Bioorganic Chemistry **29,** 156–163 (2001)
doi:10.1006/bioo.2001.1208, available online at http://www.idealibrary.com on IDEAL®

# Effect of Ginger Constituents and Synthetic Analogues on Cyclooxygenase-2 Enzyme in Intact Cells

Effie Tjendraputra, Van H. Tran, Damien Liu-Brennan, Basil D. Roufogalis, and Colin C. Duke[1]

*Herbal Medicines Research and Education Centre, Faculty of Pharmacy, University of Sydney, NSW 2006, Australia*

*Received April 4, 2001*

Seventeen pungent oleoresin principles of ginger (*Zingiber officinale*, Roscoe) and synthetic analogues were evaluated for inhibition of cyclooxygenase-2 (COX-2) enzyme activity in the intact cell. These compounds exhibited a concentration and structure dependent inhibition of the enzyme, with $IC_{50}$ values in the range of 1–25 $\mu$M. Ginger constituents, [8]-paradol and [8]-shogaol, as well as two synthetic analogues, 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decane and 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecane, showed strong inhibitory effects on COX-2 enzyme activity. The SAR analysis of these phenolic compounds revealed three important structural features that affect COX-2 inhibition: (i) lipophilicity of the alkyl side chain, (ii) substitution pattern of hydroxy and carbonyl groups on the side chain, and (iii) substitution pattern of hydroxy and methoxy groups on the aromatic moiety. 

*Key Words:* cyclooxygenase-2 inhibitors; gingerols; ginger constituents; phenolic compound structure–activity relationship.

## INTRODUCTION

The important role of cyclooxygenase (COX) enzyme in arachidonic acid (AA) metabolism has been well documented (*1–4*). Activation of COX results in the synthesis of a wide spectrum of prostaglandins (PGs) (*5,6*), the critical mediators of the inflammatory process, with various activities leading to increased vascular permeability, increased vasodilation and induction of neutrophil chemotaxis (*6*).

The COX enzyme exists as a homodimeric, heme-containing glycoprotein with a molecular weight around 70 kDa (*7,8*). In mammalian cells, two isoforms of COX have been discovered, designated COX-1 and COX-2, as described in literature (*1,8–11*). COX-1 is known as a housekeeping enzyme and is constitutively expressed in all cells (*2,3,9*), while COX-2 is the inducible enzyme (*1–3,9,12–15*), and its inducible expression is seen in cells like macrophages in the presence of inflammatory agonists (*16*).

[1] To whom correspondence and reprint requests should be addressed. Fax: (612) 93514391. E-mail: colind@pharm.usyd.edu.au.

0045-2068/01 $35.00

During inflammation or infection, induction of COX-2 expression increases the formation of prostaglandins to promote tissue repair (*17*). Additionally, COX-2 generated prostanoids were also reported to participate in induction of inflammatory pain, as an example, prostaglandins were found to sensitise pain fibers to mechanical and chemical stimuli (*9*). COX-2 thus responds more rapidly than COX-1 toward immune response, inflammation, tissue repair (*17*), and pain (*12*).

Ginger (*Zingiber officinale*) is widely used as a spice in cooking and as a medicinal herb in traditional herbal medicine. It contains several pungent constituents such as gingerols, shogaols, paradols, and gingerdiols. In traditional medicine, ginger has been used to treat many inflammatory conditions (*18*) and associated pain (*19*). Previous studies have shown that aqueous ginger extract reduced the synthesis of PG endoperoxides resulting in the reduction of prostaglandins formation as inflammatory mediators (*18*,*20*,*21*). This indicated that ginger may contain compounds that are clinically useful for treatment of inflammatory related diseases (*18*), such as asthma and arthritis. Moreover, ginger metabolites, [6]- and [8]-series of gingerol, shogaol, and paradol were shown to strongly inhibit COX-1 in disrupted rat basophilic leukemia-1 (RBL-1) cells (*22*). Additionally, [6]-gingerol was reported to reduce phorbol ester induced inflammation in mice when applied topically (*23*).

In the present study, the effect of gingerol and related substances on COX-2 was evaluated in an intact cell assay model using the cultured human airways epithelial A549 cells. The inhibitory activity of these compounds towards COX-2 enzyme occurred in a structure and dose dependent manner. Lipophilicity of the alkyl side chain, hydroxy and carbonyl groups, substitution patterns on the side chain, as well as hydroxy and methoxy groups substitution patterns on the aromatic moiety influence COX-2 inhibition. The findings that the ginger constituents and synthetic analogues act as potent COX-2 inhibitors may support the use of ginger as a medicinal herb for treatment of various inflammatory conditions and may serve to identify important lead compounds for the development of novel drugs that selectively act on COX-2 enzyme.

