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Evaluation of beagle dogs as an animal model for bioavailability testing of cinnarizine capsules

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Summary

The bioavailability of cinnarizine, 25 mg, from two commercial capsules was determined in beagle dogs, and compared with that previously found in humans given the same preparations. The gastric pH of beagle dogs, which was determined 90 min after every oral dose of test preparation by using pH test paper inserted into the stomach through a catheter, ranged from pH 1.5 to pH 8.5 with wide inter- and intra-subject variations. The gastric acidity did not affect the bioavailability of cinnarizine from capsules in beagle dogs, although the human study had shown a distinct effect of gastric acidity on the bioavailability. The discrepancy between the results for cinnarizine bioavailability in humans and beagle dogs may be ascribable to a more variable gastric pH compared with humans. In conclusion, beagle dogs cannot be used as an animal model for predicting the human bioavailability of cinnarizine, since they do not reflect the gastric acidity dependency that is observed in humans.

Introduction

The bioavailability in humans of cinnarizine from two commercial capsules, which were chosen from the results of dissolution tests as the extreme cases out of 32 commercial capsules available in Japan and its correlation with the in vitro dissolution rate were reported previously (Ogata et al., 1986). The serum levels of cinnarizine showed a wide variation among the subjects, and this was ascribable to the differences of gastric acidity. The gastric acidity-dependent bioavailability of cinnarizine was concluded to be due to pH-dependent dissolution behavior of the drug from the capsules, the rate being very fast at pH 1–2 and very slow at pH 5–6.

Beagle dogs are often used as an animal model for bioavailability tests although the relationship between bioavailability in humans and in beagle dogs has not been fully established. Similarity of gastrointestinal physiology of beagle dogs and humans is thought to be a most important factor favoring a good correlation of drug bioavailabilities after oral administration between the two species. A similarity or difference of intestinal pH

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(Crouthamel et al., 1975), intermittent HCl and biliary secretion (Barr, 1972), gastric emptying rate and gastrointestinal transit time (Cressman and Sumner, 1971; Aoyagi et al., 1982; Ogata et al., 1982b; Ogata et al., 1984) and agitating ability of the gastrointestine (Wagner et al., 1960; Ejima et al., 1982; Aoyagi et al., 1982; Aoyagi et al., 1984; Ogata et al., 1984) of beagle dogs relative to those of humans may also result in a good or poor correlation of drug bioavailabilities in the two species. Gastric acidity was also found to be one of the factors determining bioavailability (Ogata et al., 1980; Ogata et al., 1982a; Ogata et al., 1985a; Aoyagi et al., 1985). We suggested previously 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, based on the results of our studies on metronidazole (Ogata et al., 1985b).

In this paper, a study on beagle dogs was performed to investigate their suitability as an animal model for testing the bioavailability of cinnarizine capsules showing gastric acidity-dependent bioavailability in humans. For this purpose, the same cinnarizine capsules were given to beagle dogs, and the bioavailability was compared with that in humans.

Experimental

Material

Two commercial preparations of cinnarizine (25 mg), which were used in the human bioavailability test described previously and designated as capsules A and B (Ogata et al., 1986), were used. The cinnarizine contents were 96.7% and 103.8% of the labeled amount in capsules A and B, respectively. Capsules A and B were at the opposite extremes of dissolution behavior at pH 3.9 (fastest and slowest, respectively) among 32 commercial capsules as determined by the oscillating basket method with a disk (900 ml of medium; OB) and paddle method (900 ml of medium; PD). The two capsules had similar dissolution rates at pHs 1.2 and 6.0 but at other pHs the rates were markedly different. Other reagents were of analytical grade.

Beagle dog study

(i) pH of the gastric fluid

The pH of the gastric fluid was measured at each phase of the bioavailability test. A catheter was inserted into the stomach 90 min after oral administration of a capsule and vinyl tubing with pH test paper fixed at the end was inserted into the stomach through the catheter. A small volume of gastric fluid was aspirated through the tubing in order to sufficiently moisten the pH test paper with fluid, and the pH of the gastric fluid was determined from the color of the pH test paper.

