Relationship between Pharmacokinetics and the Analgesic Effect of Indomethacin in the Rat

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The relationship between the pharmacokinetic properties and the analgesic effect of indomethacin (IDM) was evaluated on a carrageenin-induced inflammation model in the rat. Rats were administered the drug in one of two ways: intravenous (i.v.) IDM bolus or i.v. IDM infusion. The analgesic activity was measured by Randall-Selitto test. No correlation was observed between the analgesic effect and the plasma IDM concentration after i.v. bolus administration of IDM. However, in the case of infusion, IDM produced a dose-dependent analgesic effect. In this paper, we demonstrated that the plasma concentration of IDM maintained by i.v. infusion had a prolonged analgesic effect on the carrageenin-induced inflammation model.

Key words indomethacin; pharmacokinetics; analgesic effect

In the use of drug treatment for pain relief, it is generally accepted that opioid analgesics and neural blockade are able to provide adequate pain management in most patients. The most effective and efficient method of achieving analgesia is to combine the routes of administration and analgesics which possess several different mechanisms of action.1–3) Because non-steroidal anti-inflammatory drugs (NSAIDs) are utilized in pain management easily and safely in combination with opioids during postoperative periods,4–9) we investigated the most effective way of administration to achieve analgesia.

Information about the relationship between plasma levels and the analgesic effect of NSAIDs is limited.9,10) The majority of clinical studies have also failed to demonstrate the relationships between plasma drug concentration and therapeutic effect of naproxen,11) flurbiprofen,12–14) phenylbutazone,15,16) or salicylate.17) We used indomethacin (IDM), one of the clinically important NSAIDs, in this study. IDM has been used extensively in several dosage forms for pain relief, arthritis and inflammation without opioid-related side effects. However, there was no correlation between the plasma concentration of IDM and the clinical response of commonly evaluated criteria.18–20)

In this report, we evaluated the relationship between plasma concentration of IDM and the analgesic effect brought about by i.v. bolus administration and by i.v. infusion in rats. We investigated the duration of the analgesic effect by i.v. infusion of IDM on a carrageenin-induced inflammation model.

MATERIALS AND METHODS

Animals Female Wistar rats (Saitama Experimental Animals Supply Co., Ltd., Saitama, Japan) were housed in stainless steel cages under conditions of controlled temperature maintained at 23±1°C and humidity of 55±10% during the investigation. Rats weighing 160–190 g were used throughout all experiments. Free access to food and water was allowed.

Chemicals IDM was a kind gift from Taisho Phama-

ceutical Co., Ltd. (Saitama, Japan). Flufenamic acid was generously given by Hokuriku Seiyaku Co., Ltd. (Fukui, Japan) and was employed as the internal standard for detection by a HPLC. All other chemicals and solvents were commercial products of analytical grade and were used without further purification.

Intravenous Bolus Administration Experiment Under light anesthesia with ethyl ether, polyethylene catheters (PE 50, i.d. 0.58 mm; o.d. 0.965 mm; Clay Adams, Parsippany, NJ, U.S.A.) were inserted into one femoral vein and one femoral artery. The cannula were routed subcutaneously in the dorsum and exteriorized on the dorsal side of the neck. The animals were left for 2 h after surgery to recover from the anesthesia. IDM was administered i.v. at a dose of 2.9, 5.8, 8.6 and 20.0 mg/kg as a saline solution after dissolving in a small amount of polyoxyethylene (20) sorbitan monooleate. After the administration, the animals were allowed freedom of movement. Blood samples were taken through the arterial catheter at 10 min, 1, 2, 4, 6 and 24 h periods. Plasma was separated from blood by centrifugation at 2000 x g for 5 min. Each plasma sample was stored at −20°C until the next experiment.

Intravenous Infusion Experiment Rats were catherized in the manner described above. IDM was administered i.v. at loading doses of 3.9, 7.0 and 10.1 mg/kg, followed immediately by 3.8, 6.8 and 9.9 µg/kg/min i.v. infusion using an infusion pump (KN-201 type, Natsume Seisakusho Co., Ltd., Tokyo, Japan). The loading doses and the infusion doses were calculated from relationships between the obtained pharmacokinetic parameters of the i.v. bolus administration as described below and injection speed of the infusion pump. Arterial blood was taken frequently to determine the concentration at 30 min, 1, 2, 3, 4, 5, 6, 7 and 8 h after the initial infusion of IDM. Each blood sample was treated in the manner described above.

