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Bioavailability of metronidazole from sugar-coated tablets in humans. I. Effect of gastric acidity and correlation with in vitro dissolution rate

H. Ogata [1], N. Aoyagi [1], N. Kaniwa [1], T. Shibazaki [1], E. Ejima [1], Y. Takagishi [2], T. Ogura [2], K. Tomita [2], S. Inoue [2] and M. Zaizen [3]

[1] Division of Drugs, National Institute of Hygienic Sciences, 18-1 Kamiyoga 1-chome, Setagaya-ku, Tokyo 158; [2] Production Division; and [3] Kaiseki Center, Shionogi and Co. Ltd., 192 Imafuku, Amagasaki, Hyogo (Japan)

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Summary

The bioavailability of five commercial sugar-coated metronidazole tablets was studied. The dissolution rates of the five test tablets were determined by rotating flask, oscillating basket, rotating basket and paddle methods at pH values of 1.2, 3, 4, 5, 6 and 7.2. Though metronidazole dissolves easily in water, with a solubility of 11.6 mg/ml at a pH of 7, some sugar-coated tablets showed very slow dissolution at a pH of 5 or 7.2, in spite of very rapid dissolution at a pH of 1.2. This may be due to the pH-dependent dissolution of their water-proof coatings. The bioavailability of metronidazole from three tablets showing extremely slow dissolution at pH 5 or 7.2 varied significantly between human subjects having high and low gastric acidity. However, correlation between bioavailability and dissolution rate were shown to be poor.

Introduction

Metronidazole, 1-(2′-hydroxyethyl)-2-methyl-5-nitroimidazole, has for many years been used in the treatment of amebiasis, giardiasis, trichromoniasis and acute

Correspondence: H. Ogata, Division of Drugs, National Institutes of Hygienic Sciences, 18-1 Kamiyoga 1-chome, Setagaya-Ku, Tokyo 158, Japan.

ulcerative gingivitis (Templeton, 1977). There have been reports of failure in therapy (Kane et al., 1961; McFadzean et al., 1969) associated with low blood levels, either due to ineffective absorption (Kane et al., 1961) or because of rapid metabolism (Stambaugh et al., 1968). In previous studies, poor bioavailability of commercial metronidazole preparations was suspected (McGilveray et al., 1978), though metronidazole is a weak base and dissolves easily in water. However, the commercial tablets were not significantly different from the reference product, and the considerable inter-subject differences in plasma metronidazole concentrations were ascribed to the individual elimination half-life differences (McGilveray et al., 1978).

In our previous paper, the inter-subject differences in diazepam bioavailability was ascribed to the gastric acidity differences of the subjects (Ogata et al., 1982). The poor bioavailability of chloramphenicol from a sugar-coated tablet was due to the very slow disintegration of the water-proof coating in a pH range between 3 and 7, although disintegration was rapid at pH 1.2 (Ogata et al., 1979b). A bioavailability study of commercial preparations after oral administration to subjects having high gastric acidity and low gastric acidity would provide useful information regarding the poor absorption of metronidazole.

The present paper describes a comparison of the bioavailability of five commercial sugar coated tablets, the effect of gastric acidity on the bioavailability and correlation of in vivo findings and in vitro dissolution rates.

Materials and Methods

Materials

A total of 5 preparations of sugar-coated tablets of metronidazole (250 mg) were obtained from manufacturers in Japan. Dimetridazole (BASF Japan) was recrystallized from water. Other reagents used were of analytical grade.

Solubility

The solubility of metronidazole at pH 1.0 (Clark-Lubs buffer), 1.2 (the 1st fluid, JP IX disintegration test), 3.0 (McIlvaine buffer), 5.0 (McIlvaine buffer), 7.0 (McIlvaine buffer) and 7.5 (the 2nd fluid, JP VIII disintegration test) was determined at 37°C according to the procedure reported previously (Ogata et al., 1979c).

Metronidazole content

Tablets were analyzed for metronidazole after grinding 10 tablets, and dissolving the powder corresponding to 250 mg of metronidazole in methanol. After filtration, the filtrate was diluted with methanol. After addition of dimetridazole as an external standard to the solution, 20 μl was subjected to HPLC using the same system and conditions as those described under Assay.

Disintegration time

Disintegration times were determined according to JP IX specifications at pH 1.2 (the 1st fluid, JP IX disintegration test), 5.0 (0.1 M potassium phosphate monobasic) and 7.2 (0.1 M sodium phosphate dibasic).

