 mM sucrose, 1 mM EDTA, 1 mM dl-dithiothreitol (DTT), 10 μg/ml leupeptin, and 2 μg/ml aprotinin at 0°C. Homogenates were centrifuged at 12,000 × g for 20 min at 4°C, and the supernatant was used to measure NOS synthase. We first determined protein concentration with the method of Bradford (1976). Enzymatic reactions were tested at room temperature for 30 min with a mixture of 40 μl of supernatant and 100 μl of 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 μl/ml L-[3H]arginine (59 Ci/mol; Amersham). For each assay three samples were run in parallel: (1) a sample prepared as described above; (2) a sample that included 1 mM NG-methyl-l-arginine (nonspecific activity); and (3) 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). A supernatant aliquot of 100 μl was added to 5 ml scintillation fluid, and counts per minute (cpm) were recorded with a β counter. Net NOS activity was calculated as the difference between sample A and sample B counts (calcium-dependent activity, equivalent to cNOS), or the difference between sample A and sample C counts (calcium-independent activity, equivalent to iNOS). Results were expressed as the production of [3H]citrulline/min/mg protein.

Plasma and Brain Levels of ASA and SA

Plasma was obtained by centrifuging samples of whole blood with 5 mg/ml potassium fluoride at 2,500 × g for 15 min at 1°C. Potassium fluoride (5 mg/ml) was added to the plasma to prevent hydrolysis of ASA, and samples were stored at −80°C. We used high-performance liquid chromatography (HPLC) to measure ASA and SA concentrations (Hewlett-Packard model 1100 integrated chromatography system) accord-

Fig. 3. Percentage of glutathione in oxidized form (%GSSG) with respect to total glutathione in brain slices. A: Time-course of percentage GSSG in oxygenated (O₂) and hypoxic (N₂) slices. B: Percentage of change with respect to pre-hypoxic value after 120 min of hypoxia, in slices incubated with ASA or SA. C: Percentage of change with respect to pre-hypoxic value, after 120 min of hypoxia, in hypoxic slices after 1 week of oral treatment with ASA or SA. *P < 0.05, **P < 0.001, with respect to oxygenated samples (A), saline-incubated (B), or saline-treated (C) animals.
ing to the method described by Rumble and Roberts (1981),
with some modifications. Perchloric acid and methanol were
used to precipitate and extract the sample. Chromatographs
were obtained with an SB-C18 column (4.6 × 25 mm, particle
size 5 μm). The mobile phase consisted of 0.03% acetonitrile,
methanol, and orthophosphoric acid (50:10:40), and ASA and
SA were measured with ultraviolet light at 237 nm.

The extraction procedure used for brain samples was a
slight modification of the method described previously by Ven-
turini and Sparber (2001). Samples were homogenized in a
solution of 21 ml/g of methanol and 4 ml/g of perchloric acid.
An aliquot of the homogenate was poured into a microcentri-
fuge filter tube, centrifuged for 15 min at 3, 200 × g, and then
50 μl of the filtrate was injected into an HPLC-UV system.
The instruments and the mobile phase were the same as that
described for plasma ASA and SA determinations.

Statistical Methods

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

RESULTS

Effects of the Hypoxia Model

In brain slices subjected to hypoxia, there was a
time-dependent increase in TBARS production (Fig. 1A)
(basal: 0.12 ± 0.006 nmol/mg protein). Total glutathione
levels decreased steadily over time during hypoxia (Fig.
2A) (basal: 8.89 ± 0.36 μmol/g tissue), and the percentage
of oxidized glutathione increased during hypoxia (Fig. 3A)
(basal: 2.68 ± 0.16%). GSHpx activity increased (basal:
4.75 ± 0.48 μmol/min/25 μg protein) (Fig. 4A), whereas
GSHtf activity decreased (basal: 2.57 ± 0.13 μmol/min/
25 μg protein) (Fig. 5A).

In brain slices, cNOS activity decreased after
120 min by 23.69% compared to the pre-hypoxia value
(Fig. 6A), and iNOS activity increased by 29.96% after
120 min (Fig. 7A).

Efflux of LDH into the incubation medium in-
creased with time during hypoxia (Fig. 8A), and reached
516% after 120 min in comparison to the pre-hypoxia
value (0.30 ± 0.04 IU/mg tissue/min).