(ii) Bioavailability study

Study I. Twelve healthy male beagle dogs, weighing 10.0 to 16.5 kg (mean 13.3 kg), were used. After overnight fasting (22 h), one capsule was given orally with 50 ml of water according to a cross-over design with an interval of at least one week. Blood samples were taken 0.5, 1, 2, 4, 6 and 8 h after drug administration. The beagle dogs were not fed during the experiment. Plasma samples were stored in a freezer (−20°C) until assayed. C_{max} (peak concentration) and T_{max} (time-to-peak concentration) were observed values and $AUC_{0\text{-}8h}$ (area under the plasma concentration–time curve from 0 to 8 h) was calculated according to the trapezoidal rule.

Study II. To determine the relationship between dose of cinnarizine and the resulting AUC, 12.5, 25 or 50 mg of cinnarizine dissolved in 20 ml of 0.1 N HCl was administered orally, followed immediately by 20 ml of water, though a catheter inserted into the stomach to six healthy male beagle dogs, weighing 10.5–14.5 kg (mean 12.4 kg), according to a Latin square design (2 × 3) with a weekly interval. Blood samples were taken at 0.5, 1, 2, 4, 6, 8 and 24 h after oral dosing. Other test procedures were the same as described in Study I.

Assay

A 50 μl aliquot of ethyl alcohol containing papaverine hydrochloride (10 μg/ml) as an internal standard, 2.0 ml of pH 6.5 phosphate (0.5 M) buffer and 5.0 ml of benzene were added to 1.0 ml of plasma and the mixture was mechanically shaken for 10 min. After centrifugation, 4.5 ml of

the organic layer was shaken with 3.0 ml of 0.1 N NaOH for 10 min and centrifuged again, then 3.5 ml of the organic layer was evaporated to dryness under a nitrogen current at 50°C. The residue was dissolved in 100 μl of ethyl alcohol and 3 μl of the solution was injected into HP 5840A gas chromatograph (Hewlett-Packard) fitted with a nitrogen selective flame ionization detector and a 150 cm × 3 mm i.d. glass column packed with 2.0% OV-101 on Gas Chrom Q (100-120 mesh). The operating conditions were as follows: oven temperature, 290°C; injection port temperature, 270°C; detector temperature, 300°C.

The gas flows were as follows: carrier (helium), 30 ml/min; hydrogen, 4 ml/min; air, 100 ml/min. Quantitation was achieved by the peak integral ratio technique. A calibration curve for the estimation of cinnarizine added in known amounts to fresh beagle dog plasma was prepared for each batch of samples. The assay limit for cinnarizine in plasma was 5 ng/ml.

The assay procedure differed from the one used for human serum assay (Ogata et al., 1986); it was simplified because cinnarizine could be successfully assayed without any interfering peak, although human serum gave an interfering peak when assayed by using the procedure described above.

Results

Gastric pH in beagle dogs

Fig. 1 shows the pH values of gastric fluid in beagle dogs. A wide inter- and intra-subject variation of pH, ranging from 1.5 to 8.5 was observed. For comparison with the human test results, the gastric acidity of beagle dogs was classified into two categories, high (less than pH 3) and low (more than pH 3), gastric pH, because the Gastrotest tablets for evaluating the gastric acidity of human subjects release dye in the stomach at a pH of 3 or less (Bianchetti and Gerber, 1958).

