Antioxidant effects of a single dose of acetylsalicylic acid and salicylic acid in rat brain slices subjected to oxygen-glucose deprivation in relation with its antiplatelet effect

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Abstract

The aim of the present study was to analyze the relative participation of the antiplatelet and the antioxidant effects of acetylsalicylic acid (ASA) and salicylic acid (SA) after a single dose (1 or 10 mg/kg i.p.) in an in vitro model of anoxia in slices of rat brain. After 20 min of drug administration, blood and brain were obtained (n = 6 rats per group). We measured: lipid peroxidation, glutathione levels and lactate dehydrogenase efflux (LDH), ASA and SA concentrations and platelet aggregation in whole blood. An increase in lipid peroxidation (80%) and in LDH efflux (520%) and a decrease in glutathione levels (35%) were observed after 120 min anoxia in saline-treated rats. SA reduced this oxidative stress and LDH efflux, but it did not modify platelet aggregation. ASA strongly inhibited platelet aggregation but exerted a poor antioxidant effect. ASA was not detectable in brain tissue. We conclude that repeated doses of ASA are necessary to obtain a tissular antioxidant effect, probably when liver generates enough SA.

Keywords: Acetylsalicylic acid; Salicylic acid; Oxidative stress; Brain anoxia

Aspirin (acetylsalicylic acid, ASA) is the most widely used drug for the secondary prevention of ischemic cerebrovascular events [2]. This effect is due mainly to its antiplatelet aggregant action [9], which prevents the formation of arterial platelet thrombi. It has been postulated that the ability of ASA to prevent cerebrovascular accidents is complemented by a possible neuroprotective effect via a direct action on brain tissue [8,12]. Several mechanisms have been invoked to explain the neuroprotective effect aspirin in brain tissue [1,4,8,15]. It has been postulated that ASA and mainly its metabolite salicylic acid (SA) inhibit the oxidative stress [19]. 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 [13]. The aim of the present study was to analyze the relative participation of the antiplatelet and the antioxidant effects of ASA and SA after a single dose, in order to mimic the acute phase of the treatment of a ischemic cerebrovascular event.

The study was done with brain tissue from male Wistar rats (body weight 300–350 g) and was performed in compliance with international guidelines for the care and handling of laboratory animals (European Community Directive 86/609 EEC). A single intraperitoneal injection of normal saline (1 ml/kg) (n = 6 animals), ASA (1 and 10 mg/kg) (n = 6 animals each) or SA (1 and 10 mg/kg) (n = 6 animals each) were given at 09:00 h. Then 20 min after the dose was given, brain was collected and slices were obtained, then the experimental model of anoxia was carried out. These doses were chosen in order to explain an acute treatment against brain damage, and they are equivalent in humans to antithrombotic (1 mg/kg) and anti-inflammatory (10 mg/kg) dose, respectively.

The rats were anesthetized with 50 mg/kg i.p. of sodium thiopental, then 5 ml blood was drawn from the cava vein and then 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 0.2-mm slices with a vibratome (Capdem Instruments, San Francisco, CA, USA) according to a previously described technique [16] with slight modification. 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) 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 perfused with a mixture of 95% N₂ and 5% CO₂ for 120 min (anoxia). One brain slice was analyzed for each of the following conditions: (1) after 30 min of incubation and before N₂ perfusion; and after (2) 30 min, (3) 60 min, (4) 90 min and (5) 120 min of perfusion with N₂. For all studies the tissues were quickly frozen in liquid nitrogen and stored at −80 °C until the day of the experiment, which was done within 7 days of freezing.

Platelet activation was measured by the platelet aggregometry method in whole blood anticoagulated with 3.8% sodium citrate, by the impedance technique [3] with a Chrono-Log method in whole blood anticoagulated with 3.8% sodium citrate. An aliquot of the homogenate was poured into a microcentrifuge filter tube and centrifuged for 15 min at 3200 × g. Fifty microliters of the filtrate was injected into an HPLC-UV system.

The data in the text, tables, and figures are expressed as the mean ± standard error of the mean from six experiments in samples from different animals. All statistical analyses were done with the Social Program for Statistical Sciences (SPSS v. 10.0). One-way analysis of variance (ANOVA) followed by Bonferroni transformation was used, and differences were calibrated at P < 0.05.

In brain slices subjected to anoxia there was a time-dependent increase in TBARS production, which had risen by 80% after 120 min of anoxia in comparison to the pre-anoxia value (0.14 ± 0.01 nmol/mg protein). Total glutathione levels decreased steadily with time under anoxia. Inhibition reached 35% after 120 min of anoxia in comparison to the pre-anoxia value (8.43 ± 0.51 μmol/g tissue). The percentage of oxidized glutathione increased during anoxia by as much as 84% after 120 min in comparison to the pre-anoxia value (2.82 ± 0.21%). Efflux of LDH to the incubation medium increased with time during anoxia, and reached 520% after 120 min in comparison to the pre-anoxia value (0.23 ± 0.02 IU/mg tissue per minute).

