Imrecoxib: a novel and selective cyclooxygenase 2 inhibitor with anti-inflammatory effect

Xiao-hong CHEN, Jin-ye BAI, Fang SHEN, Ai-ping BAI, Zong-ru GUO, Gui-fang CHENG

Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

KEY WORDS imrecoxib; cyclooxygenase 1; cyclooxygenase 2; cyclooxygenase inhibitors; inflammation

ABSTRACT

AIM: To investigate the inhibitory effect of imrecoxib, a synthetic compound of completely new structure, on cyclooxygenase 1 (COX-1) and 2 (COX-2) and its anti-inflammatory effect in vivo. METHODS: The inhibitory effects of imrecoxib on cyclooxygenase 1 and 2 were studied using whole cell assay with murine peritoneal macrophages induced by calcimycin and LPS. The inhibitory effects of imrecoxib on mRNA level of COX-1 and COX-2 in human macrophage cell line U937 were detected by reverse transcription polymerase chain reaction (RT-PCR) analysis. Effects of imrecoxib on acute and chronic inflammation were evaluated in rat carrageenan induced edema model and rat adjuvant-induced arthritis model, respectively. RESULTS: Imrecoxib was found to inhibit COX-1 and COX-2 with IC50 value of 115±28 nmol/L and 18±4 nmol/L, respectively. Imrecoxib was shown to selectively and dose-dependently inhibit COX-2 mRNA level. Imrecoxib effectively inhibited carrageenan-induced acute inflammation at the doses of 5, 10, and 20 mg·kg⁻¹·ig and adjuvant-induced chronic inflammation at the doses of 10 and 20 mg·kg⁻¹·d⁻¹·ig. CONCLUSION: Imrecoxib is a novel and moderately selective COX-2 inhibitor that possesses anti-inflammatory effect by inhibition of COX-2 mRNA expression.

INTRODUCTION

Cyclooxygenase (COX) is a rate-limiting enzyme for prostaglandin synthesis. Three isotypes of COXs (COX-1, COX-2, and COX-3) have been identified[1-2], though COX-3 activity in human has not been confirmed[3]. COX-1 is constitutively involved in actions such as platelet activation, gastrointestinal protection and kidney function. COX-2 is primarily produced in response to tissue damage and is involved in inflammatory responses. Traditional non-steroidal anti-inflammatory drugs (NSAIDs) act primarily by inhibiting COXs[4]. Inhibition of COX-1 by NSAIDs leads to heavy gastrointestinal toxicity. Newly developed COX-2 selective inhibitors, such as celecoxib (Celebrex) and rofecoxib (Vioxx) potently inhibit COX-2[5]. This type of drugs is expected to be devoided of gastric toxicity mediated primarily by inhibition of COX-1, and retain high anti-inflammatory activity.

However, there is no clear-cut division between biological functions of COX-1 and COX-2. Experimental evidence indicates that a full inflammation response is likely sustained by prostanoids generated by both enzymes[6]. In this sense, drugs inhibiting both enzymes are theoretically more effective in inflamma-
tory disease treatment. Moreover, an understanding of the physiologic features of COX-2 has led to the appreciation that COX-2 selective inhibitors may theoretically lead to problems in thrombosis, salt and water balance, and healing. The increased incidence of nongastrointestinal serious adverse events, with the COX-2 selective NSAIDs as compared with nonselective NSAIDs, in the Celecoxib Long-term Arthritis Safety Study (CLASS) and the Vioxx Gastrointestinal Outcomes Research (VIGOR) study remains a major concern[7]. With all these aspects considered, developing drugs that preferentially inhibit COX-2 with moderate selectivity may be more promising.

Imrecoxib [also known as BAP-909, 4-(4-methane-sulfonyl-phenyl)-1-propyl-3-p-tolyl-1,5-dihydropyrrol-2-one], is a synthetic compound of completely new structure (patent application No 00105899.1). In the present study, inhibitory effects of imrecoxib on cyclooxygenase 1 and 2 and its anti-inflammatory effect in vivo were described.

