Effect of (3,5,6-Trimethylpyrazin-2-yl)methyl 2-[4-(2-Methylpropyl)phenyl]propanoate (ITE), a Newly Developed Anti-inflammatory Drug, on Type II Collagen-Induced Arthritis in Mice

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The effect of (3,5,6-trimethylpyrazin-2-yl)methyl 2-[4-(2-methylpropyl)phenyl]propanoate (ITE) on type II collagen (CII)-induced arthritis in mice was studied. Mice were immunized twice with CII, ITE being given orally once a day for 40 d after the 1st immunization. Clinical assessment showed that ITE had no effect on the day of onset of arthritis but did lowered the incidence rate of arthritis and the arthritis score. And ITE had a marked suppressive effect on the mouse hind paw edema induced by CII. ITE suppressed the delayed-type mouse ear skin reaction to CII but had no effect on the level of serum anti-CII antibodies. These results suggest that ITE inhibits the development of CII-induced arthritis in mice by suppressing delayed-type hypersensitivity to CII.

Key words: anti-inflammatory drug; type II collagen-induced arthritis; mice

MATERIALS AND METHODS

Animals Male Kun-ming mice weighing 18—22 g were purchased from Shenyang Pharmaceutical University Laboratory Animal Center. Mice were housed in a room under a 12 h light-dark cycle and quarantined for one week before use. The mice were given standard laboratory chow and tap water ad libitum.

Drugs ITE synthesized at Hua-tai Institute of Pharmaceutics Science was used. ITE occurs as white powder. Purity of ITE is more than 98.5%. ITE (150, 300, 600 mg/kg) or ibuprofen (IB, 180 mg/kg) was suspended in a 0.5% carboxymethylcellulose (CMC)-saline solution and given orally to mice once a day for 40 d from the day of 1st immunization with CII. Mice in the control group were orally given 0.5% CMC-saline. Type II collagen were purchased from Sigma Co. (St. Louis, U.S.A.).

Induction of Arthritis The CII solution was dissolved overnight at 4 °C in 0.1 M acetic acid at 2 mg/mL, after which the solution was emulsified in an equal volume of complete Freund's adjuvant (CFA). This emulsion containing 100 μg of CII was injected intradermally into the base of the tail, and the mice were given an intraperitoneal injection of the same emulsion 20 d later.

Clinical Assessment The clinical symptoms of arthritis in limbs were evaluated blindly with a visual scoring system every 4 d after day 20. Arthritic lesion of a limb was graded on a scale of 0—4: 0 = no change, 1 = swelling and erythema of the digit, 2 = mild swelling and erythema of the limb, 3 = gross swelling and erythema of the digit, 4 = gross deformity and inability to use the limb. The arthritis score of each mouse was the sum of the scores of each of the limbs except the right hind limb, the maximum score being 12. A mouse that showed a score of 1 or more was regarded to be arthritic. The incidence and day of onset of arthritis also were recorded. The right hind paw's volume of each mouse was determined on days 0, 20, 24, 28, 32, 36 and 40 after 1st immunization with CII.

Measurement of Anti-CII Antibodies On day 40, blood was collected from the mice, after which the serum level of IgG antibodies to CII was determined by the enzyme-linked immunosorbent assay (ELISA). Briefly a 100 μl sample of serum (1:2000) in phosphate-buffered saline (PBS) was added to the wells of a 96 well plate that had been coated with CII overnight at 4 °C. The plate was incubated for 2 h at room temperature then, after the washing of the wells with PBS, an addition was made of 100 μl goat anti-mouse IgG (1:400) in PBS. After incubation for 1 h at room temperature, the wells were washed with PBS7, after which 100 μl of 0.05% o-phenylenediamine was added. After incubation for 1 h at room temperature, the reaction was stopped by the addition of 50 μl of 4 N H2SO4. Color was allowed to develop for 30 min, then the optical density was measured at 490 nm.

Measurement of the Delayed-Type Skin Reaction to CII The delayed-type hypersensitivity (DTH) reaction to CII was assessed by the mouse ear skin test. Briefly, on day 40, 20 μl of 0.2% CII solution in 0.1 mol/l acetic acid was injected intradermally into the right ear. The thickness of the ear was measured under ether anesthesia with a micrometer just before and 24 h after injection of the CII solution. A difference between the two values was regarded as reflecting

Fig. 1. Chemical Structure of (3,5,6-Trimethylpyrazin-2-yl)methyl 2-[4-(2-Methylpropyl)phenyl]propanoate (ITE)
edema induced by the DTH reaction.

