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Bioavailability of metronidazole from sugar-coated tablets in humans. II. Evaluation of beagle dogs as an animal model

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Summary

The bioavailability of metronidazole from five sugar-coated tablets exhibiting different dissolution behaviors relative to pH was determined in beagle dogs, and compared with that in humans administered the same preparations. The gastric pH of beagle dogs, which was determined after collecting the gastric fluid through a catheter inserted into the stomach, ranged in pH from 1.8 to 7.8. From statistical analysis of C_{max} and AUC_{0-24}, it was concluded that gastric acidity definitely affects the bioavailability of metronidazole from sugar-coated tablets. The relative values of C_{max} and AUC_{0-24}, compared with those of the tablet exhibiting the highest values, ranked similarly between corresponding gastric acidity groups of beagle dogs and humans. A significant correlation was shown between AUC_{0-24} for beagle dogs and humans having low gastric acidity ($P < 0.05$). These results suggest the possibility that beagle dogs may be used as an animal model for testing the bioavailability of a drug preparation showing gastric acidity-dependent bioavailability in humans.

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Introduction

The bioavailability of metronidazole from five commercial sugar coated tablets in humans and its relationship with in vitro dissolution rates were reported previously (Ogata et al., 1985). Although metronidazole dissolves easily in water and is absorbed rapidly, the bioavailability of metronidazole from sugar-coated tablets was strongly affected by the gastric acidity of the subject. This was ascribed to the pH-dependent dissolution behavior of the water-proof coating (Ogata et al., 1985).

Beagle dogs are often used as an animal model for bioavailability studies (Barr, 1972), although their usefulness in this regard has not been satisfactorily established. Results of studies in beagles do not always show a high correlation with humans as was shown in our previous studies (Ogata et al., 1982a; Aoyagi et al., 1982; Kaniwa et al., 1983; Ogata et al., 1984), which concentrated on the suitability of the beagle as an animal model for bioavailability testing.

The gastric acidity of humans was found to be an important determinant for drug bioavailability (Ogata et al., 1982b; Ogata et al., 1980). However, the effect of gastric acidity on drug bioavailability has not been studied in beagles and these data are indispensable for the evaluation of the beagle as an animal model for bioavailability studies. The purpose of this study was to clarify whether the bioavailability of sugar-coated metronidazole tablets was affected by gastric acidity in beagle dogs, as has been observed in humans.

Materials and Methods

Materials. Five commercial preparations of metronidazole, which were used in a human bioavailability test described previously and designated as tablets A, B, C, D and E (Ogata et al., 1985), were used.

In vitro dissolution. The dissolution rates of metronidazole from tablets were determined at 37°C using an oscillating basket with a desk (1000 ml of the dissolution medium; OB), a rotating basket (120 rpm, 900 ml of the medium; RB), a rotating flask (6.8 rpm, 1000 ml of the medium; RF) and a paddle (120 rpm, 900 ml of the medium; PD). 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) (Ogata et al., 1985). As described previously (Ogata et al., 1985), tablet A showed the fastest rate of pH independent dissolution using the OB, RB and PD methods. The dissolution rate of tablet B was slower than that of tablet A and did not demonstrate clearly pH-dependent dissolution characteristics. Tablets C and D dissolved slowly especially at pH 5, and tablet E did not dissolve at all at pH 7.2.

Beagle dog study. pH of the gastric fluid was measured once in each animal within a few weeks before the bioavailability study. Beagle dogs, which fasted overnight (about 22 h), were given 100 ml of water. Five to 7 ml of gastric fluid was collected by means of a catheter inserted into the stomach 10 min after administra-

tion of the water and the pH of the fluid was determined with a pH meter. If the fluid collection was not successful, another 100 ml of water was again administered and the procedure repeated.

Bioavailability study. Eleven healthy male beagle dogs were used in the studies.