MATERIALS AND METHODS

Reagents  LPS (E coli 055:B5) and calcimycin (A23187) were from Sigma; Brewer thioglycollate medium was from Difco; 6-keto-PGF1α and PGE2 radioimmunoassay (RIA) kit were from PLA General Hospital, Beijing, China; RPMI-1640, M-MLV reverse transcriptase, and Trizol reagent were from GIBCO-BRL; phorbol 12-myristate 13-acetate (PMA), carrageenan, and Bacillus Calmette Guerin (BCG) were all from Sigma.

Test compounds  Imrecoxib (white powder), rofecoxib, and indomethacin were synthesized by our group. Purities of them were beyond 98.5 %. For in vitro studies, all test drugs were prepared in stock solution 0.1 mol/L with Me2SO and stored at -20 ºC. Before using, the stock solution was diluted to appropriate concentrations in RPMI-1640. For COX-1 assay, all drugs were ground into fine powder and diluted to appropriate concentrations with 5 % gum arabic just before use.

Animals  Male C57BL-6J mice (17±1 g, 6-7 weeks) and Wistar rats (200±10 g, 5-6 weeks) were from the Experimental Animal Center, Institute of Experimental Animal, Chinese Academy of Medical Sciences & Peking Union Medical College (SPF, certificate No SCXK 11-00-0006). They were housed in groups under 12 : 12 h regime (lights on from 7:00 h to 19:00 h) at 23±2 ºC prior to the experiments, and were given standard laboratory chow and tap water ad libitum.

Whole cell assay with murine peritoneal macrophages induced by calcimycin and LPS  The assay was done according to the method of Shen et al[9]. Briefly, peritoneal macrophages were harvested from male C57BL-6J mice 3 d after the injection (ip) of brewer thioglycollate medium (50 mL/kg body weight), washed once in D-Hanks’ buffer and resuspended in RPMI-1640 supplemented with 5 % (v/v) newborn calf serum. For COX-1 assay, cells (5×10⁵ cells in 500 µL) were incubated with the test compounds at different concentrations or Me2SO vehicle for 1 h and were stimulated with calcimycin 1 µmol/L for 1 h. The amount of 6-keto-PGF1α (a stable metabolite of PGI2) in the supernatant was measured by radioimmunoassay. For COX-2 assay, macrophages (5×10⁵ cells in 500 µL) were incubated with the test compounds at different concentrations or Me2SO vehicle for 1 h and were stimulated with LPS of 11 mg/L for 9 h. The amount of PGE2 in the supernatant was measured by radioimmunoassay.

RT-PCR analysis with human macrophage cell line U937  Human cell line U937 was from Cell Center, Chinese Academy of Medical Sciences & Peking Union Medical College. Cells were maintained in RPMI-1640 supplemented with 10 % (v/v) newborn calf serum in a humidified environment of 5 % CO2 at 37 ºC and differentiated into macrophages by 10 nmol/L PMA according to the method of Miriam et al[9]. Macrophages were incubated with the test compounds at different concentrations or Me2SO vehicle for 1 h and were stimulated with 100 µg/L LPS for 12 h. Then cells were harvested and total RNA was extracted with Trizol reagent. First strain cDNA was synthesized from equal amount of total RNA with M-MLV reverse transcriptase and random hexamer. The cDNA of GAPDH, COX-1 and COX-2 were separately amplified by PCR with TaKaRa Tag for 28 cycles consisting of 94 ºC for 30 s, 55 ºC for 30 s, and 72 ºC for 45 s using GAPDH

Fig 1. Structure of imrecoxib (M,=369.3).
primers of 5'-ACGGATTTGGTCGTATTGGG-3' and 5'-CGCTCCTGGAAGATGGTGAT-3', COX-1 primers of 5'-GCTCAGGAGGAAGTTCATACC-3' and 5'-AGGAAGCAGCCAAACAC-3' primers, COX-2 primers of 5'-CCTGTGCCTGATGATTGC-3' and 5'-CGGTGAAACTCTGGCTAG-3'. PCR products were solved on 1% (w/v) agarose gel and visualized by ethidium bromide.