In vitro dissolution study

The dissolution rates of metronidazole from tablets were determined at 37°C by the methods previously reported, i.e. using an oscillating basket with a disk (1000 ml of the dissolution medium; OB) (Ogata et al., 1979a), a rotating basket (120 rpm, 900 ml of the medium; RB) (Ogata et al., 1979a), a rotating flask (6.8 rpm, 1000 ml of the medium; RF) (Ogata et al., 1979a) and a paddle (120 rpm, 900 ml of the medium; PD) (Ogata et al., 1984). The pHs of the dissolution media used were 1.2 (the 1st fluid, JP IX disintegration test), 3.0 (0.1 M acetate buffer), 4.0 (0.1 M acetate buffer), 5.0 (0.1 M potassium phosphate monobasic) and 6.0 (0.1 M potassium phosphate monobasic) and 7.2 (0.1 M sodium phosphate dibasic). The procedure for dissolution rate determination was the same as that reported previously (Ogata et al., 1979a).

In vivo study

Subjects. Thirteen healthy male volunteers (22–54 years; mean 33.0 years, 51–78 kg; mean 62.1 kg) participated in the studies. The gastric fluid acidity of each subject was determined after oral administration of Gastrotest tablets (Chugai Pharmaceuticals, Tokyo), which contain a protein-bound dye (3-phenylazo-2,6-diaminopyridine) that is released in the stomach by acid at a pH of 3 or less (Bianchetti et al., 1958). The amount of dye excreted in the urine in 90 min after oral administration of the tablets gives an indication of the gastric acidity. An absorbance of less than 0.170 at 520 nm was interpreted as indicating hypo- or anacidity, whereas an absorbance of more than 0.170, as indicating normal or hyperacidity. The details of procedures were reported previously (Ogata et al., 1982). The subjects evaluated as hypo- or anacidic and as normal or hyperacidic by Gastrotest were designated as low and high, respectively.

Treatment schedule

Study I. The bioavailability of metronidazole in 5 preparations was studied using a Latin square design with 5 groups of two subjects each (subject Nos. 1–10). Each subject received a 250 mg metronidazole tablet orally with 200 ml of water every 2 weeks. The subjects fasted for a period beginning from 10 h before until 4 h after taking the drug. Blood samples (5 ml) were collected at 1, 2, 3, 4, 6, 8, 24 and 32 h after drug administration, and the serum samples were stored in a freezer (−15°C) until assayed.

Study II. Three subjects (Nos. 11–13) participated in the study on the relation between the dose of metronidazole and AUC (area under serum concentration–time curve). They were given 100, 200 and 300 mg of metronidazole powder according to the procedure described for Study I.

Assay

Metronidazole in the serum was assayed by HPLC. To 0.5 ml of serum was added 0.5 ml of pH 9.2 carbonate (0.1 M) buffer and 7.0 ml of a mixed organic solvent (ether–dichloromethane, 1.5 : 1 v/v). After shaking for 10 min and centrifuging, 6.0 ml of the organic layer was separated and evaporated to dryness under a nitrogen

current at 35°C. The residue was dissolved with 200 μl of methanol containing dimetridazole (10 μg/ml) as an external standard. A sample of 20 μl of the methanol solution was subjected to HPLC at room temperature. The HPLC system consisted of a TSK GEL LS-410 (ODS) column (5 μm, 4 mm × 250 mm; Toyo Soda), a pump (LC-3A; Shimadzu Seisakusho), a variable UV-spectrophotometer (SPD-1; Shimadzu Seisakusho), an injector (SIL-1A; Shimadzu Seisakusho) and a recorder (R-112; Shimadzu Seisakusho). The mobile phase was 15% (v/v) acetonitrile in a 0.1 M potassium phosphate monobasic solution, pumped at 0.7 ml/min and monitored at 324 nm. The lower limit of the assay was 0.1 μg/ml of metronidazole in the serum. The recovery of metronidazole from the serum sample following the extraction procedure was complete.

