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Bioavailability of Griseofulvin from Tablets in Beagle Dogs and Correlation with Dissolution Rate and Bioavailability in Humans

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Abstract □ The bioavailability of four griseofulvin tablets in beagle dogs, including an ultramicrosize tablet used previously in a human bioavailability study, was investigated on the basis of the plasma 6-demethylgriseofulvin concentration. The relations with the *in vivo* findings in humans and the *in vitro* dissolution rates also were examined. Contrary to the lower bioavailability of the ultramicrosize formulation in humans, it provided the best bioavailability in beagles. The microsize griseofulvin formulations showed similar *in vivo* results to those in humans. Poor correlation of *in vivo* parameters between humans and beagles was attributed to the discrepancy of the availability of the ultramicrosize formulation between the two species. The dissolution rates determined by the pretreatment method using plastic beads were correlated more with the *in vivo* findings than those determined by the other methods. Beagles were a useful animal model for bioavailability studies of certain griseofulvin formulations but not ultramicrosize ones.

Keyphrases □ Bioavailability—griseofulvin from tablets in beagle dogs, correlation with dissolution rate and bioavailability in humans □ Dissolution rates—bioavailability of griseofulvin from tablets in beagle dogs, bioavailability in humans □ Griseofulvin—bioavailability from tablets in beagle dogs, dissolution rate and bioavailability in humans

The bioavailabilities for four lots of griseofulvin tablets in humans have been reported previously, and the relations with *in vitro* dissolution rates have been discussed (1).

Beagle dogs are often used as an animal model for bioavailability studies, but their suitability has not been clarified sufficiently. A good relation of penicillin bioavailability between humans and dogs was reported (2). Previous studies on bioavailability of diazepam formulations in humans and beagles revealed no good relations between the results from both species. The discrepancy was considered to be due to the differences of physiological states of the GI tract, especially of gastric emptying rate and GI transition time (3).

In the present study the bioavailability of griseofulvin from tablets in beagles was studied, and the relations with *in vivo* results in humans and *in vitro* dissolution rates were investigated.

EXPERIMENTAL

Formulations—Four lots of tablets containing 125 mg of griseofulvin employed in the human bioavailability study (1) were used. One formu-

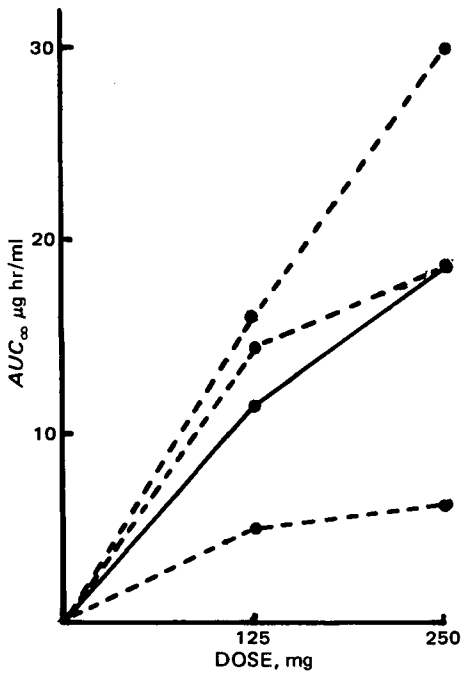

Figure 1—*Relation between griseofulvin dose and* AUC_∞ *of 6-demethylgriseofulvin. Key: (---) responses of the individual dogs; (—) average response.*

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Table I—Plasma Concentrations of Griseofulvin and 6-Demethylgriseofulvin Following Oral Administration of Formulation A to 12 Beagles