The administration of a single intraperitoneal dose of ASA did not produce any statistical significant change in the brain oxidative stress, for that reason we only compare data from ASA 10 mg/kg with SA in Figs. 1 and 2. SA reduced TBARS production after 120 of anoxia in brain slices, while ASA exerted a poor effect (Fig. 1A). The decrease in glutathione levels and the increase of its oxidized form after 120 min of anoxia in the control group was smaller in rats treated with SA or ASA (Fig. 2). The increased LDH efflux was significantly lower after 120 min of anoxia in brain slices from rats treated with ASA and mainly with SA (Fig. 1B). Maximum intensity of platelet aggregation was inhibited after a single dose of ASA, but they were no modifications after SA administration (Fig. 3). Plasma and brain levels of ASA and SA in the different groups of animals are summarized in Table 1.

In our experimental model the administration of a single dose of aspirin decreases mainly platelet activation, and SA exerted an antioxidant effect and decreased the cellular death after the anoxic period. The inhibition of collagen-induced platelet aggregation in whole blood is in agreement with previous studies in rats [6] and it is due to the platelet cyclooxygenase acetylation by ASA [17]; SA does not inhibit platelet function because it does not affect cyclooxygenase activity.
The effect of the deacetylated compound on lipid peroxidation may be explainable by the ability of SA to react with hydroxyl ions [19] and thus prevent one of the main steps in the process of peroxidation of membrane lipids. The increase in intracellular glutathione in tissues from animals treated with SA may have been a consequence of its antiperoxidant effect; if oxidizing damage were mild, the antioxidant defense system would not be activated. In other words, SA might spare glutathione stores by counteracting the factors that induce glutathione depletion. This effect of SA seems to be similar to other antioxidant drugs in experimental models of oxygen-glucose deprivation in brain [7].

In a previous study we demonstrated in the same

Fig. 1. Time-course of (A) thiobarbituric acid reactive substances (TBARS), and (B) lactate dehydrogenase efflux (LDH) in brain slices during 120 min anoxia (n = 6 rats per group) in samples obtained after 20 min of a single intraperitoneal injection of acetylsalicylic acid (ASA) or salicylic acid (SA). *P < 0.05 versus saline-treated rats.

Fig. 2. Time-course of (A) glutathione content (GSH + GSSG) and (B) percentage of glutathione in oxidized form (%GSSG with respect to GSH + GSSG) in brain slices during 120 min anoxia (n = 6 rats per group) in samples obtained after 20 min of a single intraperitoneal injection of acetylsalicylic acid (ASA) or salicylic acid (SA). *P < 0.05 versus saline-treated rats.

antioxidant defense system would not be activated. In other words, SA might spare glutathione stores by counteracting the factors that induce glutathione depletion. This effect of SA seems to be similar to other antioxidant drugs in experimental models of oxygen-glucose deprivation in brain [7].

Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</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>2.62 ± 0.16 N.D.</td>
<td>2.12 ± 0.19 0.49 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.91 ± 0.58 N.D.</td>
<td>18.76 ± 2.64 0.50 ± 0.10</td>
</tr>
<tr>
<td>SA</td>
<td>1</td>
<td>N.D. N.D.</td>
<td>5.69 ± 0.42 2.53 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>N.D. N.D.</td>
<td>30.94 ± 2.67 2.96 ± 0.12</td>
</tr>
</tbody>
</table>

Each value is the mean of six experiments. N.D., not detectable.

Fig. 3. Maximum intensity of platelet aggregation (I_max) induced with 10 µg/ml collagen in whole blood in samples obtained after 20 min of a single intraperitoneal injection of acetylsalicylic acid (ASA) or salicylic acid (SA) (n = 6 rats per group). *P < 0.05 versus saline-treated rats.
experimental model that SA plays an important role in the antioxidant effect of chronic oral administration of ASA to rats [10]. In the present study, after the administration of ASA or SA, the former was not detectable in brain tissue whereas the latter was detectable (Table 1). There is not a clear dose dependence in the brain SA concentrations; this correlated with the absence of a clear dose dependence in TBARS and glutathione concentrations after ischemia. This finding, together with greater antioxidant and cytoprotective effects of SA in the model of anoxia we tested, supports the notion that the SA formed in the liver [14] could play an important role in the cytoprotective effect on brain tissue of chronic aspirin use. However, the first dose of aspirin could protect from the early formation of arterial thrombus, but it is necessary repeated doses to obtain a tissular antioxidant and cytoprotective effect, probably when liver SA formation will be enough to produce this antioxidant effect in brain tissue.

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References