Study I. Ten healthy male beagle dogs (Nos. 1–10), weighing 8.9–11.4 kg (mean 9.98 kg), were used in the in vivo study. After overnight fasting (about 22 h), one tablet from each of the preparations described above was administered orally along with 50 ml of water according to a Latin square design (5 × 5) to each of the five groups of two dogs at weekly intervals. The beagle dogs were not fed until 10 h after drug administration. Blood samples were taken 0.5, 1, 2, 3, 4, 6, 8, 10 and 24 h after drug administration. Plasma samples were stored in a freezer (−20°C) until assayed.

Study II. To determine the relationship between the dose of metronidazole and AUC, 125, 250 and 500 mg of metronidazole diluted two-fold with corn-starch were administered orally to three beagle dogs (Nos. 5, 7 and 11), through a catheter, according to a Latin square design (3 × 3) at weekly intervals.

Assay. 0.5 ml of ethyl alcohol containing dimetridazole (5 μg/ml) was added to 0.2 ml of plasma as an internal standard and the solution was vigorously shaken and allowed to stand at room temperature for 20 min. After centrifuging at 3000 rpm for 10 min, 25 μl of the supernatant was analyzed by HPLC.

The HPLC equipment consisted of a pump (M-6000A; Waters Associates), an injector (U6K; Waters Associates), a detector (UVIDEC-100II; Japan Spectroscopic) and an integrator recorder (C-R1A; Shimadzu Seisakusho). The conditions for HPLC were as follows: column, μBondapack C18 (300 mm × 4 mm; Waters Associates) with a pre-column packed with Bondapack C18 Corasil; mobile phase, 8% acetonitrile in 0.005 M potassium phosphate monobasic (pH 4.0); flow rate, 2 ml/min; detector, 324 nm. The lower limit of the assay was 0.1 μg/ml for metronidazole in the plasma.

This assay procedure differed from the one we used in a previous study for assay of human samples (Ogata et al., 1985). The extracting and concentrating steps done for human sample were omitted for the dog assay since drug levels in the animals were about five-fold higher than those found in humans and were, therefore, unnecessary.

Results

Gastric pH of beagle dogs. The gastric acidities of beagle dogs have large inter-subject variations, pH 1.8–7.8, similar to those in humans (Ogata et al., 1985). In this study, we defined beagle dogs showing a pH lower than 3 as high gastric acidity and those showing a pH higher than 3 as low gastric acidity. This corresponds with the human gastric acidity evaluation using Gastrotest (Ogata et al., 1985).

Relationship between the dose of metronidazole and the resulting AUC. Fig. 1 shows the relationship between the dose of metronidazole and the resulting area under the plasma concentration–time curve from 0 to 24 h (AUC_{0-24}) calculated by

TABLE 1

PHARMACOKINETIC PARAMETERS OF METRONIDAZOLE AFTER ORAL ADMINISTRATION OF 250 mg METRONIDAZOLE TABLETS TO BEAGLE DOGS

Parameter	Formulation					ANOVA	Tukey's multiple range analysis
	A	B	C	D	E		
C_{max} (μg/ml)	27.9 ± 0.7 [a]	24.7 ± 2.6	18.6 ± 3.8	25.7 ± 3.0	18.2 ± 4.0	$P < 0.05$	A > D > B > C > E
T_{max} (h)	1.6 ± 0.2	4.4 ± 0.9	7.2 ± 2.8	5.1 ± 2.1	–	–	–
AUC_{0-24} (μg·h/ml)	313.8 ± 7.1	278.5 ± 24.7	216.4 ± 41.6	282.7 ± 32.1	203.0 ± 44.4	$P < 0.05$	A > D > B > C > E

[a] The figures indicate means ± standard errors.

Fig. 1.

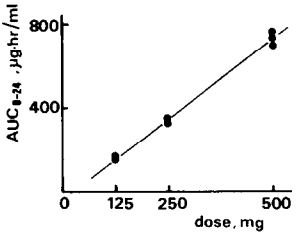


Fig. 2.

