IN VITRO METABOLISM OF SULINDAC AND SULINDAC SULFIDE: ENZYMATIC FORMATION OF SULFOXIDE AND SULFONE

Shigeyuki KITAMURA and Kiyoshi TATSUMI
Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan
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Abstract—Liver 9,000×g supernatants from guinea pigs, rabbits, and dogs could catalyze the oxidation of both sulindac sulfide and sulindac, whereas those from mice and rats could catalyze only the oxidation of sulindac sulfide. In guinea pigs, the sulindac sulfide oxidase activity was detected in the 9,000×g supernatants of kidney and lung as well as liver, whereas the sulindac oxidase activity was detected only in the liver preparation. In addition, the former activity was located in both liver microsomal and cytosolic fractions, whereas the latter activity was located only in the microsomal fraction. Both sulindac sulfide and sulindac oxidase activities of guinea pig liver microsomes were inhibited by SKF 525-A, N-ethylmaleimide, and potassium cyanide. However, carbon monoxide inhibited only the oxidation of sulindac. The microsomal sulindac oxidase activity was enhanced 4-fold by 3-methylcholanthrene treatment.

Sulindac (cis-5-fluoro-2-methyl-1-[p-(methylsulfonyl)benzylidenyl]indene-3-acetic acid) is a new non-steroid antiinflammatory agent with analgesic and antipyretic activities (1–4). In animals (5) and in man (6), the drug is mainly metabolized to sulindac sulfone (5-fluoro-2-methyl-1-[p-(methylsulfonyl)benzylidenyl]indene-3-acetic acid) by its oxidation and to sulindac sulfide (5-fluoro-2-methyl-1-[p-(methylthio)-benzylidenyl]indene-3-acetic acid) by its reduction. The former reaction is irreversible, while the latter one is reversible (7). Furthermore, Duggan et al. (8) suggested that pharmacological activities of the drug are attributable to the reduction product, sulindac sulfide.

Previously, we demonstrated that the sulfoxide reductase activity toward sulindac was located in both the microsomes and 105,000×g supernatant from guinea pig liver. In particular, the microsomal reductase was NADPH- or NADH-dependent and required the factor present in the liver soluble fraction for its activity (9). Furthermore, we showed that various kinds of flavoenzymes such as NADPH-cytochrome c reductase, NADH-cytochrome b₅ reductase, xanthine oxidase, lipoamide dehydrogenase, and NADH dehydrogenase supplemented with their electron donors exhibited the sulfoxide reductase activity in the presence of a partially purified soluble factor from guinea pig liver (10).

In the present study, the sulfoxide oxidase activity toward sulindac sulfide (the sulindac sulfide oxidase activity) and the sulfoxide oxidase activity toward sulindac (the sulindac oxidase activity) were comparatively examined in vitro.
MATERIALS AND METHODS

Chemicals: Sulindac, sulindac sulfide, and sulindac sulfone were donated by Merck, Sharp and Dohme Research Laboratories. SKF 525-A was a gift from Smith, Kline & French Laboratories. NADPH, nicotinamide, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from the Sigma Chemical Co. N-Ethylmaleimide, potassium cyanide, sodium arsenite, and monooiodoacetic acid were obtained from Nakarai Chemicals, Ltd. Phenobarbital sodium and 3-methylcholanthrene were purchased from the Tokyo Kasei Kogyo Co., Ltd and Wako Pure Chemical Industries, Ltd., respectively. Precoated silica gel thin-layer plates (Wakogel F254 plates) were obtained from Wako Pure Chemical Industries, Ltd.

Animals: Male ICR strain mice (20–25 g), Sprague-Dawley strain rats (210–250 g), Hartley strain guinea pigs (310–340 g), Japanese White strain rabbits (3 kg), and dogs (8–10 kg) were used in this study. In some experiments, guinea pigs were treated with three daily doses of phenobarbital sodium (80 mg/kg intraperitoneally) in saline solution or 3-methylcholanthrene (25 mg/kg intraperitoneally) in corn oil and killed 24 hr after the last dose.

Enzyme preparation: Animals were fasted overnight prior to use and killed. Liver and other organs shown in Table 2 were immediately removed and homogenized in four volumes of 1.15% KCl. The homogenates were centrifuged for 20 min at 9,000 × g; and in the case of liver, the supernatant fractions were centrifuged for 60 min at 105,000 × g. The microsomal fractions were homogenized in 1.15% KCl and recentrifuged for 60 min at 105,000 × g. The washed microsomal fractions were resuspended in 1.15% KCl equivalent to the original volumes of livers.