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![Fig. 4. Glutathione peroxidase activity (GSHpx) in brain slices.](image)

A: Time-course of GSHpx in oxygenated (O₂) and hypoxic (N₂) slices. B: Percentage of change with respect to pre-hypoxic value after 120 min of hypoxia, in slices incubated with ASA or SA. C: Percentage of change with respect to pre-hypoxic value, after 120 min of hypoxia, in hypoxic slices after 1 week of oral treatment with ASA or SA. *P < 0.05, **P < 0.001, with respect to oxygenated samples (A), saline-incubated (B), or saline-treated (C) animals.
Effect of ASA and SA on Rat Brain Slices Subjected to Hypoxia In Vitro

Incubation with ASA and especially with SA reduced TBARS formation in brain slices subjected to 120 min hypoxia (Fig. 1B) and glutathione depletion (maximum prevention 52.3% with ASA and 78.5% with SA, in comparison to the control group) (Fig. 2B). Moreover, ASA and especially SA reduced the percentage of glutathione in oxidized form (GSSG), which was increased in the control group (Fig. 3B). ASA also increased GSHpx activity (Fig. 4B) and reduced the decrease in GSHtf activity observed in control group (Fig. 5B).

Inhibition of cNOS activity in brain slices after 120 min of hypoxia in the control group was modified only significantly by incubation with 1,000 μM ASA or SA (Fig. 6B). The increase in iNOS activity in the control group was reduced significantly by ASA and especially by SA (Fig. 7B).

The efflux of LDH increased by 516% in the control group. The maximum increase after incubation with ASA was 488%, and the corresponding value for SA was 425% (Fig. 8B).

Effect of Oral ASA and SA Administration in Rat Brain Slices Subjected to Hypoxia

The oral administration of ASA or SA reduced TBARS production after 120 of hypoxia in brain slices; inhibition was greater with 10 mg/kg/day SA than with ASA (Fig. 1C). Both drugs reduced the decrease in glutathione levels and the fraction of glutathione in the form of GSSG (Fig. 2C and 3C). The increase in GSHpx activity after 120 min of hypoxia in the control group was reduced after ASA and especially after SA treatment (Fig. 4C). Inhibition of GSHtf activity in the treated groups was similar to that seen in the control group, except for the slight increase by ASA and SA at 10 mg/kg/day (Fig. 5C).

Inhibition of cNOS activity as a result of hypoxia was reduced by ASA only at the higher dose, whereas both doses of SA reduced inhibition (Fig. 6C). Inducible NOS activity was reduced with 10 mg/kg/day ASA and with both 1 and 10 mg/kg/day SA. (Fig. 7C).

The increased LDH efflux was significantly lower after 120 min of hypoxia in brain slices treated with ASA or with SA (Fig. 8C).

Plasma and brain levels of ASA and SA in the different animal groups are summarized in Table I.
DISCUSSION

Our results show that the administration of ASA decreases brain damage in the model of hypoxia tested, and that SA plays an important role in this effect through its antioxidant action. We analyzed two of the main final pathways of cell damage in ischemia-hypoxia: oxidative stress and nitric oxide production.

Brain Slices Without Hypoxia

We first wondered whether SA or ASA had antioxidant effects, in view of their demonstrated inhibitory effect on prostaglandin synthesis and increased production of nitric oxide (Roth et al., 1975; López-Farré et al., 1995; De La Cruz et al., 2000a, 2002a). Our results show that in brain slices without hypoxia, ASA did not lead to any significant changes in any oxidative stress parameter we measured. The effect of SA was compatible with an antioxidant profile: it inhibited lipid peroxidation and increased glutathione synthesis, but did not modify the activities of glutathione-related enzymes. The antioxidant effect in brain tissue under conditions of normal oxidation, however, was not great in quantitative terms.

The effect of the deacetylated compound (SA) on lipid peroxidation may be explainable by the ability of SA to absorb hydroxyl ions (Sagone and Husney, 1987) and thus impede a main step in the process of membrane lipid peroxidation. The increase in intracellular glutathione in tissues incubated with SA may have been a consequence of its antiperoxidant effect; if oxidizing damage was mild, the antioxidant defense system would not be activated. In other words, SA might spare glutathione stores by avoiding factors that stimulate glutathione depletion. Two observations support this notion: the percentage of GSSG was reduced, and the activities of enzymes associated with maintaining glutathione levels were not modified substantially. These findings seem to rule out a direct effect of SA on the glutathione system.

