ABSORPTION AND BIOAVAILABILITY OF CALCIUM AND MAGNESIUM SALTS OF INDOMETHACIN FROM RECTAL SUPPOSITORIES

TARO OGISO, MASAHIRO IWAKI AND EJI TAMAKI

Faculty of Pharmaceutical Sciences, Kinki University, Kowakae 3-4-1, Higashi-Osaka, 577, Japan
(Received November 18, 1983)

In order to estimate the application to rectum of calcium and magnesium salts of indomethacin (IND), the suppositories of the salts and IND were prepared using macrogol and Witepsol H-15 bases. The rectal administration of these suppositories (Witepsol H-15) was compared with the oral administration of IND and the salts. The hardness, melting point, and liquefaction time of the suppositories were similar between IND and the salts. The in vitro release of IND-Mg from the suppositories was significantly faster than that of IND, while the release of IND-Ca was slower. The area under the plasma drug concentration-time curve (AUC) after IND suppositories was significantly higher than that after oral administration of IND (p<0.01). The AUC value after IND-Ca suppositories was slightly higher than those after other suppositories, although the plasma levels in early period after IND-Ca suppositories were lower, indicating the slow absorption of IND-Ca. The multiple treatment with IND-Ca suppositories gave no histological change of the rectal mucosa. Therefore, the present results suggested that IND-Ca was favourable for the rectal administration, in high doses and repeatedly.

**Keywords**—indomethacin calcium-magnesium; rectal absorption; suppository; bioavailability; mucous membrane

INTRODUCTION

Indomethacin (IND) has been widely used in the treatment of a variety of arthritic disorders. Unfortunately, like other analgesic and anti-inflammatory agents, IND carries the risk of gastro-intestinal irritation and ulceration. The tablets which were used originally produced peptic ulceration in some patients. The introduction of suppositories and a delayed-release preparation of IND have led to a decreased incidence of side-effects. However, it is shown that the manifestations of peptic ulceration and rectal bleeding also occur with suppositories.

We find previously that calcium (IND-Ca) and magnesium salts (IND-Mg) of IND have less injury to the intestine and liver in addition to the excellent absorption after oral dosing to rats. Therefore we thought it important to assess their application to rectum and rectal absorption in the hope that the suppositories of the salts could be used for patients without gastrointestinal irritation.

In the present study, the rectal administration of IND-Ca and IND-Mg was compared with the oral, intravenous (i.v.) and rectal routes of IND in rats. In addition to the measurement of drug concentrations in blood, a histological change in the rectal tissue was estimated after repeated dosing of these suppositories.

EXPERIMENTAL

**Materials**—1) Drugs: Indomethacin (JP grade) was obtained from Sumitomo Kagaku Kogyo Co., Ltd. Didodecyl phthalate, an internal standard for gas liquid chromatography (GLC), was purchased from Gasukuro Kogyo Co., Ltd. Witepsol H-15 and macrogol 4000 and 1500 (JP grade) were obtained from Mitsuba Boueki Co., Ltd. and Kishida Chemical Co., Ltd., respectively. All other chemicals used were of special grade. 2)
Experimental Animals: Male Wistar rats weighing 200—250 g were used throughout. The animals, maintained on MF diets (Oriental Yeast Co., Ltd.) for 3—4 d prior to the experiment, were divided at random into 2—3 groups, each consisting of 4—6 rats. The animals had free access to MF diet and water before and during the experiment of oral and i.v. administrations of drugs, and were fasted for 24 h before and during the experiment of rectal administration. 3) Preparation of Calcium and Magnesium Salts of IND and Identification of the Salts: IND-Ca and IND-Mg were prepared according to the previous method and the analyses were carried out by means of proton nuclear magnetic resonance (1H-NMR) and carbon-13 nuclear magnetic resonance (13C-NMR) and infrared (IR) spectrum. 4) Preparation of Suppository: The suppositories were prepared by the fusion method at low temperature as possible, using the drug powders, sifted by a 200 mesh sieve, and macrogol (4000 : 1500 = 3 : 1) and Witrepsol H-15 as the bases. One centimeter length of the suppository (diameter, 4 mm) contained 1.5 mg IND, 1.55 mg IND-Mg or 1.58 mg IND-Ca, these are the equimolar dose.

Administration Schedules — 1) Single Intravenous and Oral Admissions: 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 or intravenously in the same dose after dissolving in a small amount of 0.01 N NaOH. IND-Ca and IND-Mg were administered orally in the equimolar dose, 6.32 and 6.20 mg respectively, as the same aqueous suspension. 2) Single Rectal Administration: Each rat received IND rectally in a 6 mg/kg dose (a 1 cm length of suppository/250 g). IND-Ca and IND-Mg were rectally administered in the equimolar dose, respectively. The suppositories were inserted to a depth of 2 cm from the anus, and the expulsion and leak of suppository were prevented by an anal holder for 4 h according to the method of Akazawa et al. 3) Multiple Rectal Administration: In multiple dose schedules, rats were treated with repeated rectal administration of IND, IND-Mg or IND-Ca (6 mg/kg as IND) from day 1 to 8, except day 3 and 6 (rats had free access of diet on day 2 and 5 after drug administration in order to prevent the weakness of physical strength caused by fasting).

