BIOAVAILABILITY OF INDOMETHACIN CALCIUM AND MAGNESIUM, AND EFFECT OF THE SALTS ON DRUG METABOLIZING ENZYME ACTIVITIES IN RATS

TARO OGISO, MASAIRO IWAKI AND EJI TAMAKI

Faculty of Pharmaceutical Sciences, Kinki University, Kowakaе-3-4-1, Higashi-Osaka, 577, Japan
(Received March 22, 1983)

To clarify the mechanism of enhanced absorption of indomethacin (IND) when dosed with magnesium silicate, reported previously, magnesium (IND-Mg) and calcium (IND-Ca) salts of IND were prepared, and the bioavailability in rat and physical properties of these salts were studied in comparison with IND. The mean plasma levels after a single oral dosing of IND-Ca and IND-Mg were significantly higher than those after IND (6 mg/kg). The absolute bioavailability calculated was 63.7% for IND, 83.2% for IND-Mg and 96.8% for IND-Ca. The area (AUC) under the plasma concentration curve after multiple oral dosing of IND-Ca and IND-Mg was also significantly larger than that after IND multiple dosing (p <0.001). Thus, the increased absorption of the salts made it possible to decrease their dosage. The mean plasma levels and the AUC after multiple dosing of the salts in the decreased doses (4.16 mg/kg for IND-Ca and 4.74 mg/kg for IND-Mg) were significantly high as compared with those in IND dose (6 mg/kg) group (p <0.05). In IND-salt multiple dose groups, the drug-metabolizing enzyme activities were only slightly decreased as compared with the control, while activities in IND dose group were substantially decreased. There was no significant change in the plasma protein binding between the IND- and the salt-treated rats. The partition coefficient (n-octanol-water) for IND-Ca and IND-Mg was higher than that of IND. The rank order of solubility in 2% taurocholate solution was IND-Mg > IND > IND-Ca, and the solubility of IND-Mg was 3 times higher than that of IND-Ca.

Therefore, the mechanism of increased absorption of these salts was probably ascribed to the enhanced lipid solubility and increased solubility in bile and intestine juice.

Keywords — magnesium–calcium salt of indomethacin; disposition; absorption; drug metabolizing enzyme; partition coefficient; solubility; bioavailability

INTRODUCTION

It is widely accepted that indomethacin (IND) has an exceptionally potent anti-inflammatory activity.1,2) However the benefit of the full therapeutic effect of IND is often limited because of gastrointestinal side effect.

In the previous paper,3) we find that a severe injury of liver and intestinal mucosa produced on repeated administration of IND are almost perfectly protected by the coadministration of magnesium silicate in rats. In addition, it is evidently shown that the amount of drug absorbed from rat intestine is significantly increased (p <0.01), and consequently high values in the area (AUC) under the plasma concentration curve and bioavailability are obtained by the coadministration of the antacid as compared with those after the administration of IND alone.3) Such an increased absorption is reported in man in the case of the concurrent administration of bishydroxycoumarin (BHC) and magnesium hydroxide.4) The reason why the tissue lesion is protected and the bioavailability is improved after coadministration of magnesium silicate is unclear, although there is a possibility that magnesium salt of IND may be partly formed in gastrointestinal, and the absorption characteristics may be improved.
In this paper, in attempt to clarify the possibility and to enhance the efficacy and safety of IND, magnesium and calcium salts of IND were prepared, and the intestinal absorption and pharmacokinetics of these salts and the effects on microsomal drug-metabolizing enzyme activities of liver and intestinal mucosa were estimated in comparison with the results obtained by IND alone, in addition to investigating the change of physicochemical property; n-octanol–water partition coefficient, plasma protein binding and solubility in bile salt solution of these salts.

