BIOAVAILABILITY OF GRISEOFULVIN PLAIN TABLETS IN STOMACH-EMPTYING CONTROLLED RABBITS AND THE CORRELATION WITH BIOAVAILABILITY IN HUMANS

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Bioavailability after giving oral doses of 62.5 mg griseofulvin tablets having different dissolution rates to stomach-emptying controlled rabbits, was estimated and compared with that in humans receiving 125 mg dose of the same griseofulvin preparations. The relative differences in $C_{\text{max}}$ and $AUC_{\infty}$ between the product with the highest availability and others tended to be greater in rabbits than in humans. The in vivo parameters correlated well between the two species. However, power analysis indicated a larger variability of $C_{\text{max}}$ in rabbits than in other species (dogs, minipigs and humans). Water volume (5 and 50 ml) coadministered with the drug did not significantly influence the bioavailability. The rabbits which were not given food after oral dosing with griseofulvin exhibited a lower $C_{\text{max}}$ than those which were fed immediately after dosing. The bioavailability of an ultramicronized formulation in rabbits was higher after the postprandial dose than after the preprandial dose. Food intake just after the drug administration seems to be an important factor for controlling the passage rate of the drug through the gastrointestinal tract in stomach-emptying controlled rabbits.

Keywords — rabbit; stomach emptying rate; human; griseofulvin; bioavailability; fluid volume effect; food effect; correlation

In principle, drug bioavailability should be estimated in humans. However, frequent use of humans is not only very difficult, but also should be restricted particularly in the development of new drugs and dosage forms, in order to avoid unpredictable responses in human volunteers. This requires an adequate model animal for bioavailability studies. There have been, however, only a few studies comparing drug bioavailability between humans and model animals, which make it difficult to estimate drug bioavailability using animals.

Our previous studies investigated the relations of the bioavailability of griseofulvin in humans with that in beagle dogs$^{1}$ and in Göttingen minipigs (minipigs G),$^{2}$ using the same four kinds of commercial plain tablets. The correlation of bioavailability between humans and dogs was poor due to a relatively higher availability of an ultramicrosize product in dogs than in humans.$^{1}$ On the other hand, the correlation between humans and minipigs G was high, but the relative differences in $C_{\text{max}}$ and $AUC_{\infty}$ between the product with the highest availability and others were smaller in pigs than in humans and, in addition, the bioavailability parameters in the pigs, as well as in dogs, were more variable than in humans.$^{2}$ This indicates that the bioavailability differences among products were less detectable in minipigs G. These unsatisfactory results necessitated further investigation on other model animals.

Recently, the usefulness of rabbits for bioavailability studies by controlling stomach-emptying has been described by Maeda et al.$^{3,4}$ The present work was undertaken to investigate the relation of bioavailability between humans and the rabbits with four types of griseofulvin tablets which were previously employed in the studies using humans,$^{5}$ dogs$^{1}$ and pigs.$^{2}$
METHODS

Formulations — Four brands of 125 mg griseofulvin tablets employed in the human,\(^5\) beagle dog\(^1\) and minipig G studies\(^6\) were used. One of them was an ultramicrosize griseofulvin tablet (A) formulated with the drug dispersed in polyethyleneglycol 6000.\(^6\) The others were microsize griseofulvin tablets (B, C and D) marketed in Japan. The drug contents for tablets A, B, C and D determined by a spectrophotometrical method\(^7\) were 123, 122, 125 and 128 mg per tablet, respectively.

Table I shows the dissolution rates of four griseofulvin tablets which were expressed as the time required for 50% of the drug to dissolve.\(^8\) The dissolution rates by a beaker method were determined in 18 l of pH 7.2 sodium phosphate buffer (0.01 m) contained in a 20 l flat-bottom beaker (29.0 cm i.d.) equipped with a three bladed screw type impeller (5 cm diameter) rotating at 512 rpm.

In method I, a tablet was gently shaken in 1 ml of water in a 100 ml round bottle flask for 1 min. After standing for 5 min, 20 g of plastic beads (8 mm diameter) were added to the bottle. The bottle, fixed at an angle of 5°, was then rotated at 3.8 rpm in a water bath (37°C) for 15 min. The contents were subsequently poured into 18 l of the pH 7.2 medium contained in the 20 l beaker along with 100 ml of water used for washing. The dissolution rate was then determined according to the beaker method procedure outlined above. In method II, a tablet was gently shaken in 20 ml of water in a 50 ml round bottle flask for 5 min and then 27 g of plastic beads were added. The bottle was rotated for 15 min as described in method I and subsequent procedures were the same as method I.

