Involvement of Prostaglandins in Kaolin-Induced Writhing Reaction in Mice

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The relationship between the kaolin-induced writhing reaction and production of arachidonate metabolites (PGs) in mice was studied. PGs were released into the peritoneal cavity after intraperitoneal injection (i.p.) of kaolin (2.5 mg/mouse) with a peak at 5 min. About 80% of the total amount was 6-keto-PGF₁α. There was a significant correlation (r = 0.8237, p < 0.001) between the number of writhes and the amount of 6-keto-PGF₁α. The writhing reaction induced by kaolin was significantly inhibited by simultaneous injection of soybean trypsin inhibitor (SBTI; 2.5 mg/mouse) and increased by simultaneous injection of captopril (50 μg/mouse). The writhing reaction induced by kaolin which was inhibited by oral administration of indomethacin (1 mg/kg) was restored by exogenous i.p. injection of PGI₂-Na (2—10 ng/mouse). Indomethacin, ibuprofen and alminoprofen inhibited the writhing reaction and reduced the level of peritoneal 6-keto-PGF₁α in parallel manner. Tiaramide, pentazocine and morphine inhibited the writhing reaction without reducing the levels of 6-keto-PGF₁α. These results differentiate the site of action of these analgesics. They suggest that the mechanism of the kaolin-induced writhing reaction in mice involves a synergic pain caused by simultaneously released bradykinin and PGI₂. This model is a useful tool which allows differentiation of mode of action of analgesics by simultaneous determination of the writhing response and peritoneal 6-keto-PGF₁α.

Keywords — kaolin; writhing; prostaglandin; bradykinin

Introduction

Recently, the authors developed a new pain model in mice by use of kaolin-induced writhing reaction for screening of antiinflammatory agents.¹ They also reported that bradykinin (BK) could be released by activation of kallikrein-kinin system through activation of factor XII and caused the writhing reaction.² The writhing response was markedly inhibited by cyclooxygenase inhibitor, suggesting involvement of arachidonate metabolites in the response. In the present study, the authors examined the role of the cyclooxygenase products in induction of the writhing response by kaolin in mice, and the relationship between them.

Materials and Methods

Animals — Male ICR mice (aged 5 weeks and weighing about 20 g) were purchased from the Shizuoka Cooperative Association for Experimental Animals.

Reagents — The reagents used were kaolin (Wako Pure Chemical Industries Co.), soybean trypsin inhibitor (SBTI; Sigma Chemical Co.), captopril (Sankyo Co., Ltd.), PGI₂-Na (Sigma), PGE₁, 6-keto-PGF₁α and thromboxan B₂ RIA kit (NEN), PGF₂α RIA kit (Baxter) and others. The drugs used were alminoprofen (Bouchara), ibuprofen (Sigma), indomethacin (Sigma), tiaramide (Fujsawa Pharmaceutical Co., Ltd.), pentazocine (Winthrop Laboratories) and morphine-hydrochloride (Takeda Chemical Industries, Ltd.). All drugs for oral administration were suspended in 5% gum arabic before use. Those for subcutaneous injection were suspended or dissolved in physiological saline solution before use. All other reagents were of the highest quality commercially available.

Procedure: 1. Time-course Changes in PGs Released into the Peritoneal Cavity in Mice

With Writhing Response Induced by Kaolin

— 1) Induction of Writhing Response with

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Kaolin: The writhing response was induced with kaolin in mice according to the method described in the previous report. That is, 0.5 ml of kaolin (5 mg/ml of saline) was intraperitoneally injected, and the number of writhing responses every 5 min were determined (early stage).

2) Collection of Peritoneal Washings after Administration of Kaolin: Mice with writhing induced by the above method were sacrificed by dislocation of cervical vertebrae, 5, 10, 30, 60, 90 and 120 min after injection of kaolin. The peritoneal cavity was washed with 2 ml of saline solution containing $1.4 \times 10^{-4}$ M indomethacin, and collected at 4°C. In some experiments, SBTI (2.5 mg/mouse) as a plasma kallikrein inhibitor or captopril (50 μg/mouse) as a kininase inhibitor was dissolved in a saline suspension of kaolin, and was administered simultaneously with kaolin.

3) Extraction and Measurement of Cyclooxygenase Products from the Peritoneal Washing: The peritoneal washings were immediately centrifuged at 4°C, 3500 rpm for 5 min, and PGs were extracted after separation of the supernatant according to the method of Powell et al. The extract was cleaned up through SEPAK C$_{18}$ (Waters Associates) by the modified method described by Kiyomiya and Ohishi. Finally, the products were eluted with ethylacetate-methanol (v/v 9:1), and evaporated to dryness. 6-Keto PGF$_{1a}$, PGE$_2$, PGF$_{2a}$ and TXB$_2$ levels were measured by radioimmunoassay using each RIA kit.

