Original Contribution

THE METABOLISM OF SULINDAC ENHANCES ITS SCAVENGING ACTIVITY AGAINST REACTIVE OXYGEN AND NITROGEN SPECIES

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Abstract—Sulindac is a sulfoxide prodrug that, in vivo, is converted to the metabolites sulindac sulfide and sulindac sulfone. It is therapeutically used as an anti-inflammatory and analgesic in the symptomatic treatment of acute and chronic rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis. In addition to its anti-inflammatory properties, sulindac and its metabolites have been shown to have an important role in the prevention of colonic carcinogenesis. Although the inhibition of prostaglandin synthesis constitutes the primary mechanism of action of sulindac, it is well known that reactive oxygen species (ROS) and reactive nitrogen species (RNS) are implicated in the pathophysiology of inflammation and cancer. Thus, the aim of this study was to evaluate the scavenging activity of sulindac and its sulfone and sulfide metabolites for an array of ROS (HO·, O₂⁻, and HOCl) and RNS (NO and ONOO⁻) using in vitro systems. The results we obtained demonstrate that the metabolism of sulindac increases its scavenging activity for all RNS and ROS studied, notably with regard to the scavenging of HOCl. These effects may strongly contribute to the anti-inflammatory and anticarcinogenic efficacy that has been shown for sulindac. © 2003 Elsevier Inc.

Keywords—NSAIDs, Sulindac, Sulindac sulfide, Sulindac sulfone, Scavenging activity, Reactive oxygen species, Reactive nitrogen species, Free radicals

INTRODUCTION

Sulindac is a sulfoxide prodrug that can be included in a therapeutical class known as nonsteroidal, anti-inflammatory drugs (NSAIDs). In vivo, sulindac is converted to the metabolites sulindac sulfide and sulindac sulfone (Fig. 1) [1,2]. Sulindac sulfide, but not sulindac sulfone, blocks prostaglandin synthesis by nonselective inhibition of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) [3]. Although the inhibition of prostaglandin synthesis constitutes the primary mechanism of the anti-inflammatory action of NSAIDs, it is well known that reactive oxygen species (ROS), such as hydroxyl radical (HO·), superoxide radical (O₂⁻), hydrogen peroxide (H₂O₂), and hypochlorous acid (HOCl), and reactive nitrogen species (RNS), such as nitric oxide (NO) and peroxynitrite (ONOO⁻), produced in excess in the inflammatory site are implicated in the pathophysiology of inflammation [4–6]. In fact, it has been suggested that the anti-inflammatory activity of NSAIDs also may be, in part, due to their ability to interfere with free radical-mediated reactions [4,6–9].

In addition to their anti-inflammatory properties, sulindac and its metabolites have been shown to have an important role in the prevention of colonic carcinogenesis. They reduce the development of premalignant colonic polyps in patients with familial adenomatous polyposis (FAP) and inhibit the occurrence of gastrointestinal cancer in rats [10–12]. The inhibition of COX by NSAIDs also provides, to a first approximation, a rationale mechanistic for the chemopreventive effect of NSAIDs in FAP [12]. However, it is well known that FAP patients suffer from chronic oxidative stress, which suggests that the antioxidant treatment may become an important therapeutic strategy in the future [13].

The free radical-scavenging activity of sulindac was only scarcely tested before, showing its scavenging effect on the trichloromethylperoxyl radical [14], hydroxyl radical [14,15], and the peroxy radical [16], without any comparison to its main metabolites sulindac sulfide and sulindac sulfone, which remain to be studied. Studies on sulindac scavenging of HOCl [17] and hydrogen peroxy-
ide [18] were also performed before, indicating an absence of effect for both ROS; but, again, the activity of sulindac’s main metabolites was not evaluated. Thus, the aim of this study was to extend the evaluation of the scavenging activity of sulindac to its sulfone and sulfide metabolites in relation to an array of ROS (HO·, O₂⁻, and HOCl) and RNS (‘NO and ONOO⁻) using in vitro systems.