MATERIALS AND METHODS

Materials — 1) Drug: Indomethacin (JP grade) was obtained from Sumitomo Kagaku Kogyo Co.,Ltd. Nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate (G-6-P), and G-6-P dehydrogenase were obtained from Oriental Yeast Co.,Ltd. Aniline was used following redistillation. Didodecyl phthalate, an internal standard for gas liquid chromatography (GLC), was purchased from Gasukuro Kogyo Co.,Ltd. All other chemicals were of special grade. 2) Experimental animals: Male Wistar rats weighing 200–250 g were used throughout. The animals had free access to MF diet (Oriental Yeast Co.,Ltd.) and water before and during the experiment.

Preparation of Calcium and Magnesium Salt (IND-Ca and IND-Mg) — IND (3g) was dissolved in a small amount of ice-cold 0.01 N NaOH, and then 35% calcium acetate (or magnesium acetate) solution (50% more than the stoichiometric amount) was gradually added to IND solution under chilling. After standing for 10–15 min on ice, the deposit was filtered, washed with ice-cold water repeatedly, and followed by drying in vacumm.

Administration Schedules — IND was administered orally in a 6 mg/kg dose as an aqueous suspension in 2% acacia in a volume of 0.5 ml/100 g. IND-Ca and IND-Mg were administered orally (p.o.) in the equimolar dose, 6.32 mg and 6.20 mg respectively, as the same aqueous suspension. The animals were divided at random into 3 or 4 groups, each consisting of 4–9 rats. One member of each pair was assigned to the control group, and received 2% acacia solution orally. In multiple p.o. dose study, animals were treated for 7 d with daily oral administration of IND or the salts in a dose described. In separate experiments, IND-Ca and IND-Mg were administered orally in a decreased dose (4.16 mg/kg and 4.74 mg/kg respectively).

Determination of Calcium and Magnesium and Identification of the Salts — The Ca and Mg salts of IND were dried on silica gel under negative pressure for 5 d. Calcium and Magnesium concentrations in the salts were measured by flame photometry, using a Hitachi 300 UHF plasma spectra scan. The preparations obtained were characterized by means of 1H-nuclear and 13C-nuclear magnetic resonance (NMR) with a JEOL, JNM-FX200 NMR spectrometer and infrared spectroscopy (IR, nujol mull method) with a Hitachi EGI-G3 IR spectrophotometer.

Determination of IND in Plasma — Plasma samples containing IND were determined by the method of Aoyama et al.5) with slight modifications, according to the GLC method, as described in a previous paper.3) Whole blood was collected at 0 (just before the dosing), 3, 6 and 12 h after the final treatment and after that at 12 h intervals up to 60 h.

Preparation of Microsomes — Animals were fasted for 12 h prior to the experiment. The microsomal fractions of liver and intestinal mucosa were prepared according to the methods of Omura and Sato6) and Shirkey et al.7) respectively. The intestinal mucosal microsomes used in the experiments were “acid” microsomes.5)

Protein Determination — Protein concentration was determined by the method described by Lowry et al.8) with bovine albumin, fraction V, as a standard.

Enzyme Assays — The concentration of cytochrome P-450 in the microsomal fraction was determined by the method of Omura and Sato.6) The activities of aniline hydroxylase, biphenyl hydroxylase, and aryl esterase were determined according to the methods described by Ikeda,9)
Bioavailability of Indomethacin Ca and Mg

Shirkey et al.,7) and Beaufay et al.,10) respectively.

Determination of Free IND Concentration in Plasma — Plasma samples obtained 6 h after a single oral dose (10 mg/kg) were subjected to ultrafiltration according to the method described by Imamura et al.31) IND concentration in the filtrate was determined by the GLC method.30)

Determination of IND Concentration in Liver — Animals were fasted for 12 h prior to the experiment. Liver homogenate was prepared in 0.25M sucrose-50 mM phosphate buffer, pH 7.5, 6 h after a single oral dose (10 mg/kg). IND concentration in the homogenate was determined by the GLC method.30)