Determination of Analgesic Activity Edema was induced by a modification of the method of Winter et al.21

Briefly, 0.05 ml of 1.0% carrageenin saline suspension (Picrin-A, Lot No. P-14, Zushi Chemical Laboratory Inc., Kanagawa, Japan) was injected into the plantar surface

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of the left hind-paw. The nociceptive sensitivity (NS) for pressure on the inflamed paw was determined by the Randall–Seilito test using an Analgesic Meter Ugo Basile (Biological Research Apparatus, Varese, Italy). The NS was determined before and at 0.5, 1, 2, 4, 5, 5.5, 6, 7, 8, 9, 10, 11, 12 and 13 h after the carrageein injection. The pain threshold (PT) was calculated as follows,

$$PT(\%) = \frac{(NS_0 - NS) / NS_0 \times 100}{(1)}$$

where $NS_0$ and $NS$ are the NS before and each time after the carrageein injection, respectively. At 5 h after the carrageein injection, IDM or a vehicle was administered by i.v. bolus or i.v. infusion. The analgesic activity (AA) was calculated as follows,

$$AA(\%) = \frac{(PT_3 - PT) / PT_3 \times 100}{(2)}$$

where $PT_3$ and $PT$ are the calculated values according to Eq. 1 at 5 h and at 5.5, 6, 7, 8, 9, 10, 11, 12 and 13 h after the carrageein injection. During the experimental period, as many variables as possible were eliminated such as temperature, humidity, etc. After final determination of the NS at 13 h, the plasma samples of the bolus administration or the infusion were stored at −20°C until the HPLC analysis.

**Analysis of IDM in Plasma** Detection of IDM in plasma was determined by a modification of the HPLC method of Skellern et al. Briefly, 100 μL of a plasma sample and 400 μL of 0.5 M citrate buffer (pH 5.0) were placed into micro-polypropylene test tubes, followed by the addition of 100 μL of the internal standard at concentrations of 80 μg/mL. The mixture was extracted with 700 μL of ethyl ether by mechanical shaking (Micro Tube Mixer MT-360, Tomy Seiko Co., Ltd., Tsukuba, Japan) for 5 min. The tubes were centrifuged at 1200 × g (3000 rpm, 4°C) for 10 min. The mixture was extracted again with 400 μL of ethyl ether. The organic phase was evaporated to dryness at 30°C under a stream of dry nitrogen. The residue was redissolved in 200 μL of the HPLC mobile phase and an aliquot of 25 μL was analyzed. For the calculation of unknown plasma samples, peak-area ratios for IDM relative to the internal standard were used. The HPLC analysis was performed using a HPLC system, Model Trirotar-VI (Japan Spectroscopic Co., Ltd., Tokyo, Japan) and monitored at 254 nm. A microprocessor (Model C-R3A Chromatopac, Shimadzu Co., Kyoto, Japan) was used for peak-area integration. The analytical column, TSK-Gel ODS-80T M (150 mm × 4.6 mm i.d., particle size 5 μm, Tosoh Co., Tokyo, Japan) was used at room temperature (20°C). The mobile phase of acetonitrile–0.1 M acetic acid (60:40, v/v) was pumped at a flow-rate of 1.0 ml/min.

**Pharmacokinetic Analysis** The plasma concentration was plotted against the time period after the i.v. bolus administration. The time-profiles of the disappearance of the 2.9, 5.8, 8.6 and 20.0 mg/kg IDM doses from the plasma were kinetically analyzed, based on the two-compartment open model using the nonlinear least-squares method (MULTI). The plasma concentration data were fitted into the two-exponential equation as follows,

$$C_t = A \exp(-\alpha t) + B \exp(-\beta t)$$

where $C_t$ is the drug concentration at time $t$, $A$ and $B$ are ordinate axis intercepts, and $\alpha$ and $\beta$ are the corresponding first-order disposition rate constants. The total body clearance ($CL_{\text{tot}}$), the steady state volume of distribution ($V_{\text{dss}}$) and the elimination half-life ($t_{1/2}$) of the β phase were calculated with the following relationships, independently,

$$CL_{\text{tot}} = D / (A + B) \beta$$

$$V_{\text{dss}} = A \beta^2 + B \beta$$

$$t_{1/2} = \ln 2 / \beta$$

where $D$ is a dose administered intravenously.