Determination of IND in Plasma — After the final treatment, 0.3 ml blood was collected at arbitrary intervals from tail vein in a heparinized syringe. The plasma was separated immediately by centrifugation at 1400 × g for 10 min. IND concentrations in plasma samples were determined by the method described in a previous paper.

Histopathology — Animals received suppository were killed by decapitation 4 h after the final treatment. The rectum (a 1.5 to 2.5 cm length from anus) was immediately removed from rats and after washing with cold saline was fixed in 10% buffered formalin for 24 h. A fragment of tissue was stained with hematoxylin and eosin, and observed by a microscope.

Pharmacokinetic Analysis — The pharmacokinetic parameters were calculated by the method described in a previous paper. The bioavailability was calculated by the following equation:

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

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

Measurement of Physical Properties — 1) Hardness: The hardness of suppository (0.8±0.1 cm length) was measured by using a Monsanto durometer. 2) Melting Point: The melting point of suppository was determined by the J.P. method. 3) Liquefaction Time: The liquefaction time was measured according to the method of Setnikar et al at 37°C. 4) Release of Drug from Suppository: In vitro release test was carried out by the method of Muranishi. Phosphate buffer (0.067M, pH 7.3) was used as the dissolution solution, and the concentration of drug in the solution was determined spectrophotometrically at 320 nm.
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

Plasma Concentrations of IND after Intravenous, Oral and Rectal Administrations

Fig. 1 is plasma levels of IND found after administration of IND by various routes. The plasma levels of IND during the first period after rectal administration (Witepsol H-15 base) were significantly higher than those after oral dosing. This indicates that rectal absorption of IND was far faster as compared with oral dosing. The AUC (509.8 ± 44.9 \( \mu \)g·h/ml) after rectal dosing was significantly greater than that (379.8 ± 33.0 \( \mu \)g·h/ml) after oral administration (\( p < 0.01 \)), although the value was less than that (596.5 ± 52 \( \mu \)g/ml) after i.v. dosing. There was no difference between the pharmacokinetic parameter, the \( \beta \), in three routes. Plasma Levels of IND after Administration of Macrogol and Witepsol H-15 Suppositories

To estimate the effect of bases on rectal absorption, the plasma concentrations were measured after a single rectal administration of IND (6 mg/kg) suppositories, prepared using a watersoluble base, macrogol and an oleaginous base, Witepsol H-15. In the case of macrogol base, the peak plasma concentration (\( C_{\text{max}} \) 329.8 ± 54.2 \( \mu \)g/ml), attained at about 3 h postadministration, was slightly, but not significant, higher than that (30.8 ± 3.89 \( \mu \)g/ml) after Witepsol suppositories.

However, in the Witepsol suppository group, plasma levels above 12 h after treatment were slightly high as compared with those after macrogol suppositories, the mean plasma concentration (\( \bar{C} \)) from 12 to 36 h was 9.18 \( \mu \)g·h/ml in the Witepsol group and 7.49 \( \mu \)g·h/ml in the macrogol group. There was no significant difference in the elimination rate (\( \beta \)) of \( \beta \) phase between both groups. The AUC after Witepsol suppositories, 520.95 ± 18.93 \( \mu \)g·h/ml, was slightly greater than that (483.30 ± 12.62 \( \mu \)g·h/ml) after macrogol suppositories (\( p < 0.05 \)), suggesting that the Witepsol suppository is superior to macrogol suppository in regard to the availability and maintenance of blood level. Therefore, after that Witepsol H-15 was used as the suppository base.

Physical Properties of Witepsol H-15 Suppository

The hardness, melting point, and liquefaction time of Witepsol H-15 suppositories of IND, IND-Mg and IND-Ca are shown in Table I. The hardness of these suppositories were similar in three groups, and the melting point was below rat body temperature (rectal temperature). The liquefaction time was 87–94 s in these suppositories. Therefore, there was no significant difference in the physical properties between the three suppositories, except for the melting point; the melting point of IND-Mg suppositories was

![Graph showing Plasma Concentrations of IND after Intravenous, Oral and Rectal Administrations](image-url)

**FIG. 1. Plasma Concentrations of IND after Intravenous, Oral and Rectal Administrations**

The animals were treated with a single administration of IND (6 mg/kg) by the routes indicated. Each point represents the mean ± S.D. of 5–6 rats. ○; i.v. (sodium salt), ■; p.o. (suspension in 2% acacia), ▲; rectal (Witepsol H-15 suppository).