Measurement of Partition Coefficient in n-Octanol-Water System — The drug at 10⁻⁴ M was dissolved with 10 ml of n-octanol (saturated with 0.001 N HCl), and was added to 10 ml of 0.001 N HCl (saturated with n-octanol). After vigorous shaking for 1 h, the phases were separated by centrifugation. The drug concentration in both phases was measured by means of a spectrophotometer at the extinction maximum (318 nm). The partition coefficient in n-octanol-0.1 M phosphate buffer (pH 6.8) system was also determined according to the same technique. The partition coefficient (P) was calculated by the following equation:

\[ P = \frac{\text{concentration of drug in } n\text{-octanol}}{\text{concentration of drug in water}} \]

Determination of Solubility in Sodium Taurocholate Solution — The solubility of drug was determined in 0.075 M Tris·HCl buffer (pH 7.4) containing 0.2 or 2% sodium taurocholate or with no taurocholate added at 37°C. The sample (300 mg) was suspended in 50 ml of the taurocholate solution and constantly shaken at 37°C in a Toyo TC-1 incubator (120 oscillations/min in 4 cm amplitude), and the concentration of drug in the supernatant, after centrifugation, obtained at arbitrary intervals was determined spectrophotometrically at 318 nm.

Pharmacokinetic Analysis — The calculation and statistical analyses were carried out with the aid of a personal computer, Sharp MZ-80B. The pharmacokinetic parameters were calculated by least-squares linear regression analysis from the appropriate part of the log plasma concentration beyond 24 h vs. time curve, as described in a previous paper.3) The absolute bioavailability was calculated by the following equation:

\[ \text{absolute bioavailability (\%)} = \frac{AUC_{\text{po}}}{AUC_{\text{iv}}} \times 100 \]

where \( AUC_{\text{po}} \) and \( AUC_{\text{iv}} \) are the area under the plasma drug concentration-time curve after oral and intravenous administrations, respectively.

Statistical Methods — The data were compared by an analysis of variance. When the analysis indicated that a significant difference existed, statistical significance between two means was determined by the Student’s t-test with \( p < 0.05 \) as the criterion of significance.

RESULTS

Identification of IND-Ca and IND-Mg and Alkaline-Earth Metal Contents in the Salts

The analyses by means of \(^1\)H-NMR, \(^{13}\)C-NMR and IR showed that no decomposed compounds and impurities were contained in the salts. The calcium content in IND-Ca was 19.83 g on an average per 1 mol of the salt, calculated as an anhydrate, this value was 99.0% of the theoretical content. The magnesium content in IND-Mg was 11.19 g on an average per 1 mol of the salt, this being 92.0% of the theoretical value. The slightly smaller value of magnesium content in IND-Mg as compared with the theoretical value indicates that IND-Mg may exist as a hydrate. This was also suggested by the decrease (8.3%) in dry weight (105°C, 4 h) (the decrease in IND-Ca was 4.1%).

Single p.o. Dose Studies (6 mg/kg)

The plasma concentrations after a single oral administration of IND (6 mg/kg), IND-Mg (6.20 mg/kg) or IND-Ca (6.32 mg/kg) are shown in Fig. 1. The plasma decay curve of the drug(s) was found to be biexponential, as shown in the previous paper.3) In IND-Ca dose group, the plasma peak concentration occurred later (maximum plasma concentration 6 to 12 h after administration) than that after IND. In both
IND-Ca and IND-Mg dose groups, the mean plasma levels were significantly higher than those in IND dose group at all points after the 6 h interval \((p < 0.05)\), suggesting the increased absorption of both the salts. The elimination rate constant \((\beta)\) of \(\beta\) phase did not change in IND and the salts, and the half-life \((t_{1/2,\beta})\) was approximately 15 h, as shown in Table I. The \(AUC\) after IND-Ca and IND-Mg dosing was increased by 52% and 31% respectively as compared with that in IND dose group. The absolute bioavailability calculated was 63.7% after IND, 83.2% after IND-Mg and 96.8% after IND-Ca.

