EFFECT OF FOOD ON BIOAVAILABILITY OF NALIDIXIC ACID FROM UNCOATED TABLETS HAVING DIFFERENT DISSOLUTION RATES

HIROYASU OGATA, NOBUO AOYAGI, NAHOKO KANIWA AND AKIRA EJIMA

Division of Drugs, National Institute of Hygienic Sciences, 18-1 Kamiyoga 1-chome, Setagaya-ku, Tokyo, 158, Japan

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The effect of food on the bioavailability of two single lots of commercial tablets and one trial tablet of nalidixic acid, varying widely in drug dissolution characteristics, was determined in healthy male subjects after oral administration. Four subjects received, in a crossover fashion, a single 250 mg dose in an uncoated tablet during periods of fasting and non-fasting. Significant differences in $C_{\text{max}}$, $T_{\text{max}}$ and $AUC_{0-8}$ were observed between the tablet having the fastest dissolution rate and the other tablets tested, when administered postprandially. Both $C_{\text{max}}$ and $AUC_{0-8}$ were significantly increased after administration of the two tablets exhibiting the poorer dissolution characteristics to the non-fasting subjects. However, food was not observed to affect any of the bioavailability parameters after postprandial administration of the tablet exhibiting the fastest dissolution rate. The improvement of bioavailability of the two tablets exhibiting the poorer dissolution by food intake was ascribable to enhanced dissolution of nalidixic acid resulting from vigorous mixing and agitation in the digestive tract. It was concluded that the effect of food on the bioavailability of nalidixic acid from uncoated tablets varies with formulation factors, especially with dissolution characteristics.

Keywords—nalidixic acid; bioavailability; human; effect of food; uncoated tablet; dissolution rate; pharmacokinetics

A large number of studies concerning the effects of food on drug bioavailability have been performed, which provide us with much information for considering the optimal time for drug administration relative to food ingestion. However, it seems very difficult to generalize the results from most of these studies since only one drug product is given without any information as to its pharmaceutical characteristics or whether it was administered with or without a standardized meal. The effect of food on drug bioavailability varies with the composition and volume of the meal and drink. The bioequivalence among products containing the same drug has received attention recently from the view point of clinical efficacy and safety, which is surely related to pharmaceutical characteristics such as dissolution and disintegration. It is reasonably assumed, therefore, that the effect of food on drug bioavailability is also dependent on formulation factors. Unfortunately, however, these factors have scarcely received any attention in studies concerning the effect of food.

Chloramphenicol bioavailability was enhanced as much as two times in non-fasting subjects as compared with fasting subjects after oral administration of a tablet having poor in vitro disintegration at pH 4, although bioavailability from the powder was not affected by food at all. Griseofulvin is one of the most well-known drug in which bioavailability is extensively enhanced by food, especially food high in fat. Crouse showed that a 1 g dose of griseofulvin achieved nearly ten times higher plasma levels when administered after fat ingestion than after fasting. However, the same blood levels of griseofulvin, as those obtained when administered after ingestion of fat in Crouse's study, were attained after administration of a Japanese commercial product containing only one eighth of
that dose (125 mg) to fasting subjects, and the area under serum concentration (AUC) was almost the same between fasting and non-fasting states. It was also suggested that nitrofurantoin bioavailability studies are required to be performed in both non-fasting and fasting subjects since the effect of food on absorption of five commercial dosage forms of nitrofurantoin varied widely in drug release and dissolution characteristics. These results represent the fact that the effect of food on drug bioavailability cannot be evaluated precisely without consideration of the formulation.

In this paper, we studied the effect of food on nalidixic acid bioavailability from two commercial tablets and one trial tablet having different dissolution rates. Nalidixic acid is a weak acid and presumed to have a bactericidal activity. The bioavailability of nalidixic acid from plain tablets in the fasting state has been studied and dissolution was shown to be a rate-limiting factor. The effect of food on bioavailability has not been studied, although it is of importance to clinical therapeutics.

EXPERIMENTAL

Materials — Two single lots of commercial nalidixic acid (250 mg) uncoated tablets designated as tablets A and B, and a slow-release tablet (tablet C) prepared in the laboratory, were used. Other reagents used were of analytical grade.