In Vivo Parameter		Griseofulvin Means ± *SD*	Griseofulvin Coefficient of Variation, %	6-Demethylgriseofulvin Means ± *SD*	6-Demethylgriseofulvin Coefficient of Variation, %
Plasma Concentration μg/ml	0.5 hr	0.209 ± 0.202	97	1.559 ± 1.128	72
	1	0.494 ± 0.481	97	2.562 ± 1.264	49
	2	0.429 ± 0.298	70	2.059 ± 1.138	55
	3	0.327 ± 0.196	60	1.519 ± 0.763	50
	4	0.203 ± 0.116	57	1.092 ± 0.468	43
	6	0.103 ± 0.036	35	0.843 ± 0.259	31
	8	0.065 ± 0.033	51	0.753 ± 0.298	40
	10	0.050 ± 0.036	73	0.544 ± 0.289	53
	24	0.003 ± 0.010	333	0.127 ± 0.199	157
C_{max}, μg/ml		0.626 ± 0.458	73	3.083 ± 1.114	36

Table II—Plasma Levels, C_{max}, t_{max}, and AUC_{24} of 6-Demethylgriseofulvin Following Oral Administration of Four Lots of 125-mg Griseofulvin Tablets to Beagles

In Vivo Parameter		Formulation[a] A	B	C	D	Result[b] of ANOVA	Tukey's[c] Test
Plasma level, μg/ml	0.5 hr	1.559 ± 0.325	1.265 ± 0.290	0.640 ± 0.123	0.699 ± 0.229	$p < 0.05$	A > B > D > C
	1	2.562 ± 0.365	1.857 ± 0.342	0.925 ± 0.112	1.222 ± 0.272	$p < 0.01$	A > B > D > C
	2	2.059 ± 0.329	1.482 ± 0.314	0.977 ± 0.135	1.234 ± 0.105	$p < 0.01$	A > B > D > C
	3	1.519 ± 0.220	1.170 ± 0.256	0.850 ± 0.138	0.951 ± 0.093	$p < 0.05$	A > B > D > C
	4	1.092 ± 0.135	0.924 ± 0.199	0.845 ± 0.202	0.813 ± 0.136	NS	
	6	0.843 ± 0.075	0.714 ± 0.135	0.540 ± 0.101	0.540 ± 0.098	NS	
	8	0.753 ± 0.086	0.584 ± 0.110	0.434 ± 0.086	0.483 ± 0.081	NS	
	10	0.544 ± 0.083	0.463 ± 0.102	0.282 ± 0.040	0.442 ± 0.102	NS	
	24	0.127 ± 0.057	0.071 ± 0.017	0.077 ± 0.029	0.053 ± 0.015	NS	
C_{max}, μg/ml		3.083 ± 0.321	2.340 ± 0.322	1.369 ± 0.156	1.749 ± 0.190	$p < 0.01$	A > B > D > C
t_{max}, hr		1.5 ± 0.2	1.3 ± 0.2	1.9 ± 0.5	1.7 ± 0.3	NS	
AUC_{24}, μg hr/ml		16.34 ± 1.38	12.86 ± 2.77	8.86 ± 1.05	10.62 ± 1.48	< 0.01	A > B > D > C

[a] The figures indicate means ± standard error. [b] NS: not significant. [c] Formulations underlined by a common line did not differ significantly ($p < 0.05$).

lation was an ultramicrosize griseofulvin tablet (A); the others were commercial microsize griseofulvin tablets (B, C, D).

Dissolution Rate—The methods to determine the dissolution rates were carried out as reported previously (1). Sink methods included nonpretreatment (18-liter beaker method and basket method) and pretreatment (Methods I and II). The dissolution rate was expressed as t_{30} the time taken for 30% of the drug to dissolve.

Bioavailability—Twelve beagles (12.0–14.0 kg; mean 12.9) were randomly divided into four groups according to a Latin square crossover design. The beagles, having fasted for 20 hr, were given a test tablet and then forced to take 30 ml of water. The beagles were not given any food until 10 hr after drug administration. Blood samples were taken at 0.5, 1, 2, 3, 4, 6, 8, 10, and 24 hr after administration, and the plasma samples were frozen and stored until assay. The experiments were repeated every week according to the dosage schedule. The bioavailability for each formulation was evaluated from the plasma concentrations of 6-demethylgriseofulvin, a metabolite of griseofulvin, at each sampling time, peak plasma concentration (C_{max}), time to C_{max} (t_{max}), and area under plasma concentration–time curves from 0 to the sampling time t (AUC_t). The value for AUC_∞ was calculated by the method of Wagner (4).