**Statistical Analysis** All results were expressed as mean±S.E.M. and they were analyzed for significance by means of the Student’s t-test. Differences were accepted as statistically significant at p<0.05.

**RESULTS**

**Effect of ITE on Clinical Assessment of CIA** The incidence of arthritis in control group was 91.7%, the mean onset occurring 25.5±5.4 d after the 1st immunization with CII (Table 1). ITE had no effect on the onset of arthritis but did decrease the incidence rate of arthritis (Table 1). And ITE lowered the arthritis score at 150 to 600 mg/kg (Fig. 2). Throughout the experiments, there were no statistical differences between the body weight in ITE-treated groups and that in arthritis control group (data not shown).

**Effect of ITE on the Development of Paw Edema of CIA** Figure 3 shows the volume change of mice’ s hind paw. In control mice, paw volume began to increase on day 28, with a peak effect on day 36. Treatment with ITE or IB significantly reduced the development of paw edema.

**Effect of ITE on the Concentration of Serum Antibodies to CII** A significant elevation of serum antibodies to CII was found in the arthritis control mice. ITE had no effect on the concentration of these antibodies (data not shown).

**Effect of ITE on Delayed-Type Skin Reaction to CII** The gain in ear thickness for the arthritis control mice was 0.15±0.07 mm 24 h after challenge with an intradermal injection of CII (Table 2). ITE at 600 mg/kg significantly suppressed the delayed-type skin reaction (Table 2).

**DISCUSSION**

The induction of immunological reactivity to CII, a major component of cartilage, has been used to establish an experimental model of arthritis in the rat.2,3 Mice were injected with CII emulsified in CFA, in experimental conditions similar to those known to be effective in the rat.2,3 This model has been widely used to evaluate antiarthritic drugs. But not all antirheumatic drugs have similar effects on murine CIA. Aura

To clarify the mechanism of ITE, we studied the effect of the drug on humoral and cellular immunity to CII in CIA mice. As CIA can be passively transferred by sera from donors immunized with CII, anti-CII antibodies function in the initiation of arthritis.12—14 ITE had no effect on the serum level of anti-CII antibodies. But ITE at 600 mg/kg significantly suppressed the delayed-type skin reaction to CII in CIA mice. Therefore ITE is believed to inhibit murine CIA

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**Table 1. Effect of ITE on the Incidence and Day of Onset of Arthritis**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>Incidence rate (%)</th>
<th>Onset (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritis control</td>
<td>—</td>
<td>12</td>
<td>91.7</td>
<td>25.5±5.4</td>
</tr>
<tr>
<td>ITE</td>
<td>600</td>
<td>12</td>
<td>50.0</td>
<td>26.7±5.5</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>12</td>
<td>61.5</td>
<td>26.5±3.7</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>14</td>
<td>85.7</td>
<td>29.5±8.6</td>
</tr>
<tr>
<td>IB</td>
<td>180</td>
<td>12</td>
<td>75.0</td>
<td>26.2±2.9</td>
</tr>
</tbody>
</table>

Each value is expressed as mean±S.E.M. *p<0.05 is considered significant as compared to arthritis control by Student’s t-test.

**Table 2. Effect of ITE on the Delayed-Type Skin Reaction to CII**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>Skin reaction (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritis control</td>
<td>—</td>
<td>12</td>
<td>0.15±0.07</td>
</tr>
<tr>
<td>ITE</td>
<td>600</td>
<td>12</td>
<td>0.08±0.04*</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>12</td>
<td>0.09±0.06</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>14</td>
<td>0.14±0.08</td>
</tr>
<tr>
<td>IB</td>
<td>180</td>
<td>12</td>
<td>0.08±0.05*</td>
</tr>
</tbody>
</table>

Each value is expressed as mean±S.E.M. *p<0.05 is considered significant as compared to arthritis control by Student’s t-test.
through suppression of cell-mediated immune responses to CII.

REFERENCES