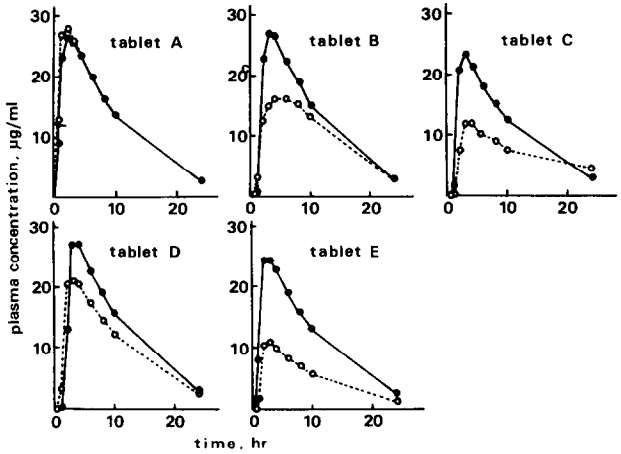


Fig. 1. Relationships between dose of metronidazole and the resulting AUC_{0-24} after oral administration of metronidazole powder to beagle dogs.

Fig. 2. Mean plasma concentration curves of metronidazole after oral administration of metronidazole (250 mg) tablets to beagle dogs having high gastric acidity (●) and low gastric acidity (○).

the trapezoidal rule. The regression line showed significant linearity between the dose and AUC_{0-24}, and intersected the abscissa near the origin, suggesting that the plasma levels of metronidazole in beagle dogs approximate the linear pharmacokinetic model within the dose range studied.

Bioavailability. Table 1 shows mean peak concentration (C_{max}), the time to peak concentration (T_{max}), and area under the plasma concentration–time curve from 0 to 24 h (AUC_{0-24}). The values were corrected for the body weight of the dogs (mean body weight = 1.0) and drug content of tablets (250 mg = 1.0). As plasma levels at 24 h showed unexpectedly high values (C_{max}) in four cases, we listed AUC_{0-24} in place of extrapolating the value of AUC ($AUC_{0-\infty}$), which would have introduced error in estimating the extent of bioavailability. The mean T_{max} of tablet E was not listed in Table 1 because two beagle dogs (Nos. 6 and 9) did not show any evidence of metronidazole absorption after tablet administration. Therefore, ANOVA for T_{max} was impossible using a complete block design.

Results of ANOVA, Table 1, show significant differences in both C_{max} and AUC_{0-24} among the tablets tested. The results of a pairwise comparison among the tablets by Tukey's multiple range analysis are also shown in Table 1. The mean values for tablets not underscored by the same line differed significantly ($P < 0.05$).

Effect of gastric acidity. A correlation between bioavailability parameters and gastric pH was not significant (data were not shown here). However, since we observed that gastric acidity appeared to affect metronidazole bioavailability in beagle dogs in the same manner observed in humans. We classified the beagle dogs into two groups according to gastric pH, approximating gastric acidity evaluation in humans.

TABLE 2

COMPARISON OF PHARMACOKINETIC PARAMETERS OF METRONIDAZOLE AFTER ORAL ADMINISTRATION OF METRONIDAZOLE TABLETS BETWEEN BEAGLE DOGS HAVING HIGH GASTRIC ACIDITY AND THOSE HAVING LOW GASTRIC ACIDITY

	Formulation				
	A	B	C	D	E
High acidity group (n = 5)					
C_{max} (μg/ml)	27.0 ± 1.2 [a]	29.3 ± 1.3	23.6 ± 5.3	29.0 ± 2.0	25.4 ± 1.7
T_{max} (h)	1.8 ± 0.4	2.8 ± 0.4	2.8 ± 0.2	3.2 ± 0.4	2.4 ± 0.2
AUC_{0-24} (μg·h/ml)	310.3 ± 13.3	316.3 ± 8.4	264.5 ± 55.7	311.7 ± 19.3	284.4 ± 11.6
Low acidity group (n = 5)					
C_{max} (μg/ml)	28.7 ± 0.6	20.0 ± 4.1	13.5 ± 4.9	22.5 ± 5.6	11.0 ± 6.7
T_{max} (h)	1.4 ± 0.2	6.0 ± 1.4	11.6 ± 5.1	7.0 ± 4.3	–
AUC_{0-24} (μg·h/ml)	317.3 ± 13.3	240.7 ± 44.4	168.2 ± 59.5	253.9 ± 62.0	121.5 ± 73.7

[a] The figures indicate means ± standard errors.