MATERIALS AND METHODS

Materials

Sulindac ([Z]-5-fluoro-2-methyl-1-[p-(methylsulfinyl)-benzylidene]indene-3-acetic acid), sulindac sulfone ([Z]-5-fluoro-2-methyl-1-[p-(methylsulfonyl)]benzylidene]indene-3-acetic acid), sulindac sulfide ([Z]-5-fluoro-2-methyl-1-[p-(methylthio)]benzylidene]indene-3-acetic acid), dihydrorhodamine 123 (DHR), 4,5-diaminofluorescein (DAF-2), sodium nitroprusside dihydrate, 30% hydrogen peroxide, β-nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), nitroblue tetrazolium chloride (NBT), anhydrous ferric chloride (FeCl₃), ethylenediaminetetraacetic acid (EDTA) disodium salt, ascorbic acid, trichloroacetic acid, thiobarbituric acid, deoxyribose, sodium hypochlorite solution with 4% available chlorine (NaOCl), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), mannitol, cysteine, lipoic acid, propylgallate, carboxy-PTIO, and sodium borohydride were all obtained from Sigma Chemical Co. (St. Louis, MO, USA). Peroxynitrite was obtained from Calbiochem (San Diego, CA, USA). Prior to each study, the concentration of the peroxynitrite stock was determined spectrophotometrically in 0.1 M NaOH (ε₃₈₀ nm = 1670 M⁻¹ cm⁻¹).

Hypochlorous acid-scavenging assay

Production of hypochlorous acid solution. HOCl was prepared immediately before use by adjusting the pH of a 1% (v/v) solution of NaOCl to 6.2 with diluted sulphuric acid. The concentration of HOCl was further determined spectrophotometrically at 235 nm using the molar absorption coefficient of 100 M⁻¹ cm⁻¹ [19]. For each assay, HOCl was appropriately diluted with 50 mM potassium phosphate buffer, pH 7.4.

Synthesis of 5-thio-2-nitrobenzoic acid (TNB). TNB was prepared according to a described procedure [20], with some modifications. Briefly, 50 mM sodium borohydride was added to a 1 mM solution of DTNB in 50 mM potassium phosphate buffer, pH 6.6, containing 5 mM EDTA. The solution was incubated at 37°C for 30 min. The concentration of TNB was determined by measuring the absorbance at 412 nm using the molar absorption coefficient of 13,600 M⁻¹ cm⁻¹. For each assay, TNB was appropriately diluted with 50 mM potassium phosphate buffer, pH 7.4.
Hypochlorous acid-scavenging assay. The assay was performed at room temperature. Reaction mixtures contained, in a final volume of 1 ml, the following reagents at the indicated final concentrations: TNB (70 μM); sulindac, sulindac sulfide, or sulindac sulfone, dissolved in ethanol, at various concentrations (0, 62.5, 125, 250, and 500 μM); and HOCl (25 μM). No direct effect was observed between ethanol and HOCl in the present assay conditions. The absorbance was measured at 412 nm, 5 min after the addition of HOCl [20]. Lipoic acid was used as a positive control. The effects are expressed as the percentage of inhibition of the TNB oxidation to DTNB (Fig. 2). Each study corresponds to four experiments performed in duplicate. 

Superoxide radical-scavenging assay

Superoxide radicals were generated by the NADH/PMS system and the O$_2^-$-scavenging activity was determined spectrophotometrically in a 96-well plate reader (Ceres 900, BioTek Instruments Inc., Winooski, VT, USA) by monitoring the effect of the tested compound on the O$_2^-$-induced reduction of NBT at 560 nm for 2 min [21].

The assay was performed at room temperature. The reaction mixtures in the sample wells contained, in a final volume of 300 μl, the following reagents at the indicated final concentrations: NADH (166 μM); NBT (43 μM); sulindac, sulindac sulfide, or sulindac sulfone, dissolved in DMSO, at various concentrations (0, 0.1, 0.2, 0.4, 0.8, and 1.6 mM); and PMS (2.7 μM). No direct effect was observed between DMSO and O$_2^-$ in the present assay conditions. All the other components were dissolved in 19 mM phosphate buffer, pH 7.4. Propylgallate was used as a positive control. The effects are expressed as the percentage of inhibition of the NBT reduction to di-formazan (Fig. 3). Each study corresponds to four experiments performed in triplicate.