Some authors have described a moderate effect of ASA and SA on GSHTf and GSHpx activities, although the effect has been observed only under oxidative stress conditions (Losche et al., 1984; van Bree et al., 1989; Endoh et al., 1996) or after in vivo administration of high ASA doses (Cai et al., 1994).

Other antioxidant drugs also show more marked effects under conditions of oxidative stress (Galvez et al., 1994; De La Cruz et al., 2000c, 2002b). This led us to wonder whether the discreet effect of SA would be enhanced in brain tissue that produced free radicals.

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**Fig. 6.** Calcium-dependent (constitutive) nitric oxide synthase (cNOS) activity in brain slices. **A:** Time-course of cNOS activity in oxygenated (O₂) and hypoxic (N₂) slices. **B:** Percentage of change with respect to pre-hypoxic value after 120 min of hypoxia, in slices incubated with ASA or SA. **C:** Percentage of change with respect to pre-hypoxic value, after 120 min of hypoxia, in hypoxic slices after 1 week of oral treatment with ASA or SA. *P < 0.05, **P < 0.001, with respect to oxygenated samples (A), saline-incubated (B), or saline-treated (C) animals.
Brain Slices With Hypoxia and Without Drugs

In this hypoxia model, we observed changes in some biochemical pathways, mainly an increase in lipid peroxidation and iNOS activity and a decrease in the glutathione antioxidant system and cNOS activity. These alterations lead to a clear increase in cell death as indicated by the accumulation of LDH activity. Moreover, these changes are in agreement with earlier data obtained with the same experimental model (Bezzi et al., 1998; De La Cruz et al., 1998; Moro et al., 1998).

Incubation of Hypoxic Brain Slices With ASA and SA

In the model we used, in vitro incubation with ASA or SA modified the biochemical pathways we investigated. Both ASA and SA curtailed the increase in oxidative stress seen in control samples; however, in all cases the effect of SA was measurably greater. The decrease in cNOS activity caused by hypoxia was attenuated to a similar degree by both compounds, and SA inhibited more effectively the iNOS increase seen in control samples. In overall terms, incubation with either salicylate derivative decreased hypoxia-induced cell death.

The greater effect of SA compared to that of ASA may be related with the ability of both to react with hydroxyl radicals (Sagone and Husney, 1987), as the production of these radicals in increased under anoxic conditions. The order of reaction intensity between these compounds and hydroxyl radicals has been shown to be SA > ASA > benzoic acid (see for example Sagone and Husney, 1987; Hall et al., 1993; Piantadosi et al., 1997; Li et al., 1999).

Our results for the effect of ASA on NOS activity were clearly compatible with the findings reported by Moro et al. (2000). The decline in cNOS activity induced by hypoxia was curtailed by ASA, although this effect was seen at millimolar (i.e., clearly supratherapeutic) concentrations. In this experimental model, the effects of SA on NOS activity were similar to those of ASA; however, the increase in iNOS activity caused by hypoxia was inhibited more strongly by SA than by ASA, as the former inhibited the increase at a concentration of 1 μM.

The effect on NOS activity may be due to direct stimulation of cNOS, as occurs in leukocytes (López-Farré et al., 1995; De La Cruz et al., 2000a) and arterial cells (De La Cruz et al., 2002a), or to a reduction in iNOS stimulation (Sánchez de Miguel et al., 1999). Regardless of the

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**Fig. 7. Calcium-independent (inducible) nitric oxide synthase (iNOS) activity in brain slices.** A: Time-course of iNOS activity in oxygenated (O2) and hypoxic (N2) slices. B: Percentage of change with respect to pre-hypoxic value after 120 min of hypoxia, in slices incubated with ASA or SA. C: Percentage of change with respect to pre-hypoxic value, after 120 min of hypoxia, in hypoxic slices after 1 week of oral treatment with ASA or SA. *P < 0.05, **P < 0.001, with respect to oxygenated samples (A), saline-incubated (B), or saline-treated (C) animals.
mechanism, excess NO production has been found in cerebral ischemia (Castillo et al., 2000), as has excess production of free radicals (Kontos, 2001). Both products react to produce hydroxyl anions (Hogg et al., 1992) and peroxynitrites (Forman et al., 1998), which cause greater cell damage than do other free radicals. Because of its greater antioxidant effect, SA may be able to reduce free radical production, which in turn would slow peroxynitrite production and decrease their potential to cause cell damage.