**Multiple p.o. Dose Studies (6 mg/kg)**

The plasma concentrations after 7 d' oral administration of IND, IND-Mg or IND-Ca in equivalent doses are shown in Fig. 2. In the multiple dose groups, the mean plasma levels were significantly higher than those in the single dose group (compare with Fig. 1). This suggests the accumulation of drug in the body during 7 d' treatment. The elimination profile from plasma was almost the same in the three groups. However, the substantially high plasma levels were shown in IND-Ca and IND-Mg dose groups as compared with those in IND-treated rats. There was no significant difference in the \(\beta\) and \(t_{1/2,\beta}\) between all groups, as shown in Table II. In all groups, however, the \(\beta\) values after multiple administration were significantly decreased as compared with those after a single administration (Table I) \((p < 0.001)\). The \(AUC\) after IND-Ca and IND-Mg dosing was extremely enhanced as compared with that after IND dosing, being a 1.7- and 1.9-fold respectively.

**TABLE I. Pharmacokinetic Parameters after a Single Oral Administration of IND (6 mg/kg), IND-Mg (6.20 mg/kg) or IND-Ca (6.32 mg/kg)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>(\beta) ((h^{-1}))</th>
<th>(t_{1/2,\beta}) ((h))</th>
<th>(AUC_{0-\infty}) ((\mu g \cdot h/ml))</th>
<th>Absolute bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IND</td>
<td>0.0449 ± 0.0022</td>
<td>15.49 ± 0.77</td>
<td>379.79 ± 33.02</td>
<td>63.7</td>
</tr>
<tr>
<td>IND-Mg</td>
<td>0.0460 ± 0.0028</td>
<td>15.11 ± 0.95</td>
<td>496.29 ± 39.81</td>
<td>83.2</td>
</tr>
<tr>
<td>IND-Ca</td>
<td>0.0469 ± 0.0009</td>
<td>14.77 ± 0.28</td>
<td>577.10 ± 74.94</td>
<td>96.8</td>
</tr>
</tbody>
</table>

*Each value represents the mean ± S.D. of 4—6 rats. a) \(p < 0.001\) compared with IND. The \(AUC_{0-\infty}\) after i.v. dosing was 596.46 ± 52.34.*

*3*
FIG. 2. Semilogarithmic Plots of Plasma IND Concentration after Multiple Oral Administration of IND, IND-Mg or IND-Ca

The animals were treated for 7 d with daily oral administration of IND (6 mg/kg), IND-Mg (6.20 mg/kg) or IND-Ca (6.32 mg/kg). Each point represents the mean ± S.D. of 6—9 rats. ○; IND, □; IND-Mg, △; IND-Ca. a) p < 0.001, b) p < 0.01 and c) p < 0.05 respectively compared with IND.

**Single p.o. Dose Studies in Decreased Dosage**

In order to obtain the AUC for IND-Ca or IND-Mg equivalent to that after a single IND dosing (6 mg/kg), the dosage of IND-Ca and IND-Mg was decreased based on the AUC described in Table I, and the animals were treated in a decreased dose (4.16 mg/kg for IND-Ca and 4.74 mg/kg for IND-Mg). The mean plasma levels and elimination rates were essentially similar in three groups, except that the peak plasma concentration of IND-Ca occurred later, as illustrated in Fig. 3. The pharmacokinetic parameters are shown in Table III. There was no significant difference in the $\beta$ and $t_{1/2,\beta}$ among the three groups, although the AUC in IND dose group was slightly higher than that after IND-Ca and IND-Mg dosing.

**Multiple p.o. Dose Studies in Decreased Dosage**

The plasma concentrations after 7 d’ oral administration of IND-Ca (4.16 mg/kg) or IND-Mg (4.74 mg/kg) in a decreased dose are shown in Fig. 4. In IND-Ca and IND-Mg multiple dose groups, the mean plasma levels were substantially high as compared with that in IND dose group. The $\beta$ after IND-Ca and IND-Mg multiple dosing were slightly, but significantly increased as compared with that after IND multiple dosing ($p < 0.001$), as shown in Table IV. There was a remarkable increase in the AUC values after IND-Ca and IND-Mg multiple dosing as compared with that after IND. These results also suggest that the amount of IND-Ca or IND-Mg absorbed was significantly larger.