Hydroxyl radical assay

The deoxyribose method for determining the scavenging effect of the potential scavenger on hydroxyl radicals was performed according to a described procedure [22]. Hydroxyl radicals were generated by a Fenton system (ascorbic acid/FeCl$_3$-EDTA). When exposed to hydroxyl radicals, the sugar deoxyribose is degraded to malonaldehyde, which generates a pink chromogen on heating with TBA at low pH.

Reaction mixtures contained, in a final volume of 1 ml, the following reagents at the indicated final concentrations: KH$_2$PO$_4$/KOH buffer, pH 7.4 (10 mM); deoxyribose (2.8 mM); H$_2$O$_2$ (1.42 mM); sulindac, sulindac sulfide, or sulindac sulfone at various concentrations (0, 6.25, 12.5, 25, 50, and 100 μM); FeCl$_3$-EDTA (20 μM and 100 μM); and ascorbic acid (50 μM). The iron salt was premixed with the chelator dissolved in water before adding to the reaction mixture. All other components were dissolved in 10 mM KH$_2$PO$_4$/KOH buffer, pH 7.4. After incubation at 37°C for 1 h, 1 ml of 2.8% trichloroacetic acid (w/v) and 1 ml of 1% thiobarbituric acid (TBA) (w/v) were added, and the mixture was
heated in a water bath at 100°C for 15 min. The absorbance of the resulting solution was measured at 532 nm. This assay was also performed without ascorbic acid or EDTA to evaluate a possible pro-oxidant and/or iron chelation activity. Mannitol was used as a positive control. The effects are expressed as the percentage of inhibition of the deoxyribose degradation to malonaldehyde (Fig. 4), with the second-order rate constants (Ks) calculated according to Halliwell and coworkers [23] (Table 1). Each study corresponds to four experiments performed in duplicate.

**Nitric oxide-scavenging assay**

The •NO-scavenging activity was measured by monitoring the •NO-induced oxidation of 4,5-diaminofluorescein (DAF-2), a specific •NO indicator, to triazolofluo-
rescein by fluorimetry, according to a described procedure [24] with some modifications.

Briefly, 1 mg of DAF-2 in 0.55 ml of DMSO was diluted 1/400-fold with 50 mM phosphate buffer, pH 7.4. Reaction mixtures contained, in a final volume of 1.5 ml, the following reagents at the indicated final concentrations: DAF-2 (3.14 μM); sodium nitroprusside (20 μM); and sulindac, sulindac sulfide, or sulindac sulfone, dissolved in DMSO, at various concentrations (0, 62.5, 125, 250, 500, and 1000 μM). No direct effect was observed between DMSO and NO in the present assay conditions. All the other components were dissolved in 50 mM phosphate buffer, pH 7.4. The reaction mixture was incubated for 10 min at 37°C under a tungsten light. Light is of prime importance for the sustained NO release by sodium nitroprusside [25]. The fluorescence signal caused by the reaction of DAF-2 with NO was measured using a spectrofluorometer (LS-50B, Perkin-Elmer Inc., Oak Brooke, IL, USA) with excitation and emission wavelengths of 495 and 521 nm, respectively, and excitation and emission slit widths of 8 nm, at 37°C. The effects are expressed as the percentage of inhibition of the DAF-2 oxidation (Fig. 5). Carboxy-PTIO was used as a positive control. Each study corresponds to four experiments performed in triplicate.

**Peroxynitrite-scavenging assay**

The ONOO−-scavenging activity was measured by monitoring the ONOO−-induced oxidation of dihydrorhodamine 123 (DHR) to rhodamine 123 by fluorimetry, according to a described procedure [26].