**Rat carrageenan-induced edema model** Effect of imrecoxib on acute inflammation was evaluated in rat carrageenan-induced edema model as described previously[10]. Briefly, 0.1 mL of 1% carrageenan in normal saline was intradermally injected into the left hindpad of male Wistar rats. Imrecoxib (5, 10, or 20 mg·kg⁻¹, ig), rofecoxib (5 mg·kg⁻¹, ig), and gum arabic vehicle were administrated 1 h before carrageenan injection. The degree of swelling was determined by measuring the circumference of the ankle joint with a ruler made of Scotch cellophane tape before and at 2, 3, 4, 5, and 6 h after the carrageenan injection. The percent degree at each time was determined by comparing with vehicle controls.

**Rat adjuvant-induced arthritis (AIA) model** Effects of imrecoxib on chronic inflammation was evaluated in AIA model as described previously[11]. Briefly, male Wistar rats were immunized on d 0 by intradermal injection of Freund's complete adjuvant containing 10 mg heat-inactivated BCG in 1 mL paraffin oil, into the left footpad in 0.1 mL for each rat. Imrecoxib (5, 10, or 20 mg·kg⁻¹·d⁻¹, ig), rofecoxib (5 mg·kg⁻¹·d⁻¹, ig), and gum arabic vehicle were started on d 7 after immunization and continued throughout the experiment. The degree of the secondary paw (noninjected paw) swelling was determined by measuring the circumference of the ankle joint with a ruler made of Scotch cellophane tape on d 14 and d 26 after immunization. The percent degree at each time was determined by comparing with vehicle controls. Rats were euthanized by carbon dioxide inhalation on d 26. The thymus and spleen of all rats were removed and weighed.

**Statistical analysis** Data were expressed as mean±SD of more than three independent experiments. Differences between groups were tested using one-way ANOVA, followed by multiple comparisons by Dunnett's test using SPSS 11.5 for Windows. A value of P<0.05 was considered statistically significant. IC₅₀ was derived from dose-inhibitory effect curves which were fit through “uphill dose response curves, variable slope” using Prism, GraphPad version 3.00.

### RESULTS

**Selective inhibition of COX-2 by imrecoxib in whole cell assay** The inhibitory effect of imrecoxib on calcimycin induced COX-1 activity was dose-dependent at the concentrations of 100-10000 nmol/L, with IC₅₀ value of 115±28 nmol/L. The inhibitory effect of imrecoxib on LPS induced COX-2 activity was dose dependent at the concentrations of 10-1000 nmol/L, with IC₅₀ value of 18±4 nmol/L. The selective ratio (IC₅₀,COX-1/IC₅₀,COX-2) of inhibition was 6.39, between that of indomethacin and rofecoxib (Tab 1).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC₅₀/nmol·L⁻¹</th>
<th>IC₅₀,COX-1/IC₅₀,COX-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imrecoxib</td>
<td>115±28</td>
<td>18±4</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>4.7±1.1</td>
<td>7.1±1.2</td>
</tr>
<tr>
<td>Rofecoxib</td>
<td>&gt;1000</td>
<td>4.7±0.5</td>
</tr>
</tbody>
</table>

**Selective decreasing of COX-2 mRNA level by imrecoxib** COX-2 mRNA level in U937 cell was increased by PMA and PMA+LPS stimulation, while COX-1 mRNA level showed no change. Imrecoxib at concentrations of 0.10-10 µmol/L selectively and dose dependently decreased COX-2 mRNA level induced by PMA+LPS, but showed no effect on COX-1 mRNA level (Fig 2). In contrast, rofecoxib and indomethacin at concentrations of 0.10-10 µmol/L showed no effect on both COX-2 and COX-1 mRNA level.

**Inhibitory effect of imrecoxib on carrageenan-induced paw edema** The administration of imrecoxib and rofecoxib 1 h before injection of carrageenan significantly inhibited the edema response at 3, 4, 5, and 6 h, and the inhibitory effect was maximal at 4 h (Tab 2). Moreover, there is no significant difference between the inhibitory potency of imrecoxib at different doses and rofecoxib (P>0.05).

**Inhibitory effect of imrecoxib on rat with AIA** Inflammatory polyarthritis was induced in all immunized rats. The peak incidence occurred on d 14 after immunization. Treatment with imrecoxib (10 or 20 mg·kg⁻¹·d⁻¹) and rofecoxib diminished the secondary paw swelling on both d 14 and d 26 after immunization (Tab 3). Moreover, there is no significant difference between
the inhibitory potency of imrecoxib at different doses and rofecoxib (P>0.05). The index of thymus and spleens were not significantly affected by both imurecoxib and rofecoxib (P>0.05, data not shown).