## MATERIALS AND METHOD

*Materials.* RPMI 1640, Dulbecco's phosphate buffered saline and penicillin streptomycin antibiotic were purchased from Life Technologies, Inc. Fetal calf serum (FCS) was purchased from CSL Diagnostic Pty. Ltd. Ethylenediamine-tetraacetic acid (EDTA) disodium dihydrate salt, indomethacin, dexamethasone, interleukin-1$\beta$ (IL-1$\beta$), and arachidonic acid were purchased from Sigma-Aldrich. Acro-cap filters were purchased from Gelman Science Australia. Sterile 12 multiwell Linbro tissue culture plates were purchased from ICN Pharmaceuticals, Inc.

*Test compounds and their sources.* [6]-, [8]-, [10]- and [12]-gingerols (**1–4**) and [8]-gingerdiol (**13**) were synthesized as described in the literature (*24–26*). [6]- and [8]-series shogaols (**5–6**) and paradols (**7–8**) were prepared according to Tanaka *et al.* (*25*) and Roufogalis *et al.* (*26*). Preparation of synthetic analogues: 5-hydroxy-1-(3-hydroxy-4-methoxyphenyl)dodecan-3-one (**9**), 5-hydroxy-1-(2-hydroxy-3-methoxyphenyl)dodecan-3-one (**10**), 2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one (**11**), 2-hydroxy-1-(3, 4-dimethoxyphenyl)dodecan-3-one (**12**), 3-hydroxy-1-(4-

hydroxy-3-methoxyphenyl)decane (**14**), 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-decan-4-ene (**15**) 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecane (**16**) and 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecane (**17**) were performed as described by Roufogalis *et al.* (*26*).

*Tissue culture conditions.* Cells (A549) were cultured in 10% FCS/RPMI 1640 (v/v) at 37°C in 5% $CO_2$ until confluent. Cells were harvested with 0.02% (w/v) EDTA/PBS, centrifuged at 1000*g* and resuspended at $5 \times 10^5$ cells/ml in fresh RPMI 1640.

*General procedure for the assay.* Cells ($5 \times 10^5$ cells/ml) were plated onto each well of a 12 multiwell plate and stimulated with IL-1$\beta$ (1 ng/ml) for 24 h, as described by Saunders *et al.* (*27*). In the dexamethasone control, cells were preincubated with dexamethasone (5 $\mu$g/ml) for 1 h, prior to IL-1$\beta$ exposure. After 24 h incubation, cells were replaced with fresh serum-free RPMI 1640 and were further incubated with dimethylsulfoxide (DMSO), test substance, or indomethacin for 5 min. Following the addition of arachidonic acid (30 $\mu$M) and further incubation for 20 min, culture supernatant was diluted in EIA buffer to measure the amount of $PGE_2$ formed using an EIA kit. The test substances were prepared in DMSO to give a dose response curve. The final concentration of DMSO in the assay was 1%. Similar assay conditions were employed to study the effects of test compounds on COX-1 enzyme activity with the exception of the step where the cells were exposed to IL-1$\beta$ (1 ng/ml).

*Data analysis.* The data was fitted to a sigmoid-$E_{max}$ model according to the equation: $E = (E_{max} * C\hat{}\gamma)/(EC_{50}\hat{}\gamma + C\hat{}\gamma)$ using the "Scientist" kinetic software to determine the $EC_{50}$ values of each of the test substances. $EC_{50}$ was expressed as the concentration that gave 50% inhibition, $E_{max}$ represents maximum percentage inhibition of the test substance, and $\gamma$ represents the cooperativity of drug molecules interactions. Log *P* values for the test compounds were calculated according to the fragment addition method using the Molecular Modelling Pro, Chemistry ChemSW Software for Windows. Data are reported as mean ± SE from duplicates of an independent assay.

## RESULTS AND DISCUSSION

The A549 cell line is known to express the COX-2 isoform in the presence of inflammatory cytokines, particularly interleukin-1$\beta$ (IL-1$\beta$). After 24 h incubation with IL-1$\beta$ (1 ng/ml), COX-2 was maximally expressed in the cells similar to that described previously (*27*).