Animals — Forty-two male albino rabbits (2.5–3.5 kg) were used in this study, eight of which were for the study of bioavailabilities of four griseofulvin tablets, three for the dose-bioavailability, eleven for the effect of water volume study and twenty for the food effect. Stomach-emptying controlled rabbits were prepared according to the cangue method.\(^8\) Gastric lavage was carried out two days before drug administration with a No. 13 Nelaton catheter (32 cm in length and 7 mm in external diameter). A soft diet given to rabbits was prepared by adding 3 parts water to 2 parts special solid diet (CR-S, Nihon Clea Co. Ltd., Tokyo).\(^9\)

Bioavailability — A tablet of griseofulvin was cut in a half and the halved product in the range of 50±5% of a tablet weight was used for the in vivo study. A halved tablet was orally given to eight rabbits having fasted overnight in a cangue, together with 20 ml of water, which was immediately followed by feeding of 50 g soft diet to the rabbits. The animals not consuming this amount of diet within 10 min were considered to be in bad health and excluded from the study.\(^9\) No other food except the soft diet was given to the rabbits until 24 h after drug administration, at which time 100 g of special solid diet (CR-S) was fed. Oral administration was carried out as follows. The tip (about 1-cm) of a No. 13 Nelaton catheter was cut off, to which a rubber tube (3 cm in length and 5 mm in internal diameter) was attached. A test product was in-

<table>
<thead>
<tr>
<th>Method</th>
<th>Tablet</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beaker</td>
<td></td>
<td>14.1</td>
<td>6.5</td>
<td>80.6</td>
<td>157.5</td>
</tr>
<tr>
<td>Method I</td>
<td></td>
<td>6.1</td>
<td>2.5</td>
<td>26.7</td>
<td>4.6</td>
</tr>
<tr>
<td>Method II</td>
<td></td>
<td>2.1</td>
<td>3.2</td>
<td>17.3</td>
<td>6.4</td>
</tr>
</tbody>
</table>
serted into the tube, which was introduced into
the stomach of rabbits. The drug was pushed out
by means of a smaller diameter (3 mm) teflon
tube. Water was also given using the same catheter.
Blood samples were taken from the ear vein
at 1, 2, 3, 5, 7, 10, 24 and 31 h after dosing, and
serum samples were stored frozen (−20°C) until
assay. The experiments were repeated every two
weeks according to a randomized block design.
The serum drug levels were normalized for 62.5
mg dose of griseofulvin with the weights of the
halved products. The bioavailability of each pro-
duct was evaluated from the observed maximum
plasma concentration of griseofulvin (Cmax),
time to Cmax (Tmax), mean residence time
(MRT)9,10 and the area under serum drug con-
centration–time curve from zero to 31 h (AUC0–31)
and zero to infinite time (AUC0–∞) which were
calculated using the trapezoidal rule and accord-
ing to the method of Wagner,11 respectively.

Assay — The concentration of griseofulvin
in serum was determined by gas chromatography
with a electron capture detector as previously
described.5 To 0.2 ml of serum were added 0.2
ml of saturated sodium chloride solution and 5
ml of ether. After shaking for 10 min, 4 ml of the
ether layer was evaporated to dryness at about
40°C under nitrogen gas stream. The residue was
dissolved in 0.1–1 ml of benzene containing 1.1
µg/ml clothiapine as an external standard and 5
µl of the solution was used for gas-liquid chro-
natography (GLC) assay. The GLC condition
was the same as previously described.5 The de-
tection limit for griseofulvin (two or three times
the noise level) was about 10 ng/ml of the assay
solution.

Dose-Bioavailability Relation — Three
stomach-emptying controlled rabbits, having
fasted overnight, were orally given one or a half
tablet of product B, corresponding to 125 or
62.5 mg of griseofulvin, respectively, which
were then followed by ingestion of 50 g of the
soft diet. The drug was administered at two
week intervals according to a cross-over design.
The other procedures were the same as described
for the bioavailability test.