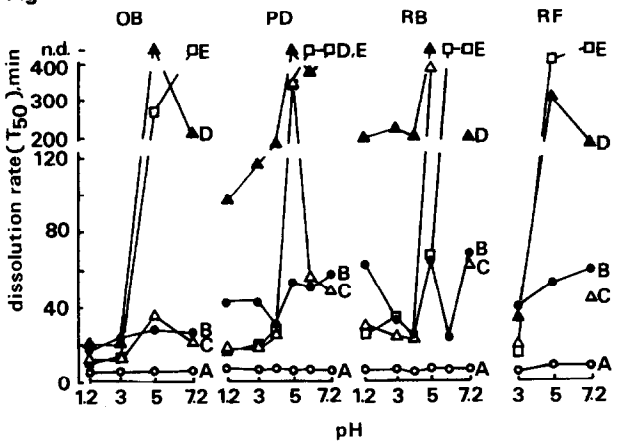
Results

Solubility

Metronidazole dissolves very easily in the acidic pH region (30.6 mg/ml at pH 1.0) and shows about 12 mg/ml solubility even in a pH range between 5 and 7 (14.1 mg/ml, 12.8 mg/ml and 11.6 mg/ml at pH 3.0, 5.0 and 7.0, respectively).

In vitro dissolution rate and disintegration time

Fig. 1 shows the dissolution rates of the 5 tablets at various pH conditions. Tablet A showed pH-independent dissolution and the fastest rate of dissolution with all methods applied. Tablet B also did not show clear pH-dependent dissolution although its dissolution was slower than that of tablet A. Tablets C and D dissolved



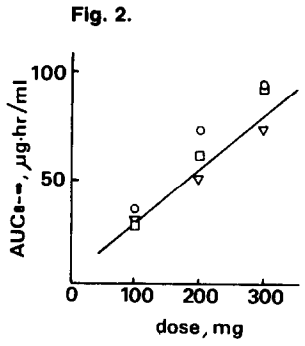


Fig. 1. Effect of pH on dissolution rates of metronidazole from 5 sugar-coated tablets. n.d. represents less than 50% dissolution within an 8-h experiment.

Fig. 2. Relationship between dose of metronidazole and the resulting $AUC_{0-\infty}$ after oral administration of metronidazole powder to humans. Same symbol represents response of the same individual.

at a very slow rate at pH 5. Tablet E did not dissolve at all at pH 7.2 regardless of the dissolution test method. As shown in Table 1, the disintegration times of the five tablets correlated with their dissolution rates with respect to the pH-dependencies. This suggests that the dissolution of metronidazole from sugar-coated tablets is mainly limited by the disintegration rate of the water-proof coating of the tablet.

Relationship between metronidazole dose and AUC

The relationship between the dose of metronidazole and the resulting $AUC_{0-\infty}$ (area under serum concentration–time curve, 0 to infinity) calculated according to Wagner (1975b) is shown in Fig. 2. The regression line passed near the origin, suggesting that the serum levels of metronidazole in humans can be treated with linear pharmacokinetics within the dose range studied, which agrees well with the findings of Amon et al. (1978).

Drug content

Metronidazole contents are 100.4, 100.5, 105.6, 101.6 and 101.8% of the labelled amount in tablets A, B, C, D and E, respectively.

Bioavailability

The mean peak concentration (C_{max}), the time to the peak concentration (T_{max}), AUC_{0-32}, $AUC_{0-\infty}$ and the corrected AUC value ($AUC_{0-\infty}$(corr)) (Wagner, 1975c) are summarized in Table 2, and the mean serum concentration–time curves are shown in Fig. 3. Subject No. 4 gave a little higher level of metronidazole at 32 h than at 24 h after tablet C administration probably because of the very slow absorption. Therefore, the mean elimination rate constant for the terminal phase was determined from the other 4 preparations given to subject No. 4 in order to estimate the $AUC_{0-\infty}$ for tablet C. The resulting error does not seem large due to the relatively low serum levels at 24 and 32 h. However, $AUC_{0-\infty}$ for tablets C, D and E may be subject to potentially significant extrapolating errors because of the slow absorption occasionally observed. Results of ANOVA (analysis of variance) (Wagner, 1975a) are also shown in Table 2. All of the parameters listed showed significant differences among the tablets tested. The results of pairwise comparisons among the tablets by the Studentized LSD (least significant differences) test (Okuno et al., 1969) are also

TABLE 1

DISINTEGRATION TIMES OF METRONIDAZOLE TABLETS

pH	Disintegration time (min)				
	Tablet A	Tablet B	Tablet C	Tablet D	Tablet E
1.2	6.4	21.8	13.0	27.1	9.5
5.0	6.3	40.9	102.5	n.d. [a]	n.d.
7.2	6.2	35.0	17.1	78.4	n.d.