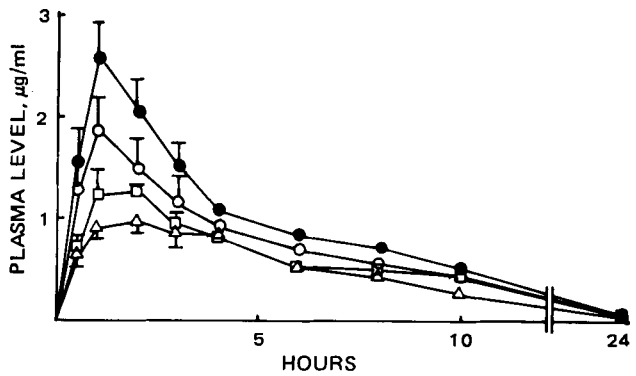

Figure 2—*Mean plasma 6-demethylgriseofulvin concentration after oral administration of 125-mg griseofulvin formulations to beagles. Key: (●) Tablet A; (○) Tablet B; (△) Tablet C; (□) Tablet D. The vertical lines show standard errors.*

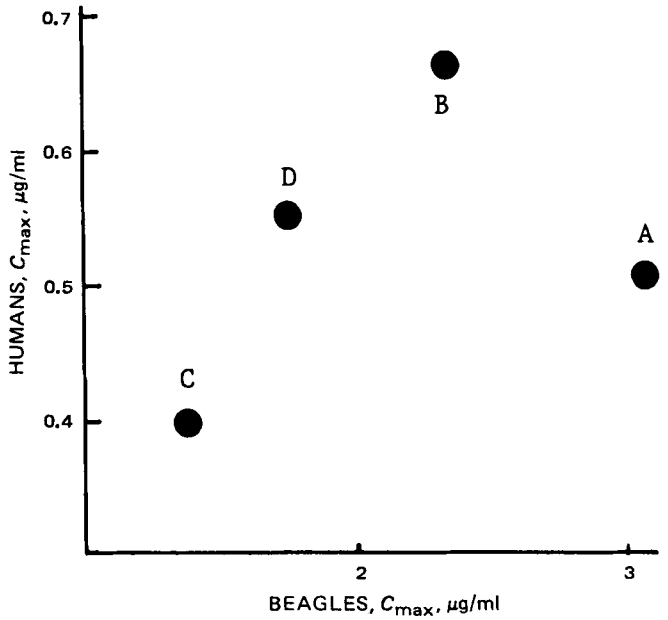

Figure 3—*Correlation of C_{max} values after oral administration of four griseofulvin formulations between humans and beagles.*

Table III—C_{max}, t_{max}, and AUC_{24} of 6-Demethylgriseofulvin Following Oral Administration of Three Griseofulvin Formulations [a]

Parameter	Formulation	Volume of Water 30 ml	Volume of Water 200 ml	Paired [b] t test
C_{max}, μg/ml	A	3.038 ± 0.287 [c]	2.608 ± 0.167	NS
	B	2.290 ± 0.490	2.067 ± 0.160	NS
	D	1.787 ± 0.270	1.388 ± 0.381	NS
t_{max}, hr	A	1.6 ± 0.2	1.8 ± 0.2	NS
	B	1.2 ± 0.2	1.7 ± 0.4	NS
	D	1.9 ± 0.4	3.6 ± 1.0	NS
AUC_{24}, μg hr/ml	A	18.89 ± 1.15	17.59 ± 0.88	NS
	B	11.13 ± 2.56	14.46 ± 1.79	NS
	D	11.68 ± 2.10	11.50 ± 1.96	NS

[a] With 30 and 200 ml of water. [b] NS: not significant. [c] Mean ± standard error.

Dose–AUC_∞ Relation—Three beagles were fasted overnight and given one and two tablets of Formulation C, corresponding to 125 and 250 mg of griseofulvin, respectively. The other procedures were the same as described for the bioavailability test.