Other cytoprotective mechanisms have been proposed for the effects of ASA in brain tissue. Riepe et al. (1997) showed that incubating ASA with rat hippocampus slices subjected to hypoxia reduced the decrease in tissue ATP content and the alteration in neuronal action potentials. In addition, ASA was reported to diminish the release of glutamate (an amino acid able to excite brain neurons), which is increased in anoxic brain tissue (Moro et al., 2000; De Cristobal et al., 2002). In both experiments, however, incubation with SA instead of ASA had no significant effect on these parameters.

Oral ASA and SA Administration

Incubation of rat brain tissue slices with either of the salicylate derivatives decreases brain damage caused by hypoxia. The slightly greater effect of SA may be due for the most part to its antioxidant effects. In humans, however, particularly persons who have had ischemic stroke, ASA is given orally to prevent further ischemic events. The beneficial effect of this treatment results from its antithrombotic effect, and possibly from its neuroprotec-

### Table 1. Plasma and Brain Concentrations of Acetylsalicylic Acid and Salicylic Acid in Rats After 7 Days of Treatment*

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg/day)</th>
<th>ASA concentrations</th>
<th>SA concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma (µg/ml)</td>
<td>Brain (µg/g tissue)</td>
<td>Plasma (µg/ml)</td>
</tr>
<tr>
<td>ASA</td>
<td>1</td>
<td>ND 0.83 ± 0.18</td>
<td>ND 2.19 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>ND 1.53 ± 0.13</td>
<td>ND 2.78 ± 0.18</td>
</tr>
<tr>
<td>SA</td>
<td>1</td>
<td>ND ND 3.48</td>
<td>ND 0.13 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>ND ND 4.51</td>
<td>ND 0.04 ± 0.04</td>
</tr>
</tbody>
</table>

*Plasma and brain concentrations of ASA and SA in rats after 7 days of treatment with 1 or 10 mg/kg/day (orally) of ASA or SA. Each value is the mean of 6-8 experiments. ASA, acetylsalicylic acid; SA, salicylic acid; ND, not detectable.

![Fig. 8. Net lactate-dehydrogenase activity (LDH) in brain slices. A: Time-course of LDH in oxygenated (O₂) and hypoxic (N₂) slices. B: Percentage of change with respect to pre-hypoxic value after 120 min of hypoxia, in slices incubated with ASA or SA. C: Percentage of change with respect to pre-hypoxic value, after 120 min of hypoxia, in hypoxic slices after 1 week of oral treatment with ASA or SA. *P < 0.05, **P < 0.001, with respect to oxygenated samples (A), saline-incubated (B), or saline-treated (C) animals.](image-url)
tive effect. According to several studies, it seems that ASA has an antiplatelet effect, and that SA is involved in modulation of the inhibition of vascular prostacyclin synthesis and the increased production of NO that ASA brings about (Peterson et al., 1981; De Gaetano et al., 1985; De La Cruz et al., 2002a, 2003). In experiments that compared the oral administration of ASA and SA, we aimed to evaluate the importance of this cytoprotective effect in brain tissue, and to determine the role of SA in this effect. We therefore tested two doses that represent prophylactic antithrombotic treatment (1 mg/kg/day), on one hand, and a dose that inhibits prostacyclin synthesis (supratherapeutic dose; 10 mg/kg/day), on the other (De La Cruz et al., 2002b).

In qualitative terms, the results were a replay of the data obtained in vitro. They confirmed that the effects of ASA and SA occurred at doses equivalent to those used for long-term treatment in humans. In addition, the effect of SA was greater than that of ASA in inhibiting oxidative stress and normalizing NOS activity. Of importance was the fact that these effects occurred even at antithrombotic doses, which implies that when ASA is taken to prevent thrombotic events, it also exerts antioxidant and cytoprotective effects in the brain.

After ASA or SA administration, the former was not and the latter was detectable in brain tissue. This finding, together with the greater antioxidant and cytoprotective effects of SA and its better ability to regulate brain NOS activity in the model of hypoxia tested, supports the notion that SA formed in the liver and blood (Levy, 1976) plays an important role in the cytoprotective effect on brain tissue seen with chronic ASA use, in addition to the effect of ASA not yet metabolized. This suggests that a single drug might be used to achieve both of the main goals of secondary prophylaxis for ischemic stroke, i.e., avoiding formation of arterial thrombi (ASA) and protecting subsidiary tissue from ischemia (SA).

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REFERENCES


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