[a] Not disintegrated within 3 h.

TABLE 2

PHARMACOKINETIC PARAMETERS OF METRONIDAZOLE

Parameter	Mean ± S.E.					Results of ANOVA		LSD test for formulation
	Tablet A	Tablet B	Tablet C	Tablet D	Tablet E	Formulation	Subject	
C_{max} (μg/ml)	5.56 ± 0.24	5.24 ± 0.36	2.39 ± 0.43	3.11 ± 0.70	2.81 ± 0.73	$P < 0.001$	N.S.	A > B > D > E > C
AUC_{0-32} (μg·h/ml)	71.70 ± 3.46	72.82 ± 5.28	43.24 ± 7.03	46.05 ± 9.90	41.73 ± 10.67	$P < 0.005$	$P < 0.05$	B > A > D > C > E
$AUC_{0-\infty}$ (μg·h/ml)	81.04 ± 5.03	84.54 ± 7.67	56.38 ± 9.41	56.00 ± 12.02	49.26 ± 12.39	$P < 0.025$	$P < 0.01$	B > A > C > D > E
$AUC_{0-\infty}$(corr) [a]	1.43 ± 0.07	1.49 ± 0.10	0.83 ± 0.09	0.98 ± 0.17	0.87 ± 0.20	$P < 0.025$	N.S.	B > A > D > E > C
T_{max}(h)	2.1 ± 0.23	3.9 ± 0.41	12.2 ± 3.30	(9.7 ± 2.8) [b]	(10.3 ± 4.0) [c]			

[a] $AUC_{0-\infty} \cdot k_{(\beta)}(h^{-1}) \cdot$ body weight (kg)/dose (mg).

[b] n = 9.

[c] n = 8.

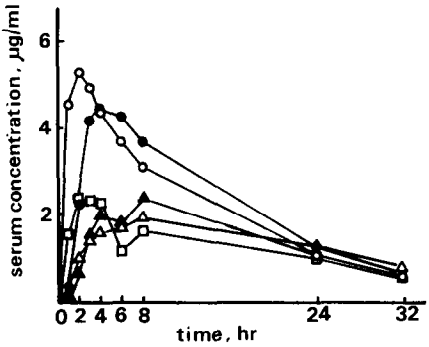


Fig. 3. Mean serum concentration of metronidazole after oral administration of metronidazole (250 mg) tablets, tablet A (○), tablet B (●), tablet C (△), tablet D (▲) and tablet E (□).

shown in Table 2. The mean values for tablets not underscored by the same line in Table 2 differed significantly ($P < 0.05$). The mean T_{max}s for tablets D and E were not listed in Table 2 because the T_{max} in 3 of the trials, tablet D to subject No. 9 and tablet E to subjects No. 6 and No. 10, could not be determined because of no evidence of metronidazole absorption. Therefore, ANOVA for T_{max} applying a complete block design could not be performed.

Very large differences in bioavailability were observed though the dissolution rate

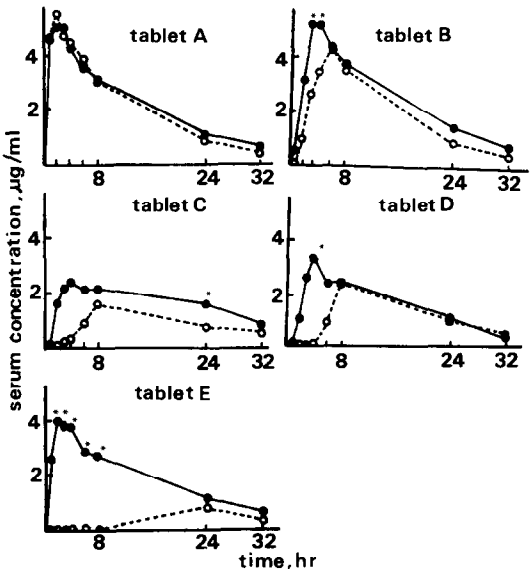


Fig. 4. Mean serum concentration of metronidazole after oral administration of metronidazole (250 mg) tablets to human subjects having high gastric acidity (●) and low gastric acidity (○). The asterisk represents a significantly different serum level of the high acidity group from the low acidity group serum level.