In Vitro Dissolution Study — The in vitro dissolution rate was determined according to two different methods: oscillating basket (OB) and beaker (BE), both using 1000 ml of pH 7.2 phosphate buffer (0.1 M). The dissolution medium was circulated through a flow-cell (0.5 cm cell length) by a micro-tube pump at 3.0 ml/min, and the absorbance of the drug was determined spectrophotometrically at 355 nm. The percent dissolved was calculated against the labeled amount.

Bioavailability Study — Four healthy male volunteers (22—52 years, 54—67 kg) participated in the study. According to a randomized block design, the subjects received a tablet along with 200 ml of water. In the fasting experiment, the subjects fasted for a period lasting from 10 h before and until 4 h after taking the drug. A standard breakfast consisting of 100 g of bread, 20 g of butter, 35 g of cucumber, 200 ml of milk and a boiled egg was consumed 15 min before drug administration. No food or liquids were permitted until 4 h after drug administration in the non-fasting experiment. Blood samples (5 ml) were collected at 0.5, 1, 2, 3, 4, 5, 6.5 and 8 h after drug administration and serum samples were stored in a freezer (−15°C) until assayed. The tests were repeated after intervals of at least 10 d.

Pharmacokinetic Analysis — Serum level versus time data were fitted to a one-compartment open model using PKM-MULTI program including the Simplex nonlinear square algorithm. The program was adapted to a PC-9801 (NEC, Tokyo) microcomputer. The pharmacokinetic fitting was performed without weighting, and the selection of appropriate model was based on AIC (Akaike’s Information Criterion) value. The models investigated were as follows: model I: one compartment with first order absorption process without lag time, model II: one compartment with first order absorption process with lag time, model III: one compartment with discontinuous two segment first order absorption processes without lag time, model IV: one compartment with discontinuous two segment first order absorption processes with lag time, model V: one compartment with abrupt absorption end before absorption ceases without lag time, model VI: one compartment with abrupt absorption end before absorption ceases with lag time.

Assay — Nalidixic acid in serum was determined by the high performance liquid chromatography (HPLC) method reported previously. Briefly, 1.0 ml of 0.03N NaOH, 0.5 ml of 0.6N HCl, 1.0 g of NaCl and 6.0 ml of chloroform were added to 1.0 ml of serum. After shaking for 10 min and centrifuging, nalidixic acid was then extracted from 4.0 ml of the organic phase using 1.0 ml of 0.03N NaOH containing piromidic acid (25 µg/ml) as an external standard.
The aqueous phase (20 μl) was subjected to HPLC. Conditions for the HPLC system were as follows: column—Hitachi Gel 3011N strong anion exchange resin (10 μm, 3.0 mm × 25 cm, Hitachi Ltd., Tokyo); monitor—335 nm wavelength (UVIDEOC-100, Japan Spectroscopic Co., Ltd., Tokyo); mobile phase—0.09% w/v of boric acid (pH 9.2), 1.3% w/v of NaCl, 0.05% w/v of sodium benzoate and 20% v/v of acetonitrile, flow rate—0.3 ml/min.

RESULTS AND DISCUSSION
Table I presents the time required to achieve 50% dissolution (T50) of nalidixic acid at pH 7.2. Dissolution of nalidixic acid from tablet A was rapid in both test methods, probably because of the rapid disintegration into fine particles. Tablet C was intentionally prepared to disintegrate into coarse particles and release nalidixic acid slowly. Dissolution rate of nalidixic acid from tablet C using the BE method was about twice that obtained by using the OB method, which is very similar to the result that benzoic acid dissolved from non-disintegrating tablet twice faster in the BE method than in extra-cylinder position of OB method.9) On the other hand, dissolution of nalidixic acid from tablet B was greatly reduced using the BE method as compared with the OB method. Tablet B did not disintegrate into small particles using the BE method but into relatively large flakes, which is suspected as the reason for the reduced dissolution rate. The OB method, however, ground the tablet into small pieces with the help of disk.

Figs. 1—4 shows the individual serum concentration of nalidixic acid versus time data and the simulated curves after oral administration during fasting and non-fasting states. Fig. 5 shows the mean serum concentration-time curves of each tablet. Table II shows the mean values of the bioavailability parameters, peak serum concentration (Cmax), time to the peak (Tmax) and area under serum concentration-time curve from 0 to 8 h (AUC0-8). Cmax and Tmax were observed values and AUC0-8 was calculated using the trapezoidal rule. Statistical analysis was performed by paired one-tail t-test.