Effect of Volume of Water Coadministered
— A group of six and a group of five stomach
emptying controlled rabbits having fasted over-
night were used for the tests of tablet A and
tablet B, respectively. A half tablet of the test
product was orally administered to a rabbit with
5 or 50 ml of water according to a randomized
block design. The rabbits were immediately
given 50 g of the soft diet. The drug was admis-
tered every two weeks according to a dosage
schedule. The other procedures were the same as
described for the bioavailability test.

Food Effect — First, the bioavailabilities
after oral doses of griseofulvin, with and without
food, were investigated using seven rabbits. Ac-
cording to a randomized block design, a half
tablet of product B was orally administered along
with 20 ml of water to a rabbit after having fasted overnight. The animals were im-
nediately given 50 g of the soft diet or kept in a
fasting state for 24 h. The other procedures and
the time interval between experiments were the
same as described for the bioavailability test.
Secondly, the bioavailabilities of griseofulvin ad-
ministered after food ingestion were investiga-
g. A group of seven and a group of six rabbits were

<table>
<thead>
<tr>
<th>TABLE II. Relations of Griseofulvin Dose with Cmax and AUC0–∞</th>
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<tbody>
<tr>
<td>Dose</td>
</tr>
<tr>
<td>Cmax (µg/ml)</td>
</tr>
<tr>
<td>AUC0–∞ (h · µg/ml)</td>
</tr>
</tbody>
</table>

a) Means ± SE (n = 3).
used for the *in vivo* studies of products A and B, and products B, C and D, respectively. A half tablet of the test product was orally administered to the rabbits having fasted overnight with 20 ml of water 30 min after ingestion of 50 g of the soft diet. The experiments were repeated every two weeks according to a randomized block design. The other procedures were the same as described for the bioavailability test.

**RESULTS**

*Dose-Bioavailability Relation*

The relations of griseofulvin dose with $C_{\text{max}}$ and $AUC_{\infty}$ were investigated using tablet B. The mean $C_{\text{max}}$ and $AUC_{\infty}$ increased in proportion to the dose of griseofulvin orally administered (Table II), which indicates that linear pharmacokinetics can be applied to the serum levels of griseofulvin within the dose range studied. A 62.5 mg dose of griseofulvin was employed for the bioavailability study to avoid over-doses in the rabbits and to compare with the griseofulvin results reported by Maeda et al. who used a 62.5 mg dose.  

*Bioavailability*

Fig. 1 shows the mean serum levels of griseofulvin after oral administration of four tablets to stomach-emptying controlled rabbits and Table III summarizes the *in vivo* parameters. The mean peak concentration, $AUC_{31}$ and $AUC_{\infty}$ after administration of the griseofulvin products were

![Graph showing serum level over time](image)

**FIG. 1. Mean Serum Levels of Griseofulvin after Oral Administration of Four 62.5 mg Griseofulvin Tablets to Stomach-Emptying Controlled Rabbits (n=8)**

Table A (●), tablet B (○), tablet C (△), tablet D (□). The vertical lines show the standard errors.

| Tablet |  |  |  |  |  |  |
|--------|---|---|---|---|---|
| $C_{\text{max}}$ ($\mu$g/ml) | 0.599<sup>a)</sup> | 0.853 | 0.406 | 0.530 | $p < 0.1$ |
|  | (0.108) | (0.164) | (0.089) | (0.101) |  |
| $T_{\text{max}}$ (h) | 8.1 | 4.5 | 4.1 | 9.1 | NS<sup>b)</sup> |
|  | (3.4) | (0.8) | (0.8) | (4.1) |  |
| MRT (h) | 11.4 | 9.0 | 13.8 | 13.0 | NS |
|  | (2.4) | (0.9) | (1.8) | (3.0) |  |
| $AUC_{31}$ ($h \cdot \mu$g/ml) | 5.139 | 7.688 | 3.716 | 4.826 | $p < 0.001$ |
|  | (0.779) | (1.032) | (0.761) | (0.687) | $B > A > D > C$ |
| $AUC_{\infty}$ ($h \cdot \mu$g/ml) | 5.276 | 7.690 | 4.119 | 5.461 | $p < 0.01$ |
|  | (0.760) | (1.028) | (0.810) | (0.857) | $B > D > A > C$ |

<sup>a</sup>) The figures in the parentheses indicate the standard errors. <sup>b)</sup> NS: not significant at $p < 0.1$. <sup>c)</sup> Formulations underlined by a common line did not differ significantly ($p < 0.05$).