A stock solution of 2.89 mM DHR in dimethylformamide was purged with nitrogen and stored at −20°C. Working solutions of DHR diluted from the stock solution were placed on ice in the dark immediately before the determinations. Buffer (90 mM sodium chloride, 50 mM sodium phosphate, and 5 mM potassium chloride, pH 7.4) was purged with nitrogen and placed on ice before use. At the beginning of the experiments, 100 μM diethylenetriaminepentaacetic acid (DTPA) was added to the buffer. The assay was performed at 37°C. Reaction mixtures contained, in a final volume of 1.5 ml, the following reagents at the indicated final concentrations: DHR (50 μM); sulindac, sulindac sulfide, or sulindac sulfone, dissolved in DMSO, at various concentrations (0, 0.3125, 0.625, 1.25, and 2.5 mM); and ONOO− (600 nM) in 0.1 M NaOH. In the present assay conditions, less than a 10% effect was found for DMSO itself, which was subtracted in the results obtained for sulindac and its metabolites. Cysteine was used as a positive control. The fluorescence signal caused by the reaction of DHR with ONOO− was measured using a spectrofluorometer (LS-50B, Perkin-Elmer Inc.) with excitation and emission wavelengths of 500 and 536 nm, respectively, and excitation and emission slit widths of 2.5 and 3.0 nm, respectively. The background and final fluorescence intensities were measured 5 min after treatment.

The effects are expressed as the percentage of inhibition of the dihydrorhodamine 123 oxidation (Fig. 6). Each study corresponds to four experiments performed in triplicate.
Statistical analysis

Results are given as mean ± SEM. Statistical comparisons were made with one-way ANOVA followed by Newman-Keuls multiple comparison test. The level of significance was set at \( p < .05 \).

RESULTS

Hypochlorous acid-scavenging activity

Figure 2 shows the results obtained in the HOCl-scavenging assay. While sulindac and sulindac sulfone were devoid of any effect, sulindac sulfide proved to be a potent HOCl scavenger in that it prevented the HOCl-induced oxidation of TNB to DTNB in a concentration-dependent manner, with an \( IC_{50} \) of 165 ± 11 \( \mu \text{M} \) (mean ± SEM, Table 1). The positive control (lipoic acid) presented an \( IC_{50} \) of 60 ± 5 \( \mu \text{M} \) (mean ± SEM, Table 1).

Superoxide radical-scavenging activity

Figure 3 shows the results obtained in the \( O_2^- \)-scavenging assay. Sulindac, sulindac sulfone, and sulindac sulfide prevented the \( O_2^- \)-induced reduction of

Table 1. ROS- and NOS-Scavenging Activities of Sulindac and Its Metabolites Sulindac Sulfide and Sulindac Sulfone

<table>
<thead>
<tr>
<th>Tested compound</th>
<th>ROS</th>
<th></th>
<th>O(_2)(^-)</th>
<th>HOCI</th>
<th>'NO</th>
<th>ONOO(^-)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>( IC_{50} ) (( \mu \text{M} ))(^a)</td>
<td>( K_s ) (M(^{-1}) s(^{-1}))</td>
<td>( IC_{50} ) (mM)(^a)</td>
<td>( IC_{50} ) (( \mu \text{M} ))(^a)</td>
<td>( IC_{50} ) (( \mu \text{M} ))(^a)</td>
<td>( IC_{50} ) (( \mu \text{M} ))(^a)</td>
</tr>
<tr>
<td>Sulindac</td>
<td>96 ± 14 (7.1 ± 0.3) ( \times 10^{10})</td>
<td>1.2 ± 0.1</td>
<td>NA</td>
<td>510 ± 82</td>
<td>1320 ± 175</td>
<td></td>
</tr>
<tr>
<td>Sulindac sulfide</td>
<td>34 ± 7(^\dagger) (21 ± 3) ( \times 10^{10})</td>
<td>0.7 ± 0.1(^*)</td>
<td>165 ± 11(^\dagger)</td>
<td>412 ± 59</td>
<td>483 ± 80(^\dagger)</td>
<td></td>
</tr>
<tr>
<td>Sulindac sulfone</td>
<td>23 ± 3(^\dagger) (23 ± 1) ( \times 10^{10})</td>
<td>1.5 ± 0.2</td>
<td>NA</td>
<td>243 ± 24(^*)</td>
<td>1021 ± 60</td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.9 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Mannitol</td>
<td>3272 ± 215 (1.5 ± 0.2) ( \times 10^{9})</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Carboxy-PTIO</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.1 ± 0.2</td>
<td></td>
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<tr>
<td>Lipoic acid</td>
<td>–</td>
<td>–</td>
<td>60 ± 5</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Propylgallate</td>
<td>–</td>
<td>0.039 ± 0.001</td>
<td>–</td>
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</tr>
</tbody>
</table>