2. Reproduction of the Writhing Response Suppressed by Pretreatment with Indomethacin by Exogenous Administration of PG1$_2$

1) Early Stage: One hour after oral administration of indomethacin (1 mg/kg, p.o.), or with 5% gum arabic as a vehicle, to mice, 0.5 ml of kaolin saline suspension (5 mg/ml) was administered intraperitoneally. Five min after the injection of kaolin, PG1$_2$-Na (2–10 ng/100 μl/mouse) was injected intraperitoneally and the number of writhing responses was determined over the following 15 min. Authentic PG1$_2$-Na was dissolved in ethanol (1 mg/ml) and kept frozen (–80°C). Immediately before use, it was diluted with 0.1 M ice-cold NaHCO$_3$ – 0.15 M NaCl buffer (pH 10.0) in an ice bath.

2) Later Stage: One hour after oral administration of indomethacin (1 mg/kg, p.o.) or 5% arabic gum as the control, kaolin was administered intraperitoneally as described above. After 60 min, 0.2 ml of captopril (100 μg/ml) and PG1$_2$-Na (2–10 ng/100 μl/mouse) were simultaneously injected intraperitoneally, and immediately after that the writhing responses in 15 min were counted.

Results

1. Time-Course Changes in the Amount of the Cyclooxygenase Products Measured in the Peritoneal Washings by Administration of Kaolin

PGs were released after injection of kaolin (2.5 mg/mouse, i.p.), with a peak at 5 min followed by a gradual decrease (Fig. 1). The amounts of PGs at the peak of release were 7.3 ng, 0.16 ng, 0.89 ng and 0.59 ng per mouse for 6-keto-PGF$_{1a}$, PGE$_2$, PGF$_{2a}$ and TXB$_2$, respectively. About 80% of the total amount was 6-keto-PGF$_{1a}$ (Table I).

2. Effects of SBTI and Captopril on the Levels

![Fig. 1. Time Course of the Amounts of Cyclooxygenase Products in the Peritoneal Washings Obtained at Indicated Time after Kaolin Injection](image-url)

Each point indicates means of measured products in the peritoneal washings from 5 mice with the S.E. indicated by vertical bars; (○), 6-keto-PGF$_{1a}$; (□), PGE$_2$; (●), TXB$_2$; (△), PGF$_{2a}$.
of the Cyclooxygenase Products in the Peritoneal Cavities of Mice 5 min after Injection of Kaolin

The amount of 6-keto-PGF$_{1\alpha}$ in the peritoneal cavity 5 min after administration of kaolin was significantly inhibited by the simultaneous injection of SBTI (2.5 mg/mouse, i.p.), whereas, augmented by simultaneous injection of captopril (50 µg/mouse, i.p.). The inhibitory or enhanced action of these inhibitors was observed approximately to the same extent even 10 min after administration of kaolin (Fig. 2). In addition, there was a significant correlation ($r = 0.8237$, $p < 0.001$) between the number of writhing responses observed for 10 min and the amount of 6-keto-PGF$_{1\alpha}$ in the peritoneal washings at 10 min after administration of kaolin (Fig. 3).

3. Restoration of the Writhing Response, Which Was Inhibited by Treatment with Indomethacin, by Administration of PGI$_2$-Na

The writhing response induced by kaolin in the early stage, which was inhibited almost completely by administration of indomethacin (1 mg/kg, p.o.), was restored by PGI$_2$-Na (2–10 ng/mouse, i.p.) dose-dependently (Fig. 4). In the later stage as well (60 min after administration of kaolin, 120 min after administration of indomethacin), the simultaneous administration of captopril (20 µg/mouse) and PGI$_2$-Na (2–10 ng/mouse) induced dose-dependent restoration of the writhing response (Fig. 4). PGI$_2$-Na (10 ng/mouse) alone did not induce the writhing reaction when injected intraperitoneally to mice without administration of kaolin.