**Statistical Analysis** Student’s t-test was used to determine significant differences. The 0.05 and 0.01 levels of probability were used as the level of significance.

**RESULTS**

The plasma concentration profiles of IDM after the i.v. bolus administration with various doses (2.9, 5.8, 8.6 and 20.0 mg/kg) of IDM are shown in Fig. 1A. The decay curve of the plasma concentration consisting of a distri-

![Fig. 1. Plasma Concentration–Time Profiles of IDM after i.v. Bolus Administration (Panel A) and after i.v. Infusion (Panel B) of IDM to Rats](image-url)

Each point represents the mean ± S.E. of 4 or 5 animals. (A): Symbols, dose administered: □, 2.9 mg/kg; ▲, 5.8 mg/kg; ▼, 8.6 mg/kg; ▶, 20.0 mg/kg in independent experiment. All of the S.E. values were smaller than the size of the symbols. (B): Symbols, loading dose and maintenance dose of infusion: ○, 3.9 mg/kg and 3.8 μg/kg/min; ●, 7.0 mg/kg and 6.8 μg/kg/min; ◆, 10.1 mg/kg and 9.9 μg/kg/min. In some cases, the S.E. values were smaller than the size of the symbols.
bution phase and a slower elimination phase was analyzed according to the two-compartment open model for each dose. The values of estimated pharmacokinetic parameters are listed in Table 1. The mean values of $CL_{tot}$, $Vd_{ss}$ and $t_{1/2}$ of IDM were 44 ml/h/kg, 0.55 l/kg and 9 h, respectively. Figure 1B shows the plasma concentration profiles of IDM after i.v. bolus (3.9, 7.0 and 10.1 mg/kg), followed immediately by 3.8, 6.8 and 9.9 $\mu$g/kg/min i.v. infusion of IDM. Steady state plasma concentration ($C_{ss}$) of IDM was observed during 2 to 8 h after starting infusion, and the $C_{ss}$ was approximately 11, 15 and 19 $\mu$g/ml depending on the three infusion doses.

Table 1. Pharmacokinetic Parameters of IDM after i.v. Administration to Intact Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>2.9</th>
<th>5.8</th>
<th>8.6</th>
<th>20.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CL_{tot}$ (ml/h/kg)</td>
<td>45.16 ± 1.14</td>
<td>44.15 ± 2.21</td>
<td>45.14 ± 1.24</td>
<td>42.07 ± 4.09</td>
</tr>
<tr>
<td>$Vd_{ss}$ (l/kg)</td>
<td>0.55 ± 0.09</td>
<td>0.53 ± 0.23</td>
<td>0.56 ± 0.10</td>
<td>0.57 ± 0.20</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>8.87 ± 0.17</td>
<td>8.58 ± 0.34</td>
<td>8.90 ± 0.19</td>
<td>9.83 ± 0.88</td>
</tr>
</tbody>
</table>

Each value represent the mean ± S.E. of 4 or 5 animals.

The time course of the $PT$ on the rat’s inflamed paw after injection of carrageen suspension is shown in Fig. 2. $PT$ clearly rose from 0 to 5 h after the injection and at 5 h had increased by 61% accompanied by increase of the edema.

Time course of the $AA$ on the carrageen-inflamed paw after the i.v. bolus administration of IDM at a dose of 2.9, 5.8, 8.6 or 20.0 mg/kg is shown in Fig. 3A. Although there were more significant effects after the administration of higher doses of IDM (8.6 and 20.0 mg/kg) than lower doses (2.9 and 5.8 mg/kg), no clear dose-dependence of $AA$ was observed during the measured time period. Figure 3B shows the time course after the i.v. bolus administration (3.9, 7.0 and 10.1 mg/kg), followed immediately by 3.8, 6.8 and 9.9 $\mu$g/kg/min i.v. infusion of IDM. During the infusion of IDM $AA$ showed a more steady and significant effect at the higher infusion dose over the measured time. After the final determination, plasma concentrations by the bolus and the infusion were consistent with the predictable and the obtained concentrations indicated in Figs. 1A and 1B, respectively.