TABLE 3

ANALYSIS OF VARIANCE OF C_{max} (TWO-WAY LAYOUT WITH REPEATED OBSERVATIONS)

Observed values:

Acidity	Dog no. (pH) [a]	Formulation					Mean ± S.D.
		A	B	C	D	E	
High	1(2.7)	27.5	29.8	27.2	27.2	23.0	
	2(1.9)	24.1	26.4	26.5	28.8	23.3	
	3(1.4)	24.6	26.4	2.6	22.6	22.2	26.86 ± 6.07
	4(2.0)	30.5	30.8	31.4	33.1	31.0	
	5(1.8)	28.2	33.2	30.4	33.3	27.3	
Low	6(6.9)	27.7	16.9	26.3	25.6	0.0	
	7(7.3)	30.0	5.9	24.4	0.5	0.3	
	8(5.8)	28.8	21.4	2.6	26.3	26.4	19.14 ± 11.81
	9(4.3)	27.3	28.9	6.1	30.3	0.0	
	10(7.8)	29.9	26.9	8.1	29.7	28.1	

ANOVA:

Source of variation	SS	Df	MS	F
Between dogs	1581.443	9		
Acidities	744.980	1	744.980	7.125 *
Dogs within groups	836.463	8	104.588	
Within dogs	3396.836	40		
Tablets	770.219	4	192.555	2.719 *
Acidities × Tablets	360.219	4	90.158	1.273
Tablets × Dogs within groups	2265.985	32	70.812	

* $P < 0.05$.

[a] pH value of gastric fluid.

Table 2 shows the mean values of C_{max}, T_{max} and AUC_{0-24} in beagle dogs having high gastric acidity (pH lower than 3; n = 5) and those having low gastric acidity (pH higher than 3; n = 5). The mean plasma concentration–time curves of the two beagle dog groups were compared for each tablet (Fig. 2). The beagle dogs classified as high acidity showed higher mean plasma levels than those classified as low acidity following tablet administration, with the exception of tablet A.

Tables 3 and 4 present the results of ANOVA on C_{max} and AUC_{0-24}, respectively, according to a two-way layout with repeated observations (Winer, 1971). Significant variations were found in two factors, gastric acidities and tablets. From statistical analysis, we conclude that gastric acidity affects the bioavailability of metronidazole from the sugar-coated tablets exhibiting different dissolution behavior relative to pH in beagle dogs, as it does in humans.

Relationship among in vivo parameters. A linear relationship existed between C_{max} and AUC_{0-24} (Fig. 3), and the values of C_{max} were almost identical within a

TABLE 4

ANALYSIS OF VARIANCE OF AUC_{0-24} (TWO-WAY LAYOUT WITH REPEATED OBSERVATIONS)

Observed values:

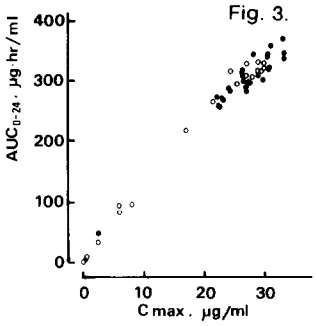
Acidity	Dog no. (pH) [a]	Formulation					Mean ± S.D.
		A	B	C	D	E	
High	1(2.7)	297.4	301.0	282.4	288.3	272.5	297.46 ± 60.20
	2(1.9)	286.6	298.3	299.1	307.8	267.9	
	3(1.4)	282.7	316.1	48.1	256.8	257.7	
	4(2.0)	342.9	321.0	355.1	368.6	317.1	
	5(1.8)	342.0	345.3	337.9	336.9	306.9	
Low	6(6.9)	292.5	217.5	307.4	295.9	0.0	220.31 ± 131.28
	7(7.3)	327.1	80.8	315.5	7.3	3.6	
	8(5.8)	316.8	266.8	32.0	314.8	298.6	
	9(4.3)	321.8	330.1	92.0	366.6	0.0	
	10(7.8)	328.2	308.4	94.2	314.7	305.2	

ANOVA:

Source of variation	SS	Df	MS	F
Between dogs	183034.535	9		
Acidities	74389.959	1	74389.959	5.478 *
Dogs within groups	108644.576	8	13580.572	
Within dogs	391929.992	40		
Tablets	89083.259	4	22270.815	2.690 *
Acidities × Tablets	37942.821	4	9485.705	1.146
Tablets × Dogs within groups	264903.912	32	8278.247	

* $P < 0.05$

[a] pH value of gastric fluid



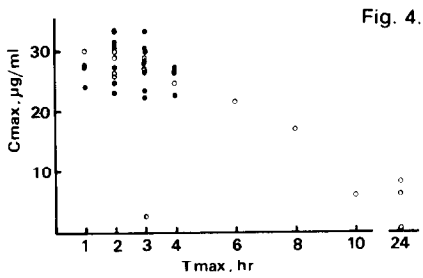


Fig. 3. Relationship between C_{max} and $AUC_{0\text{-}24}$ after oral administration of metronidazole (250 mg) tablets to beagle dogs having high gastric acidity (●) and low gastric acidity (○).

Fig. 4. Relationship between T_{max} and C_{max} after oral administration of metronidazole (250 mg) tablets to beagle dogs having high gastric acidity (●) and low gastric acidity (○).

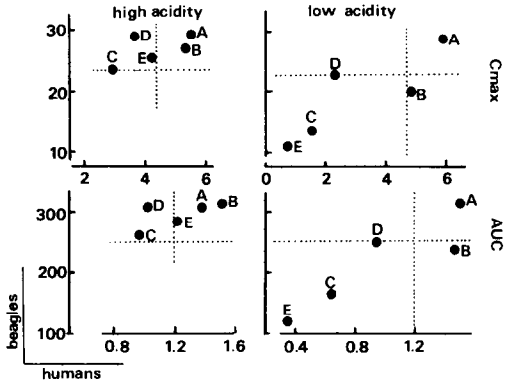


Fig. 5. Correlations of C_{max} and $AUC_{0\text{-}24}$ between corresponding subject groups with respect to the gastric acidity of humans and beagle dogs. Dotted lines represent 80% of the highest values of the parameters in each species. Correlation coefficients were as follows. In high acidity $r = 0.843$ (C_{max}) and 0.650 (AUC), and in low acidity $r = 0.856$ (C_{max}) and 0.899 ($P < 0.05$; AUC).

T_{max} of 4 h except for some observations for tablet C (Fig. 4). The data for both the high and low gastric acidity groups agreed, suggesting that absorption of metronidazole was independent of gastric acidity, but drug release from the sugar-coated tablet was affected by the acidity.

Correlation between findings in beagle dogs and humans. Human data for metronidazole bioavailability were quoted from a previous paper (Ogata et al., 1985). Fig. 5 presents the correlation of C_{max} and $AUC_{0\text{-}24}$ between corresponding subject groups with respect to the gastric acidity of humans and beagle dogs (high-high and low-low). The $AUC_{0\text{-}24}$ between beagle dogs and humans having low gastric acidities was significantly correlated ($P < 0.05$).

Discussion

Gastric pH was measured before performing the bioavailability study and ranged in pH between 1.8 and 7.8, approximating that of humans. We concluded that gastric acidity affected the bioavailability of metronidazole from sugar-coated tablets in beagle dogs using ANOVA of a two-way layout with repeated observations, although the correlation between gastric pH and C_{max} and AUC_{0-24} was not significant. The effect of gastric acidity on metronidazole bioavailability in beagle dogs is, however, not as clear cut as that observed in humans (Ogata et al., 1985) probably because of wide intra-subject gastric pH variation of beagle dogs as shown by Takahashi et al. (1983). However, according to repeated evaluations using Gastrotest (unpublished data), gastric acidity in humans appears to remain relatively constant. Gastric pH should be measured prior to each run simultaneously in order to assess accurately the effect of gastric acidity on drug bioavailability when beagle dogs are used.