Effects of Volume of Water Coadministered—Three formulations, including the ultramicrosize formulation, were used. Eight beagles were given a tablet with 30 and 200 ml of water in a crossover design. The other procedures were the same as described for the bioavailability test.

Assay—Griseofulvin and 6-demethylgriseofulvin in plasma were determined by GC (5).

RESULTS

Plasma Levels of Griseofulvin and 6-Demethylgriseofulvin—Table I shows the mean plasma griseofulvin and 6-demethylgriseofulvin concentrations after oral administration of Formulation A. The plasma griseofulvin concentrations were below one-fifth of those of 6-demethylgriseofulvin, and only a trace of griseofulvin was detected at 24 hr after administration of the drug. This can be attributed to the greater clearance of griseofulvin in dogs than in humans (6, 7), and hence, a considerable fraction of the dose administered orally will be converted to 6-demethylgriseofulvin by first-pass metabolism before reaching the blood circulation (8). The great clearance of griseofulvin probably leads to the greater coefficients of variation found in the plasma levels of griseofulvin than those of 6-demethylgriseofulvin. Considering the findings, the bioavailability of griseofulvin in beagles was estimated on the basis of the plasma level of 6-demethylgriseofulvin.

Bioavailability—The relation of the griseofulvin dose and the AUC_∞ of 6-demethylgriseofulvin is shown in Fig. 1. Large differences in the AUC_∞ values among the beagles were found. With the high dose, the unabsorbed fraction of the drug may increase by being not fully dissolved in the GI tract.

Figure 2 shows the mean plasma level–time curves of 6-demethylgriseofulvin following oral administration of four formulations. Table II lists their mean values for *in vivo* parameters. The plasma levels of 6-demethylgriseofulvin at 24 hr were very low, so the AUC_{24} can be considered as AUC_∞. Although the ultramicrosize formulation (A) showed relatively low bioavailability in humans (1), this formulation showed the highest values in the plasma concentrations, C_{max} and AUC_{24}, in beagles. The ratios of C_{max} and AUC_{24} of the ultramicrosize formulation to those of Formulation B, which showed the highest availability of all microsize formulations, were 132 and 127%, respectively. Significant differences were found between Formulation A and two microsize formulations (C and D) in the plasma levels at earlier sampling times, C_{max} and AUC_{24}.

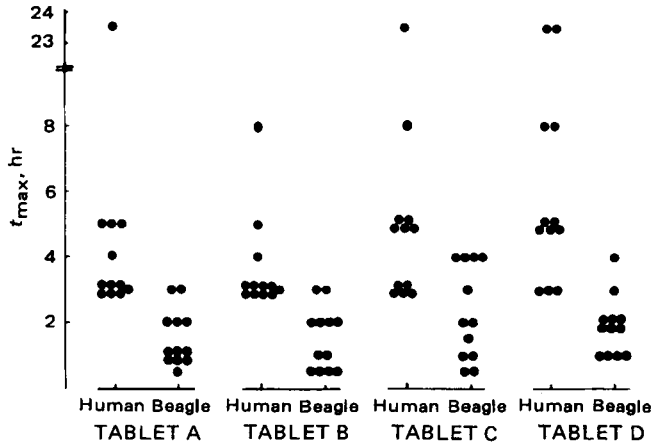

Figure 4—*Individual t_{max} values in beagles and humans after oral administration of four griseofulvin formulations.*

For microsize formulations, the *in vivo* results were similar to those in humans: Formulation B had the best bioavailability as expected from the *in vitro* dissolution rate and Formulation D showed better bioavailability than Formulation C. A significant difference was found in C_{max} between Formulations B and C but not in plasma levels and AUC_{24}. The t_{max} values in beagles, which seemed shorter than those in humans (3.6–7.9 hr), did not show significant differences among the formulations.