**TABLE II. Pharmacokinetic Parameters after Multiple Oral Administration of IND (6 mg/kg), IND-Mg (6.20 mg/kg) or IND-Ca (6.32 mg/kg)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>$\beta$ (h$^{-1}$)</th>
<th>$t_{1/2,\beta}$ (h)</th>
<th>$AUC_0-\infty$ (µg·h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IND</td>
<td>0.0361 ±0.0012</td>
<td>19.22 ±0.62</td>
<td>517.17 ±123.84</td>
</tr>
<tr>
<td>IND-Mg</td>
<td>0.0382 ±0.0016$^b$</td>
<td>18.18 ±0.74$^b$</td>
<td>885.26 ± 94.07$^a$</td>
</tr>
<tr>
<td>IND-Ca</td>
<td>0.0372 ±0.0016</td>
<td>18.67 ±0.83</td>
<td>986.46 ±194.91$^a$</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 6—9 rats. a) $p < 0.001$ and b) $p < 0.05$ respectively compared with IND.
Effect of Decreased Doses of IND-Ca and IND-Mg on Microsomal Drug-metabolizing Enzyme Activities in Liver and Intestinal Mucosa

In order to clarify the effect of 7 day treatment with IND-Ca and IND-Mg in the decreased doses on liver and intestine, hepatic and intestinal microsomal drug-metabolizing enzyme activities were determined in the multiple dose groups in comparison with the control group. As shown in Table V, multiple treatment with IND (6 mg/kg) significantly decreased all enzyme activities tested as compared with the control, while the activities after multiple treatment with IND-Ca (4.16 mg/kg) and IND-Mg (4.74 mg/kg) were slightly decreased. There was no significant change in the activities between IND-Ca and IND-Mg multiple dose groups, except cytochrome P-450 content. These results suggest that multiple treatment with IND-Ca and IND-Mg in decreased doses produced less injury of liver and intestinal mucosa.

Free Drug Concentration in Plasma and Hepatic Uptake of IND-Ca and IND-Mg

The unbound drug concentrations in plasma 6 h after a single p.o. dose of IND (10 mg/kg), IND-Ca (10.53 mg/kg) or IND-Mg (10.33 mg/kg) were determined. The binding percentages of these drugs were above 99% (99.25 ± 0.26 for IND, 99.28 ± 0.19 for IND-Mg and 99.25 ± 0.20% for IND-Ca) and no significant difference in the binding between the three groups was observed.

TABLE III. Pharmacokinetic Parameters after a Single Oral Administration of IND (6 mg/kg), IND-Mg (4.74 mg/kg) or IND-Ca (4.16 mg/kg)

<table>
<thead>
<tr>
<th>Drug</th>
<th>$B$ (h⁻¹)</th>
<th>$t_{1/2, B}$ (h)</th>
<th>$AUC_{0-\infty}$ (μg·h/ml)</th>
<th>Absolute bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IND</td>
<td>0.0449 ± 0.0022</td>
<td>15.49 ± 0.77</td>
<td>379.79 ± 33.02</td>
<td>63.7</td>
</tr>
<tr>
<td>IND-Mg</td>
<td>0.0469 ± 0.0011</td>
<td>14.78 ± 0.35</td>
<td>312.88 ± 21.50 $a$)</td>
<td>68.6</td>
</tr>
<tr>
<td>IND-Ca</td>
<td>0.0467 ± 0.0005</td>
<td>14.84 ± 0.17</td>
<td>331.03 ± 24.82 $b$)</td>
<td>84.3</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 4–6 rats. $a$) $p < 0.01$ and $b$) $p < 0.05$ respectively compared with IND.
The amounts of drug extracted by liver 6 h after a single p.o. dose of IND (10 mg/kg) and the salts (IND-Ca, 10.53 mg/kg and IND-Mg, 10.33 mg/kg) tended to increase (0.0513 ± 0.0175 for IND, 0.0753 ± 0.0039 for IND-Mg and 0.0693 ± 0.0200 µg/g liver weight for IND-Ca), but the increase may be mainly ascribed to the high plasma concentrations of these drugs.