As nalidixic acid is a weakly acidic compound (pKa = 5.9)12) and has very low solubility in a pH range between 1 to 5 (ca. 40 μg/ml10), it is reasonable to believe that nalidixic acid dissolves and is absorbed mainly in the small intestine, and that the tablet only disintegrates in the stomach. Therefore gastric emptying, in addition to dissolution, is one of the rate-limiting factors for the absorption.

Food intake is reported to retard gastric emptying of the disintegrated tablet, though the delay is dependent upon the particle size resulting from formulation of the solid preparation administered.13,14) The serum concentration of nalidixic acid showed two phases after tablets B and C were given to fasting subjects (Figs. 1—4). The similar phenomenon was observed in sulfisoxazole,15) sulfamethoxazole16) acetaminophen17) and propanol,18) which were pharmakokinetically analyzed by discontinuous two segment absorption model11,16—19) The phenomenon can be explained that the drug may be emptied from the stomach discontinuously according to the particle size distribution and/or stomach peristalsis. As shown in Figs. 1—4, serum level of nalidixic acid showing two phases was best fitted with discontinuous two segment absorption model.

Tmax was delayed by 0.75 to 1.5 h (Table I) and serum level, 0.5 h after drug administration, decreased to about one third when tablet A was administered to non-fasting as compared with fasting subjects (Fig. 5), although this was not significant. On the other hand, Tmax in tablets B and C became rather short in non-fasting as compared

<table>
<thead>
<tr>
<th>Tablet</th>
<th>T50 (min)</th>
<th>OB</th>
<th>BE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.9</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>9.7</td>
<td>107.1</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>77.0</td>
<td>389</td>
<td></td>
</tr>
</tbody>
</table>
FIG. 1. Serum Concentration of Nalidixic Acid as a Function of Time after Oral Administration of a Tablet to Subject SA during Fasting (F: ◊) and Non-fasting (NF: ♦) States

Open or solid squares represent experimental data, and the curve is the fit to a one-compartment open model. The best fitting model is as follows: tablet A in F—model IV; tablet A in NF—model II; tablet B in F—model IV; tablet B in NF—model IV; tablet C in F—model III; tablet C in NF—model II.

FIG. 2. Serum Concentration of Nalidixic Acid as a Function of Time after Oral Administration of a Tablet to Subject AO during Fasting (◊) and Non-fasting (♦) States

The best fitting model is as follows: tablet A in F—model II; tablet A in NF—model II, tablet B in F—model IV; tablet B in NF—model II, tablet C in F—model III; tablet C in NF—model III.
FIG. 3. Serum Concentration of Nalidixic Acid as a Function of Time after Oral Administration of a Tablet to Subject SH during Fasting (◇) and Non-fasting (⦿) States

The best fitting model is as follows: tablet A in F—model I; tablet A in NF—model IV, tablet B in F—model III; tablet B in NF—model IV, tablet C in F—model IV; tablet C in NF—model IV.

FIG. 4. Serum Concentration of Nalidixic Acid as a Function of Time after Oral Administration of a Tablet to Subject OG during Fasting (◇) and Non-fasting (⦿) States

The best fitting model is as follows: tablet A in F—model II; tablet A in NF—model II, tablet B in F—model I; tablet B in NF—model I, tablet C in F—model IV; tablet C in NF—model I.
with fasting subjects (Table II), probably because the particles were ground into the smaller ones in the stomach and were enhanced to dissolve in the small intestine by the food. It was also speculated that the particle sizes resulting from tablets B and C were still fairly large with slow dissolution rate compared to tablet A even when the particles were mixed with food, as the large \( T_{\text{max}} \), ca. 3 h, reflects (Table II). It is of interest that the absorption occurred abruptly after a lag time from tablet B postprandially given, although the drug seemed to be absorbed gradually with two-segment absorption in fasting state (Figs. 1-4). On the other hand, the drug seemed still to have controlled absorption rate from tablet C following postprandial administration, although the rate was enhanced (Figs. 1-4).