Enzyme assay: In the case of the 9,000 × g or the 105,000 × g supernatant, an incubation mixture consisted of 0.4 μmol of a substrate (sulindac or sulindac sulfide) in 0.1 ml of MeOH, the supernatant equivalent to 1.5 g of an organ. 2 μmol of NADPH, 25 μmol of glucose-6-phosphate, 20 μmol of nicotinamide, and 75 μmol of MgCl₂ in a final volume of 12 ml of 0.2 M phosphate buffer (pH 7.4). In the case of liver microsomes, an incubation mixture consisted of 0.4 μmol of a substrate, microsomes equivalent to 2 g of liver. 2 μmol of NADPH, 25 μmol of glucose-6-phosphate, 2 units of glucose-6-phosphate dehydrogenase, 20 μmol of nicotinamide, and 75 μmol of MgCl₂ in a final volume of 8 ml of the phosphate buffer. The incubation was carried out for 1 hr at 37°C in an open vessel. After incubation, the mixture was extracted twice with two volumes of ethyl acetate by shaking. The combined extract was evaporated to dryness in vacuo and the residue was subjected to thin-layer chromatography (TLC) using a solvent system of CHCl₃-ethyl acetate-acetic acid (16:5:1). After development, chromatograms were visualized under ultraviolet light; and the area corresponding to sulindac (Rf 0.31) or sulindac sulfone (Rf 0.47) was each scraped and eluted with 3 ml of 28% ammonia water-methanol (1:49). The amount of each compound in the eluate was determined spectrophotometrically at 327 nm for sulindac or 328 nm for sulindac sulfone.

RESULTS

Species differences of oxidase activities: Liver 9,000 × g supernatants from guinea pigs, rabbits, and dogs were capable of oxidizing both sulindac sulfide and sulindac, whereas those from mice and rats were capable of oxidizing only sulindac sulfide under the same conditions (Table 1). The highest sulindac oxidase activity was observed in the rabbit 9,000 × g supernatant. In addition, the guinea pig, rabbit, and dog 9,000 × g supernatants exhibited high sulindac
sulfide oxidase activity (the sum of the amounts of sulindac and sulindac sulfone formed from sulindac sulfide).

**Tissue localization of oxidase activities in guinea pigs:** The 9,000×g supernatants from guinea pig tissues of interest were examined for their oxidase activities under the standard assay conditions. The sulindac sulfide oxidase activity was greatest in lung, followed by kidney and liver, while the sulindac oxidase activity was detected only in liver (Table 2).

**Intracellular localization of oxidase activities:** The liver microsomes and 105,000×g supernatant from guinea pigs

| Table 1. Species differences of sulindac sulfide and sulindac oxidase activities in liver 9,000×g supernatants |
|---------------------------------------------------------------|
| Species                                           | Sulindac sulfide oxidase activity (nmol/hr/g liver) | Sulindac oxidase activity (nmol/hr/g liver) |
|                                                  | Sulindac formed | Sulindac sulfone formed | Sulindac sulfone formed |
| Mouse                                            | 82±23           | 4±3                   | 7±2                     |
| Rat                                              | 71±3            | 1±1                   | 6±1                     |
| Guinea pig                                       | 149±17          | 67±16                 | 38±9                    |
| Rabbit                                           | 82±11           | 160±19                | 159±5                   |
| Dog*                                              | 177             | 68                    | 36                      |

Values represent the mean±S.D. of four experiments.

*Values represent the mean of two experiments.

| Table 2. Tissue localization of sulindac sulfide and sulindac oxidase activities in guinea pigs |
|-----------------------------------------------------------------------------------------------|
| Tissue                                           | Sulindac sulfide oxidase activity (nmol/hr/g tissue) | Sulindac oxidase activity (nmol/hr/g liver) |
|                                                  | Sulindac formed | Sulindac sulfone formed | Sulindac sulfone formed |
| Liver                                            | 149±17          | 67±16                 | 38±9                    |
| Kidney                                           | 189±22          | 0                     | 3±2                     |
| Lung                                              | 233±15          | 0                     | 0                       |
| Testis                                           | 9±4             | 0                     | 0                       |
| Small intestine                                   | 5±1             | 0                     | 0                       |

Values represent the mean±S.D. of four experiments.

| Table 3. Sulindac sulfide and sulindac oxidase activities in liver microsomal and cytosolic fractions of guinea pigs |
|------------------------------------------------------------------------------------------------------------------|
| Preparation                                                                 | Sulindac sulfide oxidase activity (nmol/hr/g liver) | Sulindac oxidase activity (nmol/hr/g liver) |
|                                                                 | Sulindac formed | Sulindac sulfone formed | Sulindac sulfone formed |
| Microsomes                                                                 | 125±15          | 33±13                 | 32±7                    |
| Cytosol                                                                 | 35±16           | 0                     | 0                       |