Relationship between cinnarizine dose and AUC

Fig. 2 shows the relationship between dose of cinnarizine (given as a solution) and the resulting $AUC_{0\text{-}24h}$. The relationship can be regarded as

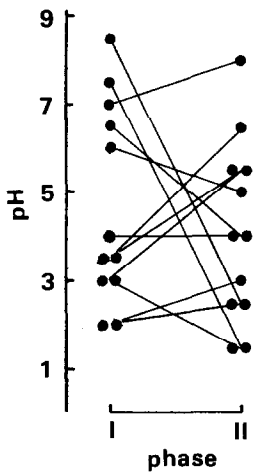

Fig. 1. Gastric pH of beagle dogs 90 min after oral administration of a cinnarizine capsule in the bioavailability test (cross-over design).

essentially linear up to a dose of 25 mg (1.72–2.38 mg/kg; mean 2.03 mg/kg) of cinnarizine, although a non-linear process does appear to exist in cinnarizine disposition in beagle dogs, because the regression line passed with negative intercept in spite of significant correlation ($r = 0.8590$ $P < 0.01$; intercept = −174.0 of AUC). Such a non-linear like relation was also shown if AUC values were plotted against doses per body weight of beagle dogs ($r = 0.8927$ $P < 0.01$; intercept = −183.7 of AUC).

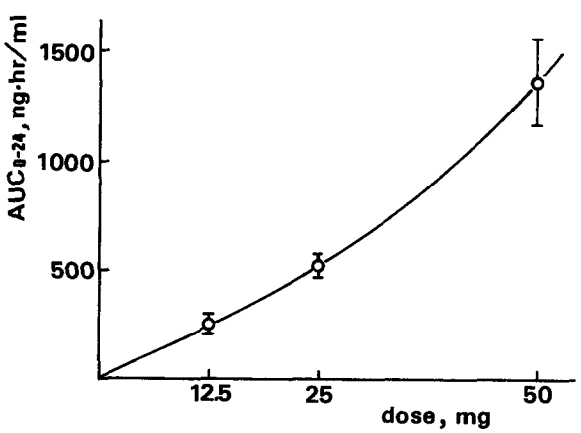

Fig. 2. Relationship between dose of cinnarizine given as aqueous solution and the resulting $AUC_{0\text{-}24h}$.

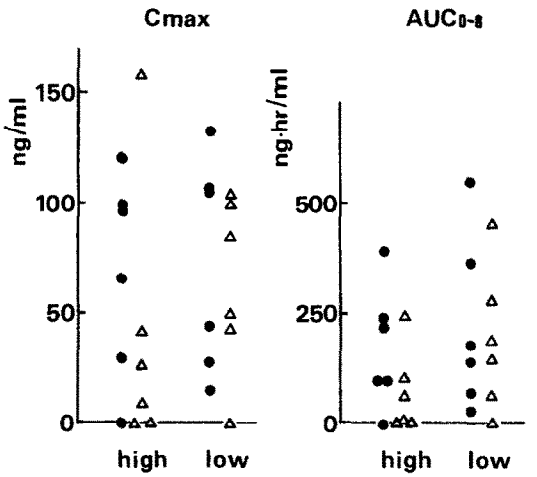

Fig. 3. Individual values of C_{max} and $AUC_{0\text{-}8h}$ after oral administration of capsules A (●) and B (△) to beagle dogs having high gastric acidity (less than pH 3) and low gastric acidity (more than pH 3).

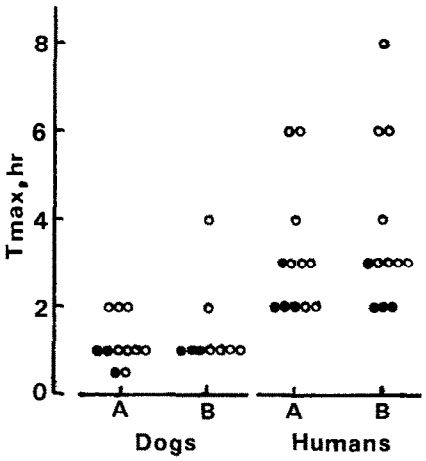

Fig. 4. Comparison of T_{max} values of cinnarizine after oral administration of capsules A and B to beagle dogs and humans. Closed and open circles represent T_{max} values of subjects showing high and low gastric acidities, respectively.

Bioavailability of cinnarizine from capsules

For estimating the extent of bioavailability, $