\( C_{\text{max}} \) of tablets B and C were significantly increased when administered postprandially, although they were still lower than that of tablet A (Table II). \( AUC_{0-\infty} \) was also enhanced when tablets B and C were administered to non-fasting subjects, especially tablet C which increased by approximately two-fold (Table II). The fact that the \( AUC_{0-\infty} \) produced by tablet A was almost constant using both administration conditions suggests that there were no interactions between the drug and food constituents such as those observed with tetracycline\(^{20}\) acetaminophen\(^{21}\) and zinc.\(^{22}\) Therefore, it was concluded that the bioavailability of nalidixic acid is not affected by food when drug release from the dosage form is rapid. The improvements of \( C_{\text{max}} \) and \( AUC_{0-\infty} \) for tablets B and C, administered postprandially, are probably due to enhanced dissolution of the drug resulting from vigorous agitation in the digestive tract. The abrupt increase of serum concentration of nalidixic acid from tablet B administered postprandially may suggest that the large flakes resulting from the disintegration of tablet B were ground into sufficiently small particles by the food for dissolving rapidly in the proximal intestine after the gastric emptying.

Significant differences were detected between tablet A and the other two formulations in the fasting state, while there were no significant differences among the three tablets under non-fasting conditions. If this experiment had been performed using tablets B or C alone, quite different conclusion concerning the effect of food on bioavailability may have been drawn. Such discrepancies relating to the effect of food on drug bioavailability are often reported.\(^{1-3}\) It is assumed that the discrepancies are probably due to

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**FIG. 5. Mean Serum Concentration of Nalidixic Acid after Oral Administration of Nalidixic Acid (250 mg) Uncoated Tablets to Fasting (○) and Non-fasting (△, △) Subjects**
### TABLE II. Effect of Food on Bioavailability Parameters of Nalidixic Acid after Oral Administration of Uncoated Tablets

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tablet</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{\text{max}} ) (( \mu \text{g/ml} ))</td>
<td>A Fasting</td>
<td>6.91 N.S.</td>
</tr>
<tr>
<td></td>
<td>Nonfasting</td>
<td>8.73</td>
</tr>
<tr>
<td></td>
<td>B Fasting</td>
<td>2.61 p &lt; 0.005</td>
</tr>
<tr>
<td></td>
<td>Nonfasting</td>
<td>2.61 p &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>C Fasting</td>
<td>5.84 p &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Nonfasting</td>
<td>1.84 p &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.51</td>
</tr>
<tr>
<td>( T_{\text{max}} ) (h)</td>
<td>A Fasting</td>
<td>0.75 N.S.</td>
</tr>
<tr>
<td></td>
<td>Nonfasting</td>
<td>1.50</td>
</tr>
<tr>
<td></td>
<td>B Fasting</td>
<td>3.50 p &lt; 0.005</td>
</tr>
<tr>
<td></td>
<td>Nonfasting</td>
<td>3.00 p &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>C Fasting</td>
<td>3.25 N.S.</td>
</tr>
<tr>
<td></td>
<td>Nonfasting</td>
<td>3.00</td>
</tr>
<tr>
<td>( AUC_{0-8} ) (( \mu \text{g} \cdot \text{h/ml} ))</td>
<td>A Fasting</td>
<td>18.04 N.S.</td>
</tr>
<tr>
<td></td>
<td>Nonfasting</td>
<td>16.54</td>
</tr>
<tr>
<td></td>
<td>B Fasting</td>
<td>10.75 N.S.</td>
</tr>
<tr>
<td></td>
<td>Nonfasting</td>
<td>13.25 N.S.</td>
</tr>
<tr>
<td></td>
<td>C Fasting</td>
<td>8.68 p &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Nonfasting</td>
<td>16.30</td>
</tr>
</tbody>
</table>

N.S.: not significant (p > 0.05).

Differences in the pharmaceutical characteristics tested, as observed in this paper and our previous series of experiments,\(^4,6\) in addition to differences in food content and water volume. Most of the reports, however, do not describe the pharmaceutical characteristics. The pharmaceutical characteristics of a dosage form should be clarified whenever an experiment testing the effect of food on bioavailability is performed.

As it is assumed that a serum level of more than 0.5 to 5 \( \mu \text{g/ml} \) following rapid absorption is more important clinically than minimum serum levels for long duration of time,\(^6\) rapid serum level elevation is preferable. Preprandial administration of a tablet having rapid dissolution will surely bring therapeutic efficacy.

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**REFERENCES**