\(^a\) \( IC_{50} \) is mean ± SEM.
\(^*\) \( p < .05 \).
\(^\dagger\) \( p < .01 \) compared to sulindac.
NA = no activity was found within the tested concentrations.
NBT to diformazan in a concentration-dependent manner. However, while the effects observed for sulindac and sulindac sulfone were only marginal, with IC$_{50}$ values of 1.2 ± 0.1 and 1.5 ± 0.2 mM (mean ± SEM), respectively, sulindac sulfide was a much more potent O$_2^*$ scavenger with an IC$_{50}$ of 0.7 ± 0.1 mM (Table 1). The positive control (propylgallate) provided an IC$_{50}$ of 0.039 ± 0.001 mM (mean ± SEM, Table 1).

Hydroxyl radical activity

Figure 4 shows the results obtained in the HO$^*$-scavenging assay. Again, sulindac, sulindac sulfone, and sulindac sulfide prevented HO$^*$-induced degradation of deoxyribose into malonaldehyde in a concentration-dependent manner. Sulindac sulfone, which revealed to be the less active scavenger in the other assays for ROS, showed to be the most potent compound in this assay. The IC$_{50}$ values were 96 ± 14, 23 ± 3, and 34 ± 7 μM (mean ± SEM) for sulindac, sulindac sulfone, and sulindac sulfide, respectively (Table 1). The second-order rate constants (Ks) calculated were (7.1 ± 0.3) × 10$^{-10}$, (23 ± 1) × 10$^{-10}$, and (21 ± 3) × 10$^{-10}$ M$^{-1}$ s$^{-1}$ (mean ± SEM) for sulindac, sulindac sulfone, and sulindac sulfide, respectively (Table 1). The positive control (mannitol) provided an IC$_{50}$ of 3272 ± 59 μM and a Ks of (1.5 ± 0.2) × 10$^{9}$ M$^{-1}$ s$^{-1}$ (mean ± SEM, Table 1). Importantly, the Ks values found for mannitol and sulindac fall within the range of values reported previously [15,23]. The assay performed without ascorbic acid or EDTA did not indicate any pro-oxidant and/or iron chelation activity, respectively (data not shown).

Nitric oxide-scavenging activity

Figure 5 shows the results obtained in the NO-scavenging assay. Sulindac, sulindac sulfone, and sulindac sulfide showed to be potent inhibitors of NO-induced oxidation of DAF-2 to triazolofluorescein in a concentration-dependent manner, with sulindac sulfide proving to be the most active and sulindac the least active scavenger. The resulting IC$_{50}$ values were 510 ± 82, 243 ± 24, and 412 ± 59 μM (mean ± SEM) for sulindac, sulindac sulfone, and sulindac sulfide, respectively (Table 1). The positive control carboxy-PTIO yielded an IC$_{50}$ of 1.1 ± 0.2 μM (mean ± SEM).

Peroxynitrite-scavenging activity

Figure 6 shows the results obtained in the ONOO$^-$-scavenging assay. Sulindac, sulindac sulfone, and sulindac sulfide showed to be potent inhibitors of ONOO$^-$-induced oxidation of DHR in a concentration-dependent manner, with sulindac sulfide proving to be the most active scavenger. The resulting IC$_{50}$ values were 1320 ± 175, 1021 ± 60, and 483 ± 80 μM (mean ± SEM) for sulindac, sulindac sulfone, and sulindac sulfide, respectively (Table 1). The positive control (cysteine) yielded an IC$_{50}$ of 4.9 ± 0.6 μM (mean ± SEM, Table 1).