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almost in proportion to their dissolution rates determined by method I. The rank orders in 
$C_{\text{max}}$ and $AUC_{31}$ among the products were $B > A > D > C$ and that in $AUC_{\infty} B > D > A > C$, 
which were similar to the rank orders in $C_{\text{max}}$ 
and $AUC_{47.5}$ in humans ($B > D > A > C$).\textsuperscript{5} The 
mean peak concentration, $AUC_{31}$ and $AUC_{\infty}$ 
after administration of the product with the 
highest bioavailability (tablet B) were approxi-
mately twice as high as those of the product 
with the lowest bioavailability (tablet C). 
Tukey's multiple range test showed statistically 
significant difference in $AUC_{31}$ between tablets 
B and C and between B and D, and in $AUC_{\infty}$ 
between tablets B and C.

Due to the difficulty of collecting the serum 
sample with a constant time interval throughout 
the experiment, $T_{\text{max}}$ is apt to more vary 
depending on the sampling time interval than 
$C_{\text{max}}$ and $AUC$. Assuming that the disposition 
and elimination parameters do not change in an 
individual, $MRT$ as well as $T_{\text{max}}$ reflects the rate 
of drug absorption when evaluating the different 
oral dosage formulations.\textsuperscript{12} $MRT$ is preferable 
to $T_{\text{max}}$ because $MRT$ is considered to be less 
dependent on the sampling time than that of 
$T_{\text{max}}$. Thus, $MRT$ was also used as the in vivo 
parameters to estimate the rate of bioavailability 
as $T_{\text{max}}$ was employed. The rank order in $MRT$ 
was well agreed with that in $C_{\text{max}}$ but the 
differences in $MRT$ among the products were not 
statistically significant.

Power analysis\textsuperscript{13,14} was employed to estimate 
the total number of rabbits required for a 20% dif-
ference in $C_{\text{max}}$, $T_{\text{max}}$ and $AUC$ to be significant 
($\alpha=0.05, 1-\beta=0.8$) in a randomized block 
design. These were compared with the numbers of 
humans, dogs and Göttingen minipigs previously 
studied\textsuperscript{20} (Table IV), which also showed 
the numbers required for randomized block 
designs. Compared with humans and other ani-
mals, the rabbit study required far more animals, 
about seventy, for a 20% difference in $C_{\text{max}}$ to be significant due to the large variabil-
ity. The power analysis also indicated that the 
parameter of $T_{\text{max}}$ was more variable than $C_{\text{max}}$ 
or $AUC$.

**Relation between Humans and Rabbits**

The correlation of mean $C_{\text{max}}$, $T_{\text{max}}$ and 
$AUC$ from zero to final sampling time between 
humans and stomach-emptying controlled 
rabbits, were investigated. As Fig. 2 shows, the $C_{\text{max}}$ 
in the rabbits was significantly correlated with 
that in humans. The correlation of $T_{\text{max}}$ between 
the two species was lower than that of $C_{\text{max}}$ 
or $AUC$. In order to compare the differences in 
$C_{\text{max}}$ and $AUC_{\infty}$ among products in rabbits 
those with in humans, the ratio of mean $C_{\text{max}}$ 
and $AUC_{\infty}$ of each product to those of tablet B 
was plotted (Fig. 3). The differences in the ratios 
of $C_{\text{max}}$ and $AUC_{\infty}$ (conveniently called as the 
relative differences) between the product with 
the highest bioavailability (tablet B) and the 
these tended to be larger in the rabbits. A con-
siderably lower $AUC_{\infty}$ in rabbits when using 
slow dissolving products as compared with 
humans seems to indicate that the drug passed 
through the absorption site without being com-

<table>
<thead>
<tr>
<th></th>
<th>$T_{\text{max}}$</th>
<th>$C_{\text{max}}$</th>
<th>$AUC$ $a)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>212</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>Dog</td>
<td>136</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>Minipig G</td>
<td>60</td>
<td>42</td>
<td>38</td>
</tr>
<tr>
<td>Rabbit</td>
<td>334</td>
<td>72</td>
<td>32</td>
</tr>
</tbody>
</table>

$a)$ $AUC$ from zero to final sampling time; $AUC_{47.5}$ for humans, $AUC_{24}$ for dogs, $AUC_{32}$ for minipigs $G$ and $AUC_{31}$ for rabbits.
FIG. 2. Correlations of $C_{\text{max}}$, $T_{\text{max}}$, and $AUC$ between Humans and Stomach-Emptying Controlled Rabbits

$AUC_{31}$ in rabbits and $AUC_{47.5}$ in humans were employed for the correlation estimation. The solid lines show the regression lines. a) $p < 0.05$.