<table>
<thead>
<tr>
<th>Injection</th>
<th>Metabolites released (ng/mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6-Keto-PGF$_{1\alpha}$</td>
</tr>
<tr>
<td>None</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>Kaolin (2.5 mg/mouse)</td>
<td>7.25 ± 1.00$^b$</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E. from 10 mice. $a$) and $b$) indicate significant differences from the untreated group at $p < 0.01$ and 0.001, respectively.
4. The Effect of Various Analgesics on Kaolin-Induced Writhing Response and the Amount of 6-Keto-PGF₁₅ in the Peritoneal Washings

Indomethacin (1 mg/kg, p.o.), ibuprofen (10 mg/kg, p.o.) and alminoprofen (5 mg/kg, p.o.) inhibited the kaolin-induced writhing response and reduced the amount of intraperitoneal 6-keto-PGF₁₅ in parallel. However, tiaramide (10 mg/kg, p.o.), morphine (10 mg/kg, s.c.) and pentazocine (30 mg/kg, s.c.) markedly inhibited the kaolin-induced writhing response, whereas they exerted no inhibitory effect on the amount of intraperitoneal 6-keto-PGF₁₅ (Table II).

Discussion

We studied the relationship between the kaolin-induced writhing response and the levels of arachidonic metabolites in the peritoneal washings. After intraperitoneal injection of

![Diagram](image-url)

**Fig. 3.** Relationship between the Number of Writhes and Intraperitoneal 6-Keto-PGF₁₅ Levels during 10 min Following Injection of Kaolin
TABLE II. The Effects of Various Analgesic and Antiinflammatory Agents on the Writhing Response and the Level of 6-Keto-PGF\textsubscript{1α}

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>No. of mice</th>
<th>No. of\textsuperscript{c} writhes</th>
<th>Inhibition%</th>
<th>6-Keto-PGF\textsuperscript{d}1α (ng/mouse)</th>
<th>Inhibition%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Untreated</td>
<td>—</td>
<td>—</td>
<td>5</td>
<td>0.0±0.0</td>
<td>—</td>
<td>0.09±0.01</td>
<td>1.0</td>
</tr>
<tr>
<td>Vehicle</td>
<td>—</td>
<td>p.o.</td>
<td>5</td>
<td>9.0±1.8</td>
<td>—</td>
<td>10.19±1.42</td>
<td>—</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>1</td>
<td>p.o.</td>
<td>5</td>
<td>1.2±0.8\textsuperscript{b}</td>
<td>87</td>
<td>1.09±0.38\textsuperscript{b}</td>
<td>89</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>10</td>
<td>p.o.</td>
<td>5</td>
<td>2.4±1.2\textsuperscript{a}</td>
<td>73</td>
<td>2.56±0.45\textsuperscript{b}</td>
<td>74</td>
</tr>
<tr>
<td>Alminoprofen</td>
<td>5</td>
<td>p.o.</td>
<td>5</td>
<td>2.7±1.2\textsuperscript{a}</td>
<td>70</td>
<td>3.53±0.69\textsuperscript{b}</td>
<td>65</td>
</tr>
<tr>
<td>Tiaramide</td>
<td>10</td>
<td>p.o.</td>
<td>5</td>
<td>2.0±0.7\textsuperscript{b}</td>
<td>78</td>
<td>9.39±0.75</td>
<td>8</td>
</tr>
<tr>
<td>B Vehicle</td>
<td>—</td>
<td>s.c.</td>
<td>5</td>
<td>6.7±1.8</td>
<td>—</td>
<td>10.81±1.38</td>
<td>—</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>1</td>
<td>s.c.</td>
<td>5</td>
<td>0.5±0.4\textsuperscript{b}</td>
<td>92</td>
<td>1.20±0.41\textsuperscript{b}</td>
<td>89</td>
</tr>
<tr>
<td>Morphone</td>
<td>10</td>
<td>s.c.</td>
<td>5</td>
<td>0.0±0.0\textsuperscript{b}</td>
<td>100</td>
<td>12.64±6.32</td>
<td>—17</td>
</tr>
<tr>
<td>Pentazocin</td>
<td>30</td>
<td>s.c.</td>
<td>5</td>
<td>0.0±0.0\textsuperscript{b}</td>
<td>100</td>
<td>14.72±4.66</td>
<td>—36</td>
</tr>
</tbody>
</table>

Each value represents mean±S.E. \textsuperscript{a} and \textsuperscript{b} indicate significantly different from vehicle at \( p < 0.05 \) and \( p < 0.01 \), respectively. The writhing was induced by intraperitoneal injection of kaolin (2.5 mg/mouse). \textsuperscript{c} Writhes were counted for 10 min after kaolin injection. \textsuperscript{d} The level was measured in the peritoneal washings obtained at 10 min after kaolin injection.