DISCUSSION

The results obtained in the present study demonstrate that sulindac and sulindac sulfone scavenge O$_2^*$, HO$^*$, NO, and ONOO$^-$, while sulindac sulfide scavenge HOCl, O$_2^*$, HO$^*$, "NO, and ONOO$^-"$. We have also clearly shown that the metabolism of sulindac increases its ROS- and RNS-scavenging activity, notably with regard to HOCl scavenging by sulindac sulfide, for which sulindac and sulindac sulfone are devoid of any activity. This is an interesting finding since sulindac sulfide is also the main anti-inflammatory and antitumoral active metabolite of sulindac.

In humans, after oral intake of sulindac, peak plasma concentration of this drug is reached after 1 h (Cmax ≈ 10–34 μM) and after 2 to 4 h for its sulfide (Cmax ≈ 3–33 μM) and sulfone (Cmax ≈ 4–19 μM) metabolites [27]. Of note, sulindac sulfide may be concentrated in the colonic epithelium at concentrations that are at least 20-fold higher than those seen in the plasma [27]. All three forms of sulindac undergo varying degrees of entero-hepatic recirculation [27]. There are little data regarding the distribution of sulindac into human tissues and fluids other than plasma, and, to date, no published studies have examined the concentrations of sulindac and its metabolites in synovial fluid or in the synovial tissue of patients with rheumatoid arthritis or osteoarthritis [27]. Importantly, the concentrations used in the present study may be considered within plasmatic and/or colonic concentrations obtained after therapeutic treatments with sulindac, notably in the HO$^*$- and HOCl-scavenging assays.

HOCl is a powerful oxidant generated by the polimorphonuclear neutrophils (PMN) enzyme myeloperoxidase from hydrogen peroxide and chloride ions [28], playing an important role in the bactericidal function [29]. However, when produced in excess, HOCl is also highly reactive toward a range of biological substrates and may cause deleterious effects in the surrounding tissue [29,30]. One of the major targets of HOCl in vivo is α$_1$-antiproteinase (α$_1$-AP), the most important inhibitor of PMN proteolytic enzymes especially elastase, which degrades elastin [31]. Other important intracellular macromolecules may be affected, namely the glutathione-S-transferase P1-1 [32], an enzyme particularly vulnerable to oxidative stress [33], and acetylcholinesterase [34].

Notably, it has been demonstrated that several sulfur-containing compounds efficiently prevent HOCl-induced deleterious effects in vitro, including reduced glutathione (GSH), oxidized glutathione (GSSG), methylglutathione (GSMe), as well as lipoic acid [34,35], N-acetylcysteine...
ity of intestinal damage-enhancing factor, especially in the treatment of injury and exogenous toxic activity. We have documented that, in acute gastrointestinal inflammation models, inhibition of endogenous NO synthase (iNOS) and NADPH oxidase [42,43,47], the involvement of ONOO− in the proinflammatory process may be of great importance. Compared to free radicals, ONOO− is a relatively stable species; but, once protonated, it gives off highly reactive peroxynitrous acid (ONOOH), which yields various cytotoxicants [48,49]. Indeed, ONOO− is a relatively long-lived cytotoxicant with strong oxidizing properties toward various cellular constituents, including sulfhydryls, lipids, amino acids, and nucleotides [50]. For example, ONOO− is able to induce oxidation of thiol groups in proteins, nitration of cysteine and tyrosine residues in proteins, nitrosation, and lipid peroxidation, causing cellular toxicity and affecting cell metabolism [26,41,50,51]. Furthermore, ONOO− directly inhibits mitochondrial respiratory enzymes, reduces cellular oxygen consumption, and inhibits membrane sodium transport [52]. The ability of ONOO− to cause severe colonic inflammation has also been documented [53], an effect shown to be potentiated with the downregulation of SOD [54], which makes the O2− more available to react with NO. Since specific endogenous scavenging enzymes responsible for ONOO− inactivation are lacking, the finding of scavengers of ONOO− is of considerable importance.