FIG. 3. Relative Differences in $C_{\text{max}}$ and $AUC_{\infty}$ among Four Tablets in Humans and Stomach-Emptying Controlled Rabbits

The ratios of $C_{\text{max}}$ and $AUC_{\infty}$ of each tablet were determined against those of tablet B.

completely dissolved, which suggested a shorter length of the absorption site for griseofulvin and/or a faster rate of passage of the drug in rabbits.

Effect of Water Volume Coadministered

The bioavailability of slightly soluble drugs, such as erythromycin stearate and amoxicillin, was shown to be enhanced by administration together with a large volume of water. The effect of water volume on the bioavailability of griseofulvin tablets A and B in rabbits was investigated using 5 and 50 ml of water. As shown in Tables V and VI, fluid volume did not significantly influence the bioavailabilities after ingestion of either product, as was previously shown in studies of dogs and minipigs.

Food Effect

According to the dosing method by Maeda et al., stomach-emptying controlled rabbits were given drugs followed by immediate intake of a
soft diet, the consumption of which was used as an index of the rabbit's state of health. Bioavailabilities were often influenced by food,\(^{16}\) and that of griseofulvin was shown to be increased due to food intake.\(^{17,18}\) Table VII lists the \textit{in vivo} parameters measured after administration of product B to rabbits with and without food. \(C_{\text{max}}\) and \(AUC_{\infty}\) after oral doses of the drug which was followed by food intake, were 44 and 26\% higher than those without food, respectively. \(T_{\text{max}}\) and \(MRT\) did not differ significantly either with or without food.

Bioavailability in rabbits when orally given griseofulvin tablets 30 min after being fed 50 g

<table>
<thead>
<tr>
<th>TABLE V.</th>
<th>Mean (C_{\text{max}}), (T_{\text{max}}), MRT and (AUC_{\infty}) after Oral Administration of Tablet A (62.5 mg) to Stomach-Emptying Controlled Rabbits (n=6) with 5 and 50 ml of Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water volume</td>
</tr>
<tr>
<td></td>
<td>5 ml</td>
</tr>
<tr>
<td>(C_{\text{max}}) ((\mu g/ml))</td>
<td>0.903±0.115(^a)</td>
</tr>
<tr>
<td>(T_{\text{max}}) (h)</td>
<td>4.4 ±0.6</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>7.7 ±1.9</td>
</tr>
<tr>
<td>(AUC_{\infty}) (h·(\mu g/ml))</td>
<td>4.763±0.483</td>
</tr>
</tbody>
</table>

\(^a\) Means ± SE. \(^b\) NS: not significant at p < 0.1 by one-tailed test.

<table>
<thead>
<tr>
<th>TABLE VI.</th>
<th>Mean (C_{\text{max}}), (T_{\text{max}}), MRT and (AUC_{\infty}) after Oral Administration of Tablet B (62.5 mg) to Stomach-Emptying Controlled Rabbits (n=5) with 5 and 50 ml of Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water volume</td>
</tr>
<tr>
<td></td>
<td>5 ml</td>
</tr>
<tr>
<td>(C_{\text{max}}) ((\mu g/ml))</td>
<td>0.524±0.059(^a)</td>
</tr>
<tr>
<td>(T_{\text{max}}) (h)</td>
<td>2.0 ±0.1</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>4.3 ±0.5</td>
</tr>
<tr>
<td>(AUC_{\infty}) (h·(\mu g/ml))</td>
<td>2.381±0.293</td>
</tr>
</tbody>
</table>

\(^a\) Means ± SE. \(^b\) NS: not significant at p < 0.1 by one-tailed test.