The relationship between the $AA$ and the plasma concentration after the i.v. bolus administration of IDM is shown in Fig. 4A; no direct correlation between them was observed in that study. Figure 4B shows the relationship between the $AA$ and the $C_{ss}$ during the infusion of IDM. When the $C_{ss}$ of IDM was maintained at approximately 11, 15 and 19 $\mu$g/ml at each infusion, the $AA$ was maintained at the mean values of 20, 52 and 67%, respectively.

DISCUSSION

The relationship between the pharmacokinetics properties and the analgesic effect of IDM was evaluated on a carrageen-induced inflammation model in the rat. The plasma concentration of IDM by the i.v. boluses (Fig. 1A) and the infusion (Fig. 1B) showed a dose-dependent time profile, and the pharmacokinetic parameters of IDM such as $CL_{tot}$, $Vd_{ss}$ and $t_{1/2}$ of the $\beta$ phase were almost the same among the four dose levels. These may suggest
that IDM at a dose of 2.9—20.0 mg/kg is eliminated by linear pharmacokinetics. We selected the carrageenin-induced inflammation model to investigate the analgesic effect of IDM. As shown in Fig. 2, local edema in combination with release of algesiogenic substances (e.g. prostaglandins, leukotrienes, bradykinin, serotonin and histamine) by the injection of carrageenin leads to inflammation and sensitization of the nociceptors, resulting in hyperalgesia.\(^5,26,27\) This suggested that the formation and release of the algesiogenic substances would be affected by the drug. As shown in Fig. 3, there were differences between the bolus and the infusion in the AA of IDM; this can be understood from the relationship between the AA and the plasma concentration of IDM by the two methods (Fig. 4). It is suggested that the AA by the infusion is kept constant in the \(C_{ua}\) of IDM. These findings suggest that the emergence of AA is delayed by the infusion in comparison with the boluses. It is known that the inflammatory sites are part of a pharmacokinetical peripheral compartment wherever inflammation might occur.\(^28\) Sugiyama reported the relationship between the inhibition of carrageenin-induced edema and the plasma concentration of NSAIDs in rats.\(^28\) In that report, the inhibition of edema was correlated with the estimated drug concentration in the peripheral compartment according to analysis by the two-compartment model against the plasma concentration after i.v. administration of the drug. In clinical studies, Duggan et al. suggest that the observed onset and duration of the therapeutic response of IDM could be explained by the kinetics of IDM in the peripheral compartment.\(^29\) The data in our study suggest that the site of action of IDM would be compatible on carrageenin-induced paw to the pharmacokinetical peripheral compartment. It can easily be deduced that the distribution from the plasma compartment to the compartment of inflammatory sites can participate in the AA. Because the compartment of the inflammatory sites is thought to be equilibrated with the plasma compartment during infusion, the \(C_{ua}\) that emerged prolonged the AA. The equilibrium of IDM will depend on the protein binding of the drug as well as other chemical properties such as \(pK_a\), molecular size, and solubility of the drug.\(^30\)

The albumin concentration at the site of inflammation will vary with both the degree of microvascular permeability and with the rate of protein removal via the lymphatic system.\(^31,32\) Many clinical investigators have determined the pharmacokinetic properties of NSAIDs in both plasma and synovial fluid.\(^33-36\) Emori et al. determined IDM concentration in serum and synovial fluid after administration of IDM for rheumatoid arthritis.\(^37\) Although the concentration of IDM in the synovial fluid was slightly higher than the drug concentration in the serum at equilibrium, elimination half-life was the same in the two compartments.\(^37\) The present study may suggest that the prolongation of AA is caused by the \(C_{ua}\) of IDM in the synovial fluid.

In conclusion, we demonstrated in an inflammation model that the infusion of IDM has the advantage of effective AA. We also showed that AA was prolonged and was dependent on drug concentration by the infusion of IDM. To clarify the contribution of pharmacokinetic factors of IDM against the AA, further studies, for example, of a more detailed analysis of the relationship between the change in measurable drug concentration in the peripheral compartment and the drug action are needed.

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