kaolin to mice 6-keto PGF\textsubscript{1α}, PGE\textsubscript{2}, PGF\textsubscript{2α}, and TXB\textsubscript{2} were released rapidly with a peak at 5 min, and thereafter the amount released decreased with time. The time-course changes in the levels of PGs in the peritoneal washings were parallel with the time course of the writhing response which was described in the previous reports\textsuperscript{1,2} in that the writhing response showed a peak at 5—10 min after kaolin-injection. There was a very close correlation (\( r=0.8237, p < 0.001 \)) between the writhing response and the amount of 6-keto-PGF\textsubscript{1α}, a major metabolite in the peritoneal cavity. PGs especially PGI\textsubscript{2} and E\textsubscript{2} are known to be closely related to inflammatory pain.\textsuperscript{5–8} Accordingly, we consider PGI\textsubscript{2} plays a very important role in induction of the writhing response by kaolin. This result is in good agreement with the report described by Doherty \textit{et al.},\textsuperscript{9} in that PGI\textsubscript{2} is the prostaglandin involved in mediation of the writhing reaction induced by zymosan in mice.

The intraperitoneal release of 6-keto-PGF\textsubscript{1α} in response to kaolin was significantly inhibited by simultaneous administration of a plasma kallikrein inhibitor, SBTI, with kaolin and was significantly increased by simultaneous administration of a kininase inhibitor, captopril. This result was in good agreement with previous reports,\textsuperscript{1,2} in that the writhing reaction induced by kaolin (early stage) was significantly suppressed or increased by simultaneous injection of SBTI or captopril, and captopril induced writhing reaction (later stage) was also significantly suppressed by simultaneous injection of SBTI. In our previous report\textsuperscript{1} we concluded that BK released via the activation of kallikrein-kinin system by activation of factor XII is greatly involved in the kaolin-induced writhing response, because the response was inhibited almost completely by SBTI and increased markedly by captopril. On the other hand, BK has been reported to stimulate production of the arachidonate metabolites by \textit{in vivo} stimulation.\textsuperscript{10–13} From the above result in consideration with these reports, the kaolin-induced release of 6-keto-PGF\textsubscript{1α} into the peritoneal cavity is considered to be induced by intrinsic BK which is produced by the activation of the kallikrein-kinin system.

The writhing response induced by kaolin, which was mostly inhibited by pretreatment with indomethacin, was distinctly restored by exogenous injection of PGI\textsubscript{2}-Na. PGI\textsubscript{2} has been reported to induce hyperalgesia at peripheral receptors and to augment markedly the pain that is induced with BK, \textit{etc}.\textsuperscript{9,14} The restoration of the indomethacin-inhibited kaolin-induced writhing response by exogenous administration of PGI\textsubscript{2} suggests that pain induced with intrinsic BK and the sensitizing activity of PGI\textsubscript{2} released.
by stimulation with BK provide the main mechanism of the kaolin-induced writhing response.

The effects of drugs on the writhing response and the amount of 6-keto-PGF$_{1\alpha}$ released into the peritoneal cavity were studied by administration of various analgesics. Indomethacin, ibuprofen and alminoprofen, i.e., acidic non-steroidal anti-inflammatory drugs which are considered to act by inhibition of cyclooxygenase, inhibited in parallel both the kaolin-induced writhing response and the amount of intraperitoneal 6-keto-PGF$_{1\alpha}$. On the other hand, tiaramide as a basic non-steroidal drug, morphine and pentazocine as a narcotic and non-narcotic drugs, i.e., showed no significant inhibitory effect on the amount of intraperitoneal 6-keto-PGF$_{1\alpha}$ even at dose of the drugs that almost completely inhibited the writhing response. These effects on writhing reaction could be responsible to their central action, since tiaramide is also reported to have central analgesic action in addition to its peripheral action. From these results, the present model of writhing response was considered to enable the differentiation of the sites of the effect of various analgesics, to some degree, by investigation of the relationship between the inhibitory activity against the writhing response and the inhibitory activity against the release of 6-keto-PGF$_{1\alpha}$ into the peritoneal cavity.

As stated above, the mechanism of induction of the writhing response by kaolin in mice involves a correlation between the induction of pain by BK produced via the activation of the kallikrein-kinin system by activation of factor XII, and pain by the sensory nerve-sensitizing activity of mainly PGI$_2$ released by stimulation with intrinsic BK. Furthermore, it is suggested that the sensory nerve-sensitizing effect of PGI$_2$ could be a main cause of pain reaction in this writhing model, since this writhing reaction was markedly inhibited by administration of acidic non-steroidal anti-inflammatory drugs, such as ibuprofen, indomethacin and alminoprofen. In addition, the present model is considered to be a new and useful one which allows the differentiation of mode of action of various analgesic drugs by simultaneous determination of writhing response and the amount of 6-keto-PGF$_{1\alpha}$ released into the peritoneal cavity.

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