<table>
<thead>
<tr>
<th>TABLE VII.</th>
<th>Mean (C_{\text{max}}), (T_{\text{max}}), MRT and (AUC) after Oral Administration of Tablet B (62.5 mg) to Rabbits (n=7) with and without Food</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Treatment</td>
</tr>
<tr>
<td></td>
<td>With food(^a)</td>
</tr>
<tr>
<td>(C_{\text{max}}) ((\mu g/ml))</td>
<td>0.920±0.173(^c)</td>
</tr>
<tr>
<td>(T_{\text{max}}) (h)</td>
<td>4.1 ±0.8</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>9.1 ±1.0</td>
</tr>
<tr>
<td>(AUC_{31}) (h·(\mu g/ml))</td>
<td>8.227±1.016</td>
</tr>
<tr>
<td>(AUC_{\infty}) (h·(\mu g/ml))</td>
<td>8.232±1.014</td>
</tr>
</tbody>
</table>

\(^a\) 50 g of soft diet was given just after dosing. \(^b\) one tailed significance level. \(^c\) Means ± SE. \(^d\) NS: not significant at p < 0.1.
of the soft diet, was investigated. Figs. 4 and 5 compare the mean serum drug levels between tablets A and B, and B, C and D, respectively. Oral administration of slow dissolving products, C and D, gave rise to an abnormal rise of the serum levels in some rabbits at 31 h after dosing, probably due to the effect of food given 24 h after dosing. Tables VIII and IX list the in vivo parameters. Contrary to the in vivo results after preprandial dose (Table III, Fig. 1), the serum levels at earlier time and the $C_{\text{max}}$ after a postprandial dose of tablet A were higher than those of tablet B, although the differences were not statistically significant due to the large variabili-

**DISCUSSION**

Previous studies investigated the usefulness of beagle dogs and Göttingen minipigs as model animals for bioavailability studies, using griseofulvin as the test drug. Since it is a poorly water soluble compound and because of its long residence time in the gastrointestinal tract, its in vivo

**FIG. 4.** Mean Serum Levels after Oral Administration of Griseofulvin Tablets A (●) and B (○) to Rabbits ($n=7$) 30 min after Food Ingestion

The vertical lines show the standard errors.

**FIG. 5.** Mean Serum Levels after Oral Administration of Griseofulvin Tablets B (○), C (△), and D (□) to Rabbits ($n=6$) 30 min after Food Ingestion

The vertical lines show the standard errors.

**TABLE VIII.** Mean $C_{\text{max}}$, $T_{\text{max}}$ and $AUC_{31}$ after Oral Administration of Griseofulvin Tablets A and B (62.5 mg) to Rabbits ($n=7$) after Food Ingestion

<table>
<thead>
<tr>
<th>Tablet</th>
<th>A</th>
<th>B</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (µg/ml)</td>
<td>1.067 ±0.250$^{a)}$</td>
<td>0.646 ±0.130</td>
<td>NS$^{b)}$</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>6.7 ±4.2</td>
<td>7.5 ±4.0</td>
<td>NS</td>
</tr>
<tr>
<td>$AUC_{31}$ (h·µg/ml)</td>
<td>6.399 ±0.727</td>
<td>5.359 ±1.572</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^{a)}$ Means ± SE. $^{b)}$ NS: not significant at $p < 0.1$. 

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dissolution and absorption is considered to be influenced to a greater extent by the physiological conditions existing in the gastrointestinal tracts of humans and animals than are the more soluble drugs. In addition, the chemically neutral character of the drug can eliminate pH influence on its in vivo dissolution, which simplifies dissolution behavior in the gastrointestinal tract and make it easier to investigate the physiological factors affecting dissolution except for pHs.

The previous studies, however, did not provide satisfactory results. The correlations of the in vivo parameters between humans and dogs were poor \( r = 0.388 \) for \( C_{\text{max}} \) and \( r = 0.306 \) for \( AUC \) due to a relatively higher availability of the ultramicroized formulation in dogs than in humans.\(^{11}\) On the other hand, the correlation between humans and pigs was high \( r = 0.934 \) for \( C_{\text{max}} \) and \( r = 0.937 \) for \( AUC \), but the relative differences in \( C_{\text{max}} \) and \( AUC \) between the product with the highest availability and the others tended to be smaller in pigs than in humans, and the variability of \( C_{\text{max}} \) and \( AUC \) in pigs, as well as in dogs, was larger than that in humans.\(^{2} \) This indicates more difficulty in detecting bioavailability difference between products in minipigs G than in humans. Those unsatisfactory results necessitate further study on other model animals.