Values represent the mean±S.D. of four experiments.
Table 4. Influences of various chemicals on sulindac sulfide and sulindac oxidase activities in guinea pig liver microsomes

| Addition              | Concentration (M) | Sulindac sulfide oxidase activity (%) of control | Sulindac oxidase activity (%) of control |
|-----------------------|-------------------|-----------------------------------------------|----------------------------------------|
| None                  |                   | 100                                           | 100                                    |
| Carbon monoxide       |                   | 85                                            | 36                                     |
| SKF 525-A             | 10^{-3}           | 30                                            | 16                                     |
| N-Ethylmaleimide      | 10^{-4}           | 47                                            | 16                                     |
| Potassium cyanide     | 10^{-3}           | 35                                            | 11                                     |
| Sodium arsenite       | 10^{-4}           | 99                                            | 100                                    |
| Monoiodoacetic acid   | 10^{-3}           | 87                                            | 78                                     |

Values represent the mean of four experiments.

Table 5. Effects of 3-methylcholanthrene and phenobarbital treatments on sulindac sulfide and sulindac oxidase activities in guinea pig liver microsomes

| Treatment               | Sulindac sulfide oxidase activity (nmol/hr/g liver) | Sulindac oxidase activity (nmol/hr/g liver) |
|-------------------------|-----------------------------------------------------|---------------------------------------------|
|                         | Sulindac formed | Sulindac sulfone formed | Sulindac sulfone formed |
| Control                 | 132±3          | 20±2                       | 32±7                       |
| 3-Methylcholanthrene    | 68±10          | 118±2                      | 129±2                      |
| Phenobarbital           | 125±4          | 24±2                       | 35±6                       |

Values represent the mean of four experiments.

were assayed for their oxidase activities in the presence of NADPH. The sulindac sulfide oxidase activity was located in both the microsomes and 105,000 x g supernatant, but the activity of the former fraction was much higher than that of the latter one. However, the sulindac oxidase activity was detected only in the microsomal fraction (Table 3).

Effect of chemicals on oxidase activities: Carbon monoxide inhibited the oxidation of sulindac by guinea pig liver microsomes. SKF 525-A (a potent inhibitor of microsomal drug metabolizing enzymes), N-ethylmaleimide (a SH reagent), and potassium cyanide exhibited an inhibitory effect on the oxidation of both sulindac sulfide and sulindac. However, sodium arsenite and monoiodoacetic acid had a little or no effect on these reactions (Table 4).

Effect of 3-methylcholanthrene or phenobarbital treatment on oxidase activities: The sulindac oxidase activity in guinea pig liver microsomes was enhanced 4-fold by 3-methylcholanthrene treatment, whereas no significant alteration was observed in the sulindac sulfide oxidase activity. On the other hand, phenobarbital treatment did not alter sulindac sulfide and sulindac oxidase activities in the liver microsomes (Table 5).

DISCUSSION

The present study demonstrated that enzyme systems responsible for the oxidation of sulindac sulfide and sulindac are primarily located in the liver microsomal fraction. The
results are consistent with other reports that sulfide compounds such as chlorpromazine (11), 4,4'-diaminodiphenyl sulfide (11), S-n-propyl-L-cysteine (12), S-carboxymethyl-alkyl mercaptan (13), and methyl tetrahydrofurfuryl sulfide (14) and sulfoxide compounds such as dimethyl sulfoxide (15) and methyl tetrahydrofurfuryl sulfoxide (14) can be also oxidized by microsomal enzymes to the corresponding sulfoxides and sulfones, respectively. However, SKF 525-A did not affect the sulfoxidation of chlorpromazine (11), 4,4'-diaminodiphenyl sulfide (11), and S-n-propyl-L-cysteine (12); whereas this chemical exhibited an inhibitory effect on the oxidation of sulindac sulfide as shown in Table 4. To the contrary, the oxidation of S-n-propyl-L-cysteine was markedly inhibited by carbon monoxide (12), whereas only a little inhibition was observed in the oxidation of sulindac sulfide.

It is known that microsomal oxidase activities toward dimethyl sulfoxide (15) and methyl tetrahydrofurfuryl sulfoxide (16) could be induced by phenobarbital, but not 3-methylcholanthrene. On the other hand, the sulindac oxidase activity could be induced by 3-methylcholanthrene, but not phenobarbital, as shown in Table 5. The enzymes responsible for the oxidation of dimethyl sulfoxide (15) and methyl tetrahydrofurfuryl sulfoxide (14) resided in rat liver microsomes, whereas the sulindac oxidase was not located in liver microsomes of rats and mice, but in those of guinea pigs, rabbits, and dogs. These facts suggest that several different enzyme systems in the microsomal fraction are involved in oxidation reactions of sulfide and sulfoxide compounds.

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