**Partition Coefficient**

In order to gain insight into the mechanisms by which IND-Ca and IND-Mg dosing increased intestinal absorption of the drugs, the partition coefficient (n-octanol–water) for these drugs was measured in comparison with that for IND. As shown in Table VI, when 0.001 N HCl was used as water layer, the partition coefficient for IND was 161.40 ± 36.05, suggesting that IND is a relatively high lipid-soluble drug. However, the partition coefficient for IND-Ca and IND-Mg was much higher. On the other hand, the partition coefficient for these salts in n-octanol-0.1 M phosphate buffer (pH 6.8) system was also significantly higher than that of IND. These results indicate that the lipodic properties of IND were enhanced by the formation of magnesium or calcium salt.

**Dissolution Behavior in Sodium Taurocholate Solution or Buffer**

To confirm the possibility that the solubility of IND-Ca or IND-Mg may be enhanced in bile or intestinal juice, their dissolution profiles in 0.2 or 2% sodium taurocholate solution or

---

**TABLE IV. Pharmacokinetic Parameters after Multiple Oral Administration of IND (6 mg/kg), IND-Mg (4.74 mg/kg) or IND-Ca (4.16 mg/kg)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>$\beta$ (h$^{-1}$)</th>
<th>$t_{1/2, \beta}$ (h)</th>
<th>$AUC_{0-\infty}$ (µg·h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IND</td>
<td>0.0361 ± 0.0012</td>
<td>19.22 ± 0.62</td>
<td>517.17 ± 123.84</td>
</tr>
<tr>
<td>IND-Mg</td>
<td>0.0412 ± 0.0007$^a$</td>
<td>16.82 ± 0.29$^a$</td>
<td>766.31 ± 195.80$^a$</td>
</tr>
<tr>
<td>IND-Ca</td>
<td>0.0402 ± 0.0016$^a$</td>
<td>17.24 ± 0.68$^a$</td>
<td>773.40 ± 154.09$^b$</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 4–9 rats. $a$) $p < 0.001$, $b$) $p < 0.01$ and $c$) $p < 0.05$ respectively compared with IND.
buffer (pH 7.0) were estimated. The dissolution curves for free drug and the salts are shown in Fig.5. The rank order of solubility in 0.075 M Tris-HCl buffer (pH 7.0) was IND-Mg > IND > IND-Ca. It was also shown that the rank order of solubility in 2% sodium taurocholate solution was IND-Mg > IND > IND-Ca. The solubility of free drug and the salts in the bile salt solutions was extremely enhanced as compared with that in the buffer alone. The

### TABLE V. Effect of Multiple Oral Administration of IND (6 mg/kg), IND-Mg (4.74 mg/kg) or IND-Ca (4.16 mg/kg) on Liver and Intestinal Microsomal Drug-metabolizing Enzyme Activities