Rabbits have not been considered to be useful for bioavailability studies because of slow gastric emptying times.\(^{19}\) For example, the rabbits after an oral dose of griseofulvin exhibited a prolonged plateau in the plasma concentration–time curves in a fasting state.\(^{20}\) However, Maeda et al. has developed a new technique for promoting the stomach emptying rate of rabbits, which is promising to be useful for drug absorption studies.\(^{8,4} \) In his study, there was a high correlation in the mean plasma levels from three experimental griseofulvin tablets prepared in their laboratory between humans and rabbits.\(^{40} \) In the procedure by Maeda et al., drug administration is designed to be followed by feeding. Food, however, influences the in vivo dissolution and absorption of drugs in various manner.\(^{16} \) Thus, the effect of food in stomach-emptying controlled rabbits was investigated.

The bioavailability of griseofulvin administered with food tended to be higher than that without food (Table VII), which suggested that food increased the in vivo dissolution and absorption of griseofulvin in the rabbits as previously shown in humans.\(^{17,18} \) The drug level in the serum rose abnormally at 31 h in some animals, when the slow dissolving products were administered after feeding (Fig. 5). The drug given after feeding will have a tendency to remain in the digestive tract longer than that given prior to feeding, and dissolution and absorption of the remaining drug will be again enhanced by food given at 24 h after dosing. While, the drug given immediately prior to feeding, according to the dosing method of Maeda et al.,\(^{3} \) might move together with the food through the gastrointestinal tract, and thus, the food seemed to control the transition rate of the drug through the tract of the stomach-emptying controlled rabbits.
The bioavailability of an ultramicrosize griseofulvin tablet (A) was relatively higher than that of tablet B when the drug was administered after feeding (Table VIII) but lower when administered prior to feeding (Table III). The differences in $C_{\text{max}}$ and $AUC_{31}$ of the ultramicrosize product between pre- and post-prandial doses were significant by Student's $t$-test at $p < 0.1$ and $p < 0.05$, respectively. In vitro, tablet A had tendency to form a paste-like agglomerate in the presence of small quantity of water which did not disperse well in the dissolution medium and led to its relatively slow dissolution rate (method I). Food given prior to the drug administration probably promoted the disintegration and dissolution of the ultramicrosize product but not that given after the drug. The promoting effect of food on the disintegration of tablets was also observed in a chloramphenicol study involving humans.\(^\text{21}\)

The correlations of $C_{\text{max}}$ and $AUC$ between humans and stomach-emptying controlled rabbits were high (Fig. 2) and the relative differences in $C_{\text{max}}$ and $AUC$ between the product with the highest availability and the others tended to be larger in rabbits than in humans (Fig. 3). However, the variability of the in vivo parameters in the rabbits, as well as in other animals, especially that of $C_{\text{max}}$ was larger than those in humans (Table IV). This suggests that even though the relative differences of $AUC$ and $C_{\text{max}}$ between products were larger in rabbits than in humans, the difference in rabbits may not be statistically significant unless many rabbits were used—more than three times the number of human subjects. The great variation of $C_{\text{max}}$ in stomach emptying controlled rabbits might be due to the variation of their gastric emptying rate, namely, even if the gastric emptying of rabbits was artificially improved by the procedure of gastric lavage and using a cangue, their gastric emptying rate might not be completely controlled, which seems to be more variable than that of humans, dogs and minipigs. Also, the increase of the body weight of rabbits during the experiment might contribute to the variation of the parameters. The variabilities of the in vivo parameters in rabbits will be, however, able to be decreased by improvement of the experimental conditions, since, in the rabbit study, a skill of gastric lavage of rabbits and a stress which would be given to rabbits due to the forced drug administration must affect the gastric emptying and the in vivo absorption of the drug, which leads to the variation of the in vivo parameters.

The successive studies on the relation of griseofulvin bioavailability between humans and animals lead to the following conclusion. Minipigs G and stomach-emptying controlled rabbits may be useful to predict relative bioavailabilities of an ultramicrosize griseofulvin tablet and microsize ones in humans, judging from the high correlation in the bioavailability between humans and those animals for the four products. While the use of beagle dogs may be restricted to microsize formulations only. Furthermore, those animals including rabbits have a disadvantage for bioequivalence testing from the view of a sample size, because at least two to six-fold number of the animals than that of humans are required to attain the same statistical power for $C_{\text{max}}$ or $AUC$.

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