\( a) p < 0.01 \) in i.v. vs. p.o. \( b) p < 0.01 \) in p.o. vs. rectal, \( c) p < 0.05 \) in i.v. vs. rectal, \( d) p < 0.05 \) in p.o. vs. rectal.
TABLE I. Physical Properties of Witexsol H-15 Suppositories

<table>
<thead>
<tr>
<th></th>
<th>Hardness (kg/cm²)</th>
<th>Melting point (°C)</th>
<th>Liquefaction time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IND</td>
<td>2.58±0.21</td>
<td>34.56±0.11</td>
<td>91±4</td>
</tr>
<tr>
<td>IND-Ca</td>
<td>2.39±0.18</td>
<td>34.38±0.11</td>
<td>94±7</td>
</tr>
<tr>
<td>IND-Mg</td>
<td>2.40±0.19</td>
<td>34.16±0.14</td>
<td>87±7</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.D. of 6–10 experiments. 

a) p < 0.01 in IND vs. IND-Ca. b) p < 0.001 in IND vs. IND-Mg. c) p < 0.01 in IND-Ca vs. IND-Mg.

The lowest of three suppositories. The results of the in vitro release test are shown in Fig. 2. The released amount of IND-Mg from suppositories was significantly larger than that of IND during the first hour, while the release of IND-Ca from the suppository was smaller. The differences observed seemed to be attributed to the solubility of drugs; IND-Mg exerting the highest water-solubility, as reported previously. The release rate constants (v), calculated from the slope of the log residual ratio vs. time curve by least-squares regression analysis (slope = −v / 2.303), were 1.09 for IND, 1.66 for IND-Mg and 0.80 h⁻¹ for IND-Ca, respectively.

Single Rectal Administration

The plasma concentrations after a single rectal administration of IND-Mg (6.20 mg/kg) or IND-Ca (6.32 mg/kg) in Witexsol H-15 suppositories were compared with those after a single oral dose. In the rectal dose groups, the plasma concentrations up to 6 h after administration were higher than those of oral dosing, as shown in Fig. 3. This indicates the rapid absorption of the salts from rat rectum. However, the plasma concentrations beyond 6 h after rectal and oral dosing were similar each other, and there was no significant difference between the AUC values of the rectal and oral administrations in either IND-Ca or IND-Mg. Some pharmacokinetic parameters calculated from the data are shown in Table II along with those of IND. No significant difference in the parameters was shown among three groups, although the AUC value after IND-Ca was slightly higher, but not significant. The absolute bioavailability calculated was 85.5% for IND, 87.9% for IND-Mg and 89.1% for IND-Ca.

Multiple Rectal Administration

The effect of multiple rectal administration of IND-Ca on the disposition, bioavailability and rectal membrane was compared with that of IND. The plasma concentrations after multiple rectal administration of IND-Ca and IND are
shown in Fig. 4. The peak plasma level occurred later in IND-Ca dose group than in IND group, however the mean maximal plasma concentrations ($C_{\text{max}}$) were almost the same in both groups.

**FIG. 3.** Plasma Concentrations of IND during the First Period after Oral and Rectal Administrations of IND Salts

The animals were treated with a single oral or rectal administration of IND salts (6 mg/kg as IND). (A) IND-Ca, (B) IND-Mg. Each point represents the mean± S.D. of 4—5 rats. ●; oral dosing, ○; rectal dosing. a) $p < 0.01$ and b) $p < 0.05$ respectively in p.o. vs. rectal.

**TABLE II.** Pharmacokinetic Parameters of IND after Single Rectal Administration of the Drug and Its Salts

<table>
<thead>
<tr>
<th></th>
<th>$C_{\text{max}}$ (μg/ml)</th>
<th>$\beta$ (h$^{-1}$)</th>
<th>$AUC_{0-\infty}$ (μg·h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IND</td>
<td>31.45±5.34</td>
<td>0.0466±0.0009</td>
<td>509.79±44.91</td>
</tr>
<tr>
<td>IND-Ca</td>
<td>28.30±3.88</td>
<td>0.0464±0.0011</td>
<td>531.42±45.46</td>
</tr>
<tr>
<td>IND-Mg</td>
<td>32.73±4.27</td>
<td>0.0464±0.0019</td>
<td>524.45±20.97</td>
</tr>
</tbody>
</table>

Each value represents the mean± S.D. of 5 rats.
The AUC (721.2 ± 33.7 µg·h/ml) of IND-Ca was slightly greater than that (661.5 ± 43.7 µg·h/ml) of IND, but not significant like other pharmacokinetic parameters.

**Histopathology of Epithelium**

To clarify the histological change in the tissue of rectum, the epithelium was observed with optical microscope following multiple dosing of the agents. In the group treated with IND, there was an only partial removal of the mucous epithelium from lamina propria and a slight membrane perturbation, as shown in Fig. 5, while in IND-Ca dose group significant histopathological changes were not shown.