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Treatment</th>
<th>Activities</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome P-450&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Control</td>
<td>0.830±0.062</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>IND</td>
<td>0.498±0.076&lt;sup&gt;d&lt;/sup&gt;</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>IND-Mg</td>
<td>0.729±0.085&lt;sup&gt;f,g&lt;/sup&gt;</td>
<td>87.8</td>
</tr>
<tr>
<td></td>
<td>IND-Ca</td>
<td>0.606±0.047&lt;sup&gt;d,h,i&lt;/sup&gt;</td>
<td>73.0</td>
</tr>
<tr>
<td>Aniline hydroxylase&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Control</td>
<td>0.774±0.054</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>IND</td>
<td>0.504±0.060&lt;sup&gt;d&lt;/sup&gt;</td>
<td>65.1</td>
</tr>
<tr>
<td></td>
<td>IND-Mg</td>
<td>0.738±0.110&lt;sup&gt;g&lt;/sup&gt;</td>
<td>95.3</td>
</tr>
<tr>
<td></td>
<td>IND-Ca</td>
<td>0.670±0.039&lt;sup&gt;e,g&lt;/sup&gt;</td>
<td>86.6</td>
</tr>
<tr>
<td>Biphenyl hydroxylase&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Control</td>
<td>0.175±0.005</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>IND</td>
<td>0.126±0.005&lt;sup&gt;d&lt;/sup&gt;</td>
<td>72.0</td>
</tr>
<tr>
<td></td>
<td>IND-Mg</td>
<td>0.162±0.010&lt;sup&gt;e,g&lt;/sup&gt;</td>
<td>92.6</td>
</tr>
<tr>
<td></td>
<td>IND-Ca</td>
<td>0.167±0.004&lt;sup&gt;f,g&lt;/sup&gt;</td>
<td>95.4</td>
</tr>
<tr>
<td>Aryl esterase&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Control</td>
<td>7.048±0.756</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>IND</td>
<td>5.588±0.357&lt;sup&gt;d&lt;/sup&gt;</td>
<td>79.3</td>
</tr>
<tr>
<td></td>
<td>IND-Mg</td>
<td>5.972±1.232</td>
<td>84.3</td>
</tr>
<tr>
<td></td>
<td>IND-Ca</td>
<td>6.507±0.822&lt;sup&gt;h&lt;/sup&gt;</td>
<td>92.3</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 4—9 rats. a) The content of cytochrome P-450 is expressed as nmol per mg of protein. b) The activities of enzyme are nmol of product per min per mg of protein. b) The activities is expressed as mmol of product per min per mg of protein. d) p < 0.001, e) p < 0.01 and f) p < 0.05 respectively compared with Control. g) p < 0.001 and h) p < 0.05 compared with IND. i) p < 0.05 compared with IND-Mg.

### TABLE VI. Partition Coefficient of IND, IND-Mg and IND-Ca

<table>
<thead>
<tr>
<th>Drug</th>
<th>Partition coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n-Octanol-0.001 N HCl</td>
</tr>
<tr>
<td>IND</td>
<td>161.40±36.05</td>
</tr>
<tr>
<td>IND-Mg</td>
<td>311.04±139.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IND-Ca</td>
<td>475.04±101.42&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The partition coefficient was measured in n-octanol—water system. Each value represents the mean ± S.D. of 7—9 experiments. a) p < 0.001 and b) p < 0.01 compared with IND. c) p < 0.05 compared with IND-Mg.
Bioavailability of Indomethacin Ca and Mg

solubility of IND-Mg and IND in 2% sodium taurocholate solution was 3 and 2 times respectively higher than that of IND-Ca.

DISCUSSION

IND has been widely used in the treatment of a variety of arthritic disorders, however, gastrointestinal side effects and hematopoietic injury by the drug are well documented. In order to prevent the undesirable side effects, some countermeasures, such as coadministration of antacids and the use of sustained release preparations and suppository, are taken in clinical. We have shown in the previous paper that when IND is coadministered with magnesium silicate, the plasma concentrations and the AUC of the anti-inflammatory drug are significantly enhanced and the hepatic and intestinal injury is protected.

Ambre and Fischer suggest that chelate formation may play a role in the increased absorption of BHC when it is given together with magnesium hydroxide. Therefore, in an attempt to clarify the mechanisms of increased absorption and reduced injury after coadministration of antacid, magnesium and calcium salts of IND were prepared, and the bioavailability in rats and physical properties of these salts were estimated. The following assumptions concerning the mechanism may be conceived: First, the formation of poorly water-soluble salts of IND may cause the decrease in direct lesion of the drug to tissues, as suggested by aluminium salt of aspirin. Second, the physical properties altered owing to the formation of alkaline-earth metal salts may lead to the enhanced solubility in intestinal juice and bile, and consequently increasing the intestinal absorption. Third, the alkaline-earth metal salts formed may possess the high affinity of drug for the lipid membrane of epithelial cells and may be accelerated drug absorption by passive diffusion.