All active COX-2 inhibitors in this study exhibit a full inhibition of the enzyme in a dose-dependent manner. Compounds that inhibit COX-2 activity by more than 80% at 10 $\mu$M were selected for determination of $IC_{50}$ values. The results showed that [10]-gingerol (**3**), [6]-shogaol (**5**), [8]-paradol (**8**), and several synthetic analogues **11**, **14**, **15**, and **17** with $IC_{50}$ values of 3.7, 2.1, 3.4, 5.5, 2.9, 4.1, and 1.4 $\mu$M, respectively (Fig. 1; Tables 1 and 2) had the greatest inhibitory activity towards this enzyme. While other compounds, such as [8]-gingerdiol (**13**) ($IC_{50}$ = 12.5 $\mu$M), [8]-gingerol (**2**) ($IC_{50}$ = 10 $\mu$M) and its positional isomer, (**9**) ($IC_{50}$ = 15.8 $\mu$M), had moderately strong COX-2 inhibitory activity (Fig. 1; Tables 1 and 2).

Interestingly, our results showed that [6]-gingerol (**1**), a homologue of [8]-gingerol

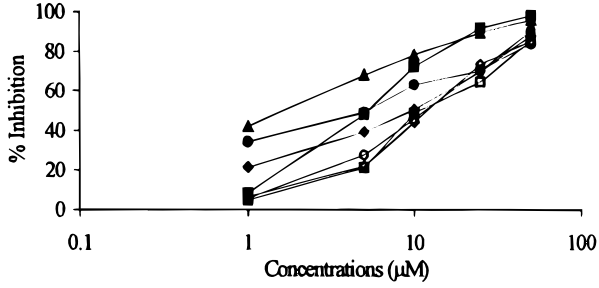


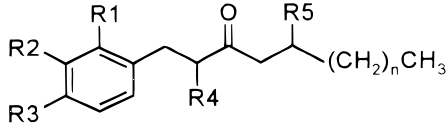

**FIG. 1.** The concentration dependent inhibition study of **2** (♦), **6** (■), **8** (▲), **13** (○) and synthetic analogues **15** (◇), **16** (●), and **17** (□). Measurement of COX-2 inhibitory activity of the test substance was performed as described under Materials and Methods.

(**2**), with two carbons less in the side chain, has no significant inhibitory effect on COX-2 enzyme.

A preliminary study on the selectivity of the synthetic gingerols analogues **11** and **14** revealed that these compounds assayed under similar conditions with the same

TABLE 1

Chemical Structures, Log *P*, and $IC_{50}$ Values of Gingerol Analogues and Related Substances Studied in A549 Intact Cells for COX-2 Inhibitory Effects




| No. | R1 | R2 | R3 | R4 | R5 | Double bond | *n* | $IC_{50}$ ($\mu$M) | Log *P* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | H | OMe | OH | H | OH | — | 4 | >50 | 1.8 |
| 2 | H | OMe | OH | H | OH | — | 6 | 10.0 ± 1.3 | 2.9 |
| 3 | H | OMe | OH | H | OH | — | 8 | 3.70 ± 0.02 | 3.9 |
| 4 | H | OMe | OH | H | OH | — | 10 | 6.6 ± 1.6 | 5.0 |
| 5 | H | OMe | OH | H | H | (C4–C5) | 4 | 2.1 ± 0.3 | 3.9 |
| 6 | H | OMe | OH | H | H | (C4–C5) | 6 | 7.2 ± 0.4 | 4.9 |
| 7 | H | OMe | OH | H | H | — | 4 | 24.5 ± 3.8 | 4.1 |
| 8 | H | OMe | OH | H | H | — | 6 | 3.4 ± 0.9 | 5.1 |
| 9 | H | OH | OMe | H | OH | — | 6 | 15.8 ± 2.0 | 2.9 |
| 10 | OH | OMe | H | H | OH | — | 6 | >50 | 2.9 |
| 11 | H | OMe | OH | OH | H | — | 6 | 5.5 ± 0.9 | 3.9 |
| 12 | H | OMe | OMe | OH | H | — | 6 | >50 | 4.3 |

*Note*. Data are reported as mean ± average deviation of duplicates from one determination. $IC_{50}$ values were calculated by fitting data to the Sigmoidal-$E_{max}$ model using the Scientist Software as described under Material and Methods. Log *P* values were calculated by the addition method using the Molecular Modelling Pro Software as described under Material and Methods.