**DISCUSSION**

In the previous study, we have shown that IND-Ca with higher lipid-solubility and IND-Mg with higher bile salt-solubility are satisfactorily absorbed with less intestinal irritation, as compared with IND, and consequently a high bioavailability was observed. Thus, the application of the salts to rectum is thought to be of value in view of IND therapy with less side-effects.

![Graph showing plasma concentrations of IND after multiple rectal administration of Witepsol H-15 Suppositories](image)

**FIG. 4. Plasma Concentrations of IND after Multiple Rectal Administration of Witepsol H-15 Suppositories**

The animals were treated for 6 d with daily rectal administration of IND (6 mg/kg/d) or IND-Ca (6.32 mg/kg/d). Each point represents the mean±S.D. of 4 rats. ●: IND, ○: IND-Ca. a) p < 0.05 in IND vs. IND-Ca.

![Rectal lumen after multiple rectal administration of IND and IND-Ca Suppositories](image)

**FIG. 5. Rectal Lumen after Multiple Rectal Administration of IND and IND-Ca Suppositories**

Hematoxylin-eosin stain was used. Magnification 100 ×. A: Control (no administration), B: IND, C: IND-Ca.
that the serum level after 100 mg of IND by rectum is 75% of that when given by mouth in patients.4) Our study in rats, however, indicated that the plasma peak levels after the rectal and oral doses of IND (6 mg/kg) were attained about 3 h after dosing, and that the AUC after IND suppositories was significantly greater than that following oral route. The difference between human and rat may be explained by the differences of species and suppository bases used. The much more absorption of IND after rectal dosing than after oral administration seems to be ascribed to the increased permeation across the mucosal membrane, probably due to partial changes of the membrane structure, as suggested by a microscopic observation of the epithelium after the repeated dosing of IND. This speculation is of interest in comparison with the reports that absorption of digoxin and insulin from rectum is accelerated by the addition of Tween 6014 and salicylates,15 respectively, and that the histological change of the rectal tissue is produced by Tween-type surfactants16 and transient changes in the transmembrane potential across the rectal mucosa occur following salicylate type adjuvant administration.17

The present results on IND-Ca and IND-Mg demonstrated that the AUC values of the salts following rectal administration to rat, like rectal IND dosing, were far greater than those following oral dosing of IND (Table II). This indicates that the salts were also satisfactorily absorbed from the rectum, as well as their oral doses.7) The pharmacokinetic parameters obtained following a single rectal dosing of IND-Ca and IND-Mg were extremely similar to those after their oral administration. The AUC of IND-Ca (531.4 ± 45.5 µg·h/ml) after rectal administration, however, was slightly smaller, but not significant, than that (577.1 ± 74.9 µg·h/ml) after oral route of the salt. The relatively small AUC and slow absorption of IND-Ca from Witexsol H-15 suppositories appear to be partly due to its poor water-solubility, as demonstrated previously.7) This was clearly proved by the slow release of IND-Ca from Witexsol suppository (Fig. 2). This view is also partly strengthened by the fact that the AUC of IND-Mg after rectal dosing was greater than that when dosed orally, because the salt has the higher water-solubility7) and larger release rate constant (Fig. 2). Another cause producing a small AUC after rectal dosing is probably attributed to the partial leak of IND-Ca from rectum, since IND-Ca, of which absorption is slow, can stay in the rectum for a longer time, and there is a possibility that the drug would be partly leaked out from the anus after taking off the anal holder.

It is noticeable that side effects are more marked with higher plasma concentrations and that the effects are seen above 6 µg/ml in healthy volunteers.4) If the disturbance is due solely to local damage to the mucosa, it should be avoided by rectal administration. As mentioned above, indigestion and occasional peptic ulceration is reported to occur with suppositories of IND.11 That repeated dosing of IND-Ca gave no histological alteration of rectal mucosa (Fig. 5) suggests that the application of IND-Ca to rectum is favourable for patients who require large doses. The result obtained leads us to speculate that IND has a direct irritative action to the membrane, as demonstrated by some workers,7) while the calcium salt has a less action. Since the dose (6 mg/kg) used in this study was much higher than the clinical doses in human, the high plasma levels (> 6 µg/ml) were maintained for many hours, but it would be reasonable to assume that the administration of IND-Ca or IND-Mg in the decreased doses can diminish the side effects, including headache and the effect on nervous system.13

In conclusion, the present results lead us to postulate that IND-Ca, and possibly IND-Mg too, are favourable for the rectal dosing as judging from that a great AUC was obtained with negligible irritation on the mucous membrane following application of suppositories.

REFERENCES
Rectal Absorption of Indomethacin Ca and Mg