The very small slope of the regression line for both C_{max} and AUC between humans and beagles classified as having high gastric acidity, seems to suggest that using beagle dogs in place of humans for bioavailability testing is not indicated. On the contrary, bioavailability of metronidazole in beagle dogs classified as having low gastric acidity corresponded well with that in humans also classified as having low gastric acidity (Fig. 5).

The data presented here suggest that the absorption of metronidazole from sugar-coated tablets in beagle dogs occurs in a manner similar to that humans, and that the effect of gastric acidity in beagle dogs on bioavailability of metronidazole from sugar-coated tablets is analogous to that humans, though the effect is somewhat variable.

This report is the first one showing that gastric acidity affects drug bioavailability in beagle dogs and that the relationship between gastric acidity and drug bioavailability in beagle dogs corresponds to that in humans. We therefore suggest that beagles may be used as an animal model for bioavailability testing of a drug preparation showing gastric acidity-dependent bioavailability in humans, although further studies will be needed on other drug preparations.

References

Aoyagi, N., Ogata, H., Kaniwa, N., Koibuchi, M., Shibazaki, T., Ejima, A., Tamaki, N., Kamimura, H., Katougi, Y. and Omi, Y., Bioavailability of griseofulvin from tablets in beagle dogs and correlation with dissolution rate and bioavailability in humans. J. Pharm. Sci., 71 (1982) 1169–1172.

Barr, W.H., The use of physical and animal models to assess bioavailability. Pharmacology, 8 (1972) 55–101.

Kaniwa, N., Ogata, H., Aoyagi, N., Shibazaki, T., Ejima, A., Watanabe, Y., Motohashi, K., Sasahara, K., Nakajima, E., Morioka, T. and Nitanai, T., The bioavailability of flufenamic acid and its dissolution rate from capsules. Int. J. Clin. Pharmacol. Ther. Toxicol., 21 (1983) 56–63.

Ogata, H., Aoyagi, N., Kaniwa, N., Koibuchi, M., Shibazaki, T., Ejima, A., Watanabe, Y., Motohashi, K., Tsuji, S. and Kawazu, Y., Relation between drug bioavailability from solid dosage form in human and dissolution rate: acidity of human gastric fluid and drug bioavailability. J. Pharm. Dyn., 3 (1980) s-17.

Ogata, H., Aoyagi, N., Kaniwa, N., Koibuchi, M., Shibazaki, T., Ejima, A., Shimamoto, T., Yashiki, T., Ogawa, Y., Uda, Y. and Nishida, Y., Correlation of the bioavailability of diazepam from uncoated tablets in beagle dogs with its dissolution rate and bioavailability in humans. Int. J. Clin. Pharmacol. Ther. Toxicol., 20 (1982a) 576–581.

Ogata, H., Aoyagi, N., Kaniwa, N., Koibuchi, M., Shibazaki, T. and Ejima, A., The bioavailability of diazepam from uncoated tablets in humans. I. Effect of gastric fluid acidity. Int. J. Clin. Pharmacol. Ther. Toxicol., 20 (1982b) 166–170.

Ogata, H., Aoyagi, N., Kaniwa, N., Shibazaki, T., Ejima, A., Takasugi, T., Mafune, E., Hayashi, T. and Suwa, K., Bioavailability of nalidixic acid from uncoated tablets in humans. Part II: Bioavailability in beagles and its correlation with bioavailability in humans and in vitro dissolution rates. Int. J. Clin. Pharmacol. Ther. Toxicol., 22 (1984) 240–245.

Ogata, H., Aoyagi, N., Kaniwa, N., Shibazaki, T., Ejima, A., Takagishi, Y., Ogura, T., Tomita, K., Inoue, S. and Zaizen, M., Bioavailability of metronidazole from sugar-coated tablets in humans. I: Effect of gastric acidity and correlation with in vitro dissolution rate. Int. J. Pharm., 23 (1985) 277–288.

Takahashi, T., Uezono, Y. and Fujioka, H., Gastric-acidity controlled rabbits for evaluation of bioavailability. Yakuzaigaku, 43 (1983) 61–67.

Winter, B.J., Statistical Principles in Experimental Design, 2nd edn, McGraw-Hill-Kogakusha, Tokyo, 1971, p. 514.