Effects of Volume of Water Coadministered—Thirty milliliters of water was given to beagles; however, 200 ml was used in the human bioavailability study (1). The large volume of water enhanced the *in vivo* absorption of erythromycin stearate and amoxicillin which are poorly soluble in water (9). The different volumes of water used in the human and beagle tests might lead to a discrepancy in the *in vivo* results, especially for the ultramicrosize formulation. To clarify this, the bioavailabilities were tested with 30 and 200 ml of water. As shown in Table III, the fluid volume did not significantly affect the bioavailabilities, which leads to the conclusion that the discrepancy of bioavailabilities between humans and beagles is not due to the difference of the volume of water administered. As another explanation, the physiological differences in the GI tract between the two species may be considered.

Correlation Between the Bioavailability and Dissolution Rates—The correlation coefficients between the *in vivo* parameters and *in vitro* dissolution rates (t_{30}) determined by sink methods are shown in Table IV. The *in vivo* parameters are correlated more in the log–log and normal–reciprocal regressions with t_{30} determined by Method II (Table IV), in which the tablets were treated in 20 ml of water with plastic beads before the determination of dissolution rates.

Correlation Between Humans and Beagles—As shown in Table V, low correlation coefficients were found between humans and beagles. The poor relations are attributed mainly to the discrepancy of the ultramicrosize formulation (A) between them. Figure 3 shows that the microsize formulations (B, C, D) showed a good relation (r = 0.976) in C_{max} values between humans and beagles, which suggests that the bioavailabilities of microsize formulations can be evaluated in beagles instead of humans.

DISCUSSION

The *in vivo* findings in beagles did not correlate well with those in humans. The ultramicrosize formulation provided the best availability

Table IV—Correlation Coefficients Between *In Vivo* Parameters (X) and t_{30} (Y) Determined by Sink Methods

In Vitro Test Method	Condition	X − Y^{-1} Serum Level 1 hr	3 hr	5 hr	C_{max}	AUC_{24}	log X − log Y Serum Level 1 hr	3 hr	5 hr	C_{max}	AUC_{24}
Beaker	—	0.747	0.631	0.514	0.613	0.579	−0.872	−0.760	−0.655	−0.731	−0.701
	Polysorbate 80	0.714	0.595	0.474	0.577	0.541	−0.859	−0.748	−0.638	−0.719	−0.686
	Diastase	0.776	0.672	0.562	0.658	0.625	−0.916	−0.846	−0.751	−0.824	−0.793
	848 rpm	0.803	0.697	0.587	0.680	0.648	−0.903	−0.802	−0.704	−0.775	−0.747
	pH 1.2	0.556	0.423	0.292	0.405	0.366	−0.788	−0.656	−0.535	−0.623	−0.587
	pH 1.2 Polysorbate 80	0.641	0.510	0.382	0.491	0.453	−0.793	−0.663	−0.542	−0.630	−0.594
Basket	—	0.872	0.790	0.695	0.777	0.749	−0.948	−0.896	−0.815	−0.878	−0.851
Method I	—	0.472	0.422	0.341	0.429	0.404	−0.589	−0.636	−0.575	−0.635	−0.602
Method II	—	0.943	0.932	0.889	0.933	0.903	−0.910	−0.945	−0.910	−0.943	−0.927

Table V—Correlation Coefficients of Bioavailability Parameters Between Humans and Beagles

Beagle	Human	r
$C_{0.5}$[a]	C_1	0.789
	C_3	0.505
C_1	C_1	0.678
	C_3	0.448
C_{max}	C_{max}	0.388
t_{max}	t_{max}	0.711
AUC_{24}	$AUC_{47.5}$	0.306

[a] C_t shows the plasma or serum concentration at time t.

in beagles contrary to its lower absorption in humans. Considering the insignificant effects of the volume of water on the bioavailability, the ultramicrosize formulation discrepancy may be attributed to the physiological differences in the GI tract. None of the *in vitro* dissolution methods indicated such a superiority of the ultramicrosize formulation in beagles. These findings suggest that this formulation may disintegrate into the original ultramicrosize particulate state of the drug and allow rapid dissolution in the GI tract in beagles beyond the expectation from the *in vitro* dissolution findings.