At the end of the study, a general necropsy was performed, and abdominal, peritoneal, and thoracic cavities were normal in all rats.

**DISCUSSION**

Activated macrophages express COX-2 and produce excessive amounts of PGE$_2$, which plays a key role in the process of inflammation. The present study demonstrated that imrecoxib, a synthetic compound of completely new structure, was a selective inhibitor of COX-2 in two *in vitro* assays based on macrophages. In whole cell assay with murine peritoneal macrophages induced by calcimycin and LPS, imrecoxib selectively inhibited COX-2 activity with moderate selectivity, and the IC$_{50}$ value of COX-2 inhibition by imrecoxib was comparable to that of indomethacin. This provided further consideration that imrecoxib might be a novel COX-2 inhibitor with moderate selectivity. RT-PCR analysis with human macrophage cell line U937 presents the difference between imrecoxib and other COX-2 inhibitors. Imrecoxib was shown to selectively and dose dependently inhibit COX-2 mRNA expression in the present study, and showed no effect on COX-1 mRNA expression. Indomethacin and rofecoxib, however, showed no such effect, which conforms to the result of Barrios-Rodiles *et al* [12]. We also investigated effect of imrecoxib on mRNA expression of other inflammatory mediators, such as interleukin-1, interleukin-6, interleukin-8, and MMP-9 in PMA-differentiated U937 cell. However, the compound did not affect their mRNA expression (data not shown).

To investigate anti-inflammatory effect of imrecoxib *in vivo*, we evaluated the inhibitory effects of imrecoxib on rat carrageenan-induced paw edema and rat adjuvant-induced arthritis, respectively. Imrecoxib at different doses were shown to effectively

| Tab 2. Inhibitory effects of compounds on carrageenan-induced paw edema in rat at different time. n=10. Mean±SD. *P<0.05, †P<0.01 vs control. |
|---|---|---|---|---|
| Time/h | 5 | 10 | 20 | Control |
| | BAP-909/mg·kg$^{-1}$ | Rofecoxib/mg·kg$^{-1}$ |  |
| 2 | Paw edema/∆cm | 0.46±0.05 | 0.51±0.04 | 0.51±0.04 | 0.49±0.06 | 0.51±0.06 |
| Inhibition/% | 9.8 | 0 | 0 | 4.9 | - |
| 3 | Paw edema/∆cm | 0.54±0.05 | 0.51±0.08 | 0.48±0.07 | 0.51±0.06 | 0.65±0.12 |
| Inhibition/% | 17.3b | 21.2c | 26.9c | 21.2c | - |
| 4 | Paw edema/∆cm | 0.63±0.07 | 0.54±0.05 | 0.54±0.07 | 0.59±0.06 | 0.75±0.12 |
| Inhibition/% | 16.7b | 28.3c | 28.3c | 21.7c | - |
| 5 | Paw edema/∆cm | 0.65±0.09 | 0.65±0.08 | 0.56±0.07 | 0.64±0.05 | 0.75±0.05 |
| Inhibition/% | 13.3b | 13.3b | 25.0c | 15.0b | - |
| 6 | Paw edema/∆cm | 0.64±0.07 | 0.66±0.07 | 0.63±0.05 | 0.63±0.05 | 0.75±0.05 |
| Inhibition/% | 15.0b | 11.7b | 16.7c | 16.7c | - |
inhibit carrageenan-induced paw edema and adjuvant-induced secondary paw swelling. Moreover, there was no significant difference between inhibitory efficacy of imrecoxib at different doses and rofecoxib in two inflammation models. The results suggest that imrecoxib may effectively relieve acute and chronic inflammation in treatment of inflammatory disease.

In conclusion, the present study clearly demonstrated that imrecoxib was a novel and moderately selective COX-2 inhibitor that possessed anti-inflammatory effect by inhibiting COX-2 mRNA expression. It has potential therapeutic role for acute and chronic inflammatory disease.

REFERENCES