TABLE 3

PHARMACOKINETIC PARAMETERS OF METRONIDAZOLE IN HUMANS HAVING HIGH GASTRIC ACIDITY

Parameter	Mean ± S.E.					Results of ANOVA		LSD test for formulation
	Tablet A	Tablet B	Tablet C	Tablet D	Tablet E	Formulation	Subject	
C_{max} (μg/ml)	5.35 ± 0.17	5.52 ± 0.39	2.39 ± 0.61	3.64 ± 0.94	4.18 ± 0.74	$P < 0.05$	N.S.	B > A > E > D > C
$AUC_{0-\infty}$ (μg·h/ml)	85.69 ± 4.90	95.79 ± 7.22	73.23 ± 9.96	63.95 ± 15.39	73.78 ± 12.11	N.S.	N.S.	
$AUC_{0-\infty}$(corr) [a]	1.38 ± 0.08	1.51 ± 0.15	0.96 ± 0.11	1.02 ± 0.23	1.21 ± 0.20	$P < 0.05$	$P < 0.05$	B > A > E > D > C
T_{max} (h)	2.3 ± 0.33	3.2 ± 0.31	11.0 ± 4.20	(7.8 ± 4.1) [b]	6.0 ± 3.62			

[a] $AUC_{0-\infty} \cdot k_{(\beta)}(h^{-1}) \cdot$ body weight(kg)/dose(mg).
[b] n = 5.

TABLE 4

PHARMACOKINETIC PARAMETERS OF METRONIDAZOLE IN HUMANS HAVING LOW GASTRIC ACIDITY

Parameter	Mean ± S.E.					Results of ANOVA		LSD test for formulation
	Tablet A	Tablet B	Tablet C	Tablet D	Tablet E	Formulation	Subject	
C_{max} (μg/ml)	5.89 ± 0.55	4.83 ± 0.71	1.57 ± 0.33	2.33 ± 1.04	0.76 ± 0.53	$P < 0.001$	N.S.	A > B > D > C > E
$AUC_{0-\infty}$ (μg·h/ml)	74.06 ± 10.12	67.66 ± 12.36	31.11 ± 7.87	44.08 ± 20.30	13.53 ± 8.68	$P < 0.01$	N.S.	A > B > D > C > E
$AUC_{0-\infty}$(corr) [a]	1.49 ± 0.11	1.46 ± 0.16	0.64 ± 0.13	0.94 ± 0.26	0.35 ± 0.24	$P < 0.005$	N.S.	A > B > D > C > E
T_{max} (h)	1.8 ± 0.25	5.0 ± 0.58	14.0 ± 6.00	12.0 ± 4.00	(24.0 ± 0.0) [b]			

[a] $AUC_{0-\infty} \cdot k_{(\beta)}(h^{-1}) \cdot$ body weight (kg)/dose (mg).
[b] n = 2.

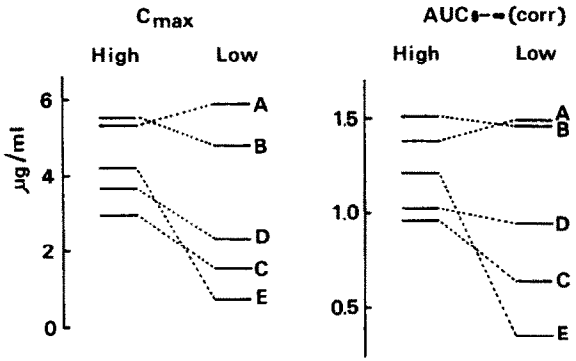


Fig. 5. Comparison of mean C_{max} and $AUC_{0-\infty}$(corr) of 5 metronidazole tablets between high gastric acidity subject group (High) and low gastric acidity subject group (Low).

of metronidazole at pH 1.2 was fast in all of the tablets tested. The bioavailabilities of tablets C, D and E, which showed pH-dependent dissolution especially slow at pH 5 or 7.2, gave poor values (about 0.6 of that of tablet A), which is very similar to bioavailabilities for diazepam (Ogata et al., 1982) and chloramphenicol (Ogata et al., 1979b) previously reported. The bioavailability of diazepam, which is released at a pH-dependent rate, has been shown to be affected by the gastric acidity of the subject (Ogata et al., 1982). It is suspected that the bioavailability of metronidazole also differs with respect to the gastric acidity of the subjects since metronidazole was also released at a pH-dependent rate from the sugar-coated tablets tested.

Tables 3 and 4 show the mean values of C_{max}, $AUC_{0-\infty}$, $AUC_{0-\infty}$(corr) and T_{max} in the subject group showing high gastric acidity (n = 6) and that having low gastric acidity (n = 4). Results of ANOVA and pairwise comparisons are also shown in Tables 3 and 4. Statistical analysis was done according to a randomized block design.