The t_{max} values in beagles for different formulations were smaller than those in humans (Fig. 4). This suggests rapid transition of the drug to the absorption site, namely, fast gastric emptying of the drug in beagles. The good bioavailability of the ultramicrosize formulation may be related to the rapid gastric emptying of the drug which leads to dissolution of the drug in the small intestinal tract.

Formulation D provided higher C_{max} and plasma levels at earlier sampling times than Formulation C as observed in the human test. The *in vitro* dissolution rate of the drug from Formulation D was enhanced over that from Formulation C by pretreatment with plastic beads. These findings suggest that in beagles and humans there is a strong intensive deaggregation action on the particles or aggregates of the drug during their transition into the GI tract.

Although there was no significant difference in AUC_∞ values among the formulations in humans, the AUC_{24} (considered as AUC_∞) of Formulations C and D were significantly lower than that of Formulation A in beagles. This suggests their incomplete dissolution during passage through the GI tract and also suggests the short absorption site and/or fast transition of the drug in the GI tract in beagles as previously shown for the bioavailability of diazepam in beagles (3).

Although the *in vivo* findings of the ultramicrosize formulation in beagles did not agree with those in humans, the bioavailabilities of the microsize formulations showed good agreement. Considering this, beagles may serve as a useful animal model for bioavailability studies of certain griseofulvin tablet formulations, but not ultramicrosize ones.

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NOTES

Antibradykinin Active Material in *Aloe saponaria*

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Abstract □ A material having antibradykinin activity on isolated guinea pig ileum was partially purified from the nondialysate of the pulp of *Aloe saponaria* by repetition of gel chromatography using a hydrophilic polyvinyl gel and dextran gels. From the results of amino acid and carbohydrate analyses, the antibradykinin-active material was estimated to be a glycoprotein. It was found that this material catalyzes the hydrolysis of bradykinin at pH 7.4. The results of peptide analysis using reversed-phase high-performance liquid chromatography coupled with amino acid analysis indicate that this glycoprotein cleaves the Gly^4-Phe^5 and Pro^7-Phe^8 bonds of the bradykinin molecule.

Keyphrases □ Antibradykinin—active material in *Aloe saponaria*, guinea pig ileum, glycoprotein, high-performance liquid chromatography □ Glycoprotein—antibradykinin active material in *Aloe saponaria*, high-performance liquid chromatography, guinea pig ileum □ *Aloe saponaria*—antibradykinin active material, glycoprotein, high-performance liquid chromatography, guinea pig ileum

Cardiac stimulant action of the constituents in the dialysate of the pulp from *Aloe saponaria*[1] on isolated cardiac muscles has been reported (1). Antibradykinin activity of the nondialysate of the pulp has been examined here to obtain pharmacological evidence for its anti-inflammatory action (2). In this report, the results of partial purification of material having antibradykinin activity from *A. saponaria* on isolated guinea pig ileum and its proteolytic property against bradykinin are presented.

EXPERIMENTAL

Materials—The following materials were purchased from suppliers: dextran gel[2,3], hydrophilic polyvinyl gel[4], dialysis membrane[5], synthetic bradykinin[6], and bromelain[7]. The gel filtrations were performed at room temperature at a flow rate of 21 ml/hr using a microtube pump[8].

Methods of Analysis—Protein and carbohydrate contents in samples

[1] *Aloe saponaria* is also known as white spotted *aloe* or soap *aloe*.

[2] Sephadex G-100, Pharmacia Fine Chemicals, Uppsala, Sweden.
[3] Sephadex G-25, Pharmacia Fine Chemicals, Uppsala, Sweden.
[4] Toyopearl HW 40, Toyo Soda Mfg., Co. Ltd., Tokyo, Japan.
[5] Visking tube, Visking Co., Union Carbide Corp.
[6] The Protein Research Foundation, Osaka, Japan.
[7] Nakarai Chemical Co., Ltd., Kyoto, Japan.
[8] Tokyo Riakkikai Co., Ltd., Tokyo, Japan.

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