We have found that the plasma levels after IND-Ca or IND-Mg were significantly higher than those after IND in both single and multiple dosing (Fig. 1 and 2). This demonstrates that the intestinal absorption of the salts was highly enhanced. The bioavailability of IND-Ca was 96.8% and was almost equivalent to the i.v. administration of IND. Thus, the increased intestinal absorption of the salts made it possible to decrease their dosage. This is of very significant, since most adverse effects of IND are dose related. When animals were treated with the decreased dosage of IND-Ca (4.16 mg/kg) and IND-Mg (4.74 mg/kg) for 7 d, in which dosage the similar AUC was obtained after a single dose of each drug, the plasma concentrations of drug were substantially higher in IND-Ca and IND-Mg dose groups than in IND dose group (Fig. 4). In addition, the decreased doses of the salts showed significantly less injury of the liver and intestinal mucosa of rats as compared with IND after 7 d' treatment (Table V). The increased absorption and the decreased injury of

FIG. 5. Dissolution Profiles of IND, IND-Mg and IND-Ca in 0.075 M Tris-HCl Buffer and Taurocholate Solution at pH 7.4

Each point represents the mean of 4 experiments. Sodium taurocholate was dissolved in 0.075 M Tris-HCl buffer, pH 7.4.

-IND, O ; IND-Mg, △ ; IND-Ca.
- - - - - - ; 2% sodium taurocholate,
- - - - - - ; 0.2% sodium taurocholate,
- - - - - - - ; 0.075 M Tris-HCl buffer (pH 7.0).
liver and intestinal mucosa, in spite of high plasma and hepatic concentrations, after IND-Ca and IND-Mg indicated that most of the salts were directly absorbed from intestine and might be transported to the organs in the form of salts during the early hours after dosing. The salts absorbed would be gradually dissociated into the free form in blood and tissues, this was demonstrated by the data that the β values, in vivo plasma protein binding and hepatic uptake of IND-Ca and IND-Mg, the latter two were measured 6 h after dosing, were almost the same as those of IND.

An attempt has been made to clarify the mechanism of the increased absorption of IND-Ca and IND-Mg, by means of measuring the partition coefficient and solubility in bile salt solution or buffer. Bates et al.15 have shown that bile salts markedly increased the solubility and dissolution rate of poorly water-soluble drugs. It is shown that bile salts considerably enhanced the dissolution of IND,16 and affected its absorption processes.17 In this study, the solubility of IND and the salts were remarkably increased in 2% sodium taurocholate solution as compared with that in buffer. Particularly, the solubility of IND-Mg was high, and it seems probable that the relatively high solubility was highly involved in the enhanced absorption in vivo. When IND-Ca was administered orally, the mean plasma peak time was delayed as compared with those of IND-Mg and IND (Fig. 1). This is explained by the relatively lower solubility of IND-ca in bile salt solution and buffer. The cause in which the amount of IND-Ca absorbed was significantly greater than that of IND is probably ascribed to the high lipid solubility of the salt. Thus, poor but slight solubility and increased lipid solubility of IND-Ca and IND-Mg seem to favor the intestinal absorption.

It is shown that the plasma level of IND correlates closely to the degree of intestinal injury.18 However, the directly stimulative action to the cell membrane, as presented by Roth,19 should be partly considered, as demonstrated by the less decrease in microsomal drug-metabolizing enzyme activities of intestinal mucosa in IND salt dose groups (Table V).

In conclusion, the intestinal absorption of IND-Ca and IND-Mg was highly increased as compared with that of IND, and substantially high plasma levels and bioavailability after the salts were obtained. These made it possible to decrease the dosage of IND-Ca and IND-Mg. In such decreased dosage, the higher plasma levels and much less decrease in microsomal drug-metabolizing enzyme activities in intestinal mucosa and liver were produced. The mechanism of increased absorption of the salts was mainly attributed to the enhanced lipid solubility and increased solubility in bile salt solution. These results suggest that most of IND-Ca and IND-Mg administered were absorbed in the salt form from the intestine. Therefore, this study throws some light on the improvement of safety and efficacy of IND.

REFERENCES
Bioavailability of Indomethacin Ca and Mg

Biochem., 93, 73—81 (1979).