The mean serum concentration–time curves of the two subject groups for each tablet are given in Fig. 4. The serum levels of metronidazole for each tablet were quite different between the two subject groups.

The mean values of C_{max} and $AUC_{0-\infty}$(corr) were compared between the two subject groups (Fig. 5). For both parameters, the differences between tablet A and the other tablets were larger in the low acidity group compared with those in high acidity group, and the ranking of the tablets for both parameters were also different between the two subject groups.

Correlation between in vivo and in vitro findings

The correlations between in vivo parameters (Y; C_{max}, AUC_{0-32}, $AUC_{0-\infty}$ and $AUC_{0-\infty}$(corr)) in both subject groups and in vitro dissolution rates (X; T_{lag}, T_{30}, T_{50} and T_{70}, representing the lag times, 30%, 50% and 70% of dissolution, respectively) were tested statistically with X-Y, X^{-1}-Y and log X–log Y relations. Unfortunately, we could not get significant correlations between the in vivo and in vitro parameters, except for the combinations of AUC_{0-32} and $AUC_{0-\infty}$ in the high acidity group and

T_{70} at pH 5 using the PD method (X-Y). Although in vivo findings seem to agree well with the dissolution rate–pH relationship, the relative values and the ranking between the tablets tested did not agree with those of dissolution rates. The disintegration times also showed poor correlation with in vivo parameters.

Discussion

In the high acidity subject group, tablets B and E showed almost the same bioavailability as tablet A (within 0.8 of tablet A in $AUC_{0-\infty}$(corr)) and tablets C and D showed values of 0.70 and 0.74 of tablet A for the same parameter, respectively. However, in the low acidity group only tablet B gave the same $AUC_{0-\infty}$(corr) (0.98 of tablet A). Tablets C, D and E showed very poor values (0.43, 0.63 and 0.23 of tablet A, respectively). The discrepancy of the bioavailability evaluation between the two subject groups relative to gastric acidity is very important. If the bioavailability was evaluated using a subject group in which subjects having high gastric acidity are at a high rate, subjects having hypo- or anacidity appears to suffer from unexpectedly low bioavailability of metronidazole, especially from tablet E.

Such gastric acidity-dependent bioavailability is probably due to the pH-dependent dissolution of tablets C, D and E which showed especially slow dissolution at a pH between 5 and 7.2. Several types of coating material which exhibit such a pH-dependent dissolution are used in Japanese and international marketplaces. These include shellac, Eudragid E (Lehmann, 1968, 1969), MPM 47 (Ida et al., 1962), AEA, etc. These may provide gastric acidity-dependent bioavailability.

No adequate explanation can be given for the poor correlation between in vivo parameters and in vitro dissolution rates. The disintegration of the water-proof coating and/or the core of the tablet are required for drug absorption from a sugar-coated tablet at the initial stage. It is, however, not clear how this process occurs in the gastrointestinal tract or how it is affected by physiological factors. The process seems to follow a rather complicated pathway (Aoyagi et al., 1982; Alpsten et al., 1979; Hunter et al., 1980; Hunter et al., 1981). Although correlation between in vivo parameters and dissolution rates was not observed, it appears that the dissolution rates determined at various pHs, ranging from 1 to 7, gave useful information on the bioavailability. However, data determined at pH 1.2 according to official pharmacopoeias did not provide predictive information.

From the above-described results, we can suggest that the gastric acidity of a subject significantly influences the bioavailability of a drug which is formulated in a sugar-coated tablet having a water-proof coating showing pH-dependent dissolution. The concept that the main ingredients for a water-proof coating should dissolve easily at pH 1.0 for good bioavailability because human gastric acidity is near pH 1.0 has long been accepted. The official pharmacopoeias also seem to adopt that concept (JP X, USP XX, BP 1980), since disintegration for coated tablets is tested in 0.1 N HCl. However, as shown here, often a film consisting of insoluble coating materials at pH 5–7.2 does not disintegrate in the subjects having low gastric acidity.

Therefore, the coating materials for a water-proof coating should be selected carefully. The in vitro dissolution and disintegration tests should be done at 2 or 3 different pHs, for example, at 1.0, 5.0 and 7.0, in order to predict the bioavailability of a drug from a specific tablet formulation and coating.

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