Sulindac and its metabolites scavenge ONOO− and prevent the formation of ONOO− via the scavenging of O2− and NO. Sulindac sulfide was found to be the most potent ONOO− scavenger, which is probably due to the reactivity of its thioether. In line with these results, it has been shown that the thioether methionine is converted by ONOO− to the correspondent sulfoxide or is fragmented to ethylene and dimethyl sulfoxide [55]. Nevertheless, in the present study we observed that the sulfone form of sulindac is also more potent than sulindac itself. Although the scavenging mechanism needs to be further studied, the present study, which showed an increase in the scavenging of NO and SOD, suggests that sulindac may be an antioxidant drug.

Peroxynitrite (ONOO−), a potent oxidizing, nitrating species, can be produced in vivo by the rapid interaction between NO and O2− [46]. Since these three reactive species are known to be generated during inflammatory reactions with the concomitant activation of inducible NO synthases (iNOS) and NADPH oxidase [42,43,47], the involvement of ONOO− in the proinflammatory process may be of great importance. Compared to free radicals, ONOO− is a relatively stable species; but, once protonated, it gives off highly reactive peroxynitrous acid (ONOOH), which yields various cytotoxicants [48,49]. Indeed, ONOO− is a relatively long-lived cytotoxicant with strong oxidizing properties toward various cellular constituents, including sulfhydryls, lipids, amino acids, and nucleotides [50]. For example, ONOO− is able to induce oxidation of thiol groups in proteins, nitration of cysteine and tyrosine residues in proteins, nitrosation, and lipid peroxidation, causing cellular toxicity and affecting cell metabolism [26,41,50,51]. Furthermore, ONOO− directly inhibits mitochondrial respiratory enzymes, reduces cellular oxygen consumption, and inhibits membrane sodium transport [52]. The ability of ONOO− to cause severe colonic inflammation has also been documented [53], an effect shown to be potentiated with the downregulation of SOD [54], which makes the O2− more available to react with NO. Since specific endogenous scavenging enzymes responsible for ONOO− inactivation are lacking, the finding of scavengers of ONOO− is of considerable importance.

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During inflammation, the activation of mast cells, macrophages, eosinophils, and neutrophils generates O2−, with NADPH oxidase playing an important role [29,56]. Besides the reaction with NO, O2− is also rapidly converted in H2O2 by superoxide dismutase (SOD). Much of the damage caused by increased generation of O2− and H2O2 arises from their transition metal-dependent conversion into the highly reactive hydroxyl HO radical [57]. Although the HO radical-scavenging effect of sulindac was already observed before [14], we have clearly demonstrated that sulindac sulfide
and sulindac sulfone exhibit a much more potent effect than their parent compound, which emphasizes the contribution of the metabolism for the antioxidant effect of this molecule. The importance of $O_2^\cdot$ -scavenging activity in the therapy of inflammation has been established [4]. The fact that all the tested compounds scavenged this radical and that sulindac sulfide is a much more potent $O_2^\cdot$ scavenger than sulindac and sulindac sulfone reinforce our interest in this molecule.

Sulindac is effective in treating intestinal adenomas in humans with familial adenomatous polyposis (FAP) and in preventing intestinal tumors in the C57Bl/6J-Min+ (Min) mouse, an animal model of FAP [58]. Although there is a clear demonstration that sulindac sulfide is the main metabolite responsible for the protective effects [58,59], it has been shown that sulindac sulfone, which is the main metabolite responsible for the antioxidant effect of this molecule. The importance of $O_2^\cdot$ -scavenging activity in the therapy of inflammation has been established [4]. The fact that all the tested compounds scavenged this radical and that sulindac sulfide is a much more potent $O_2^\cdot$ scavenger than sulindac and sulindac sulfone reinforce our interest in this molecule.

In conclusion, the results obtained in this study demonstrate that, besides the trichloromethylpro pyl radical, hydroxyl radical, and peroxyl radical scavenging activity that was reported before for sulindac, its metabolism increases its RNS- (NO and ONOO$^-$) and ROS- (HOCl, $O_2^\cdot$,$\cdot$HO) scavenging activity, especially in the case of HOCl. This effect may contribute strongly to the anti-inflammatory and anticarcinogenic efficacy that has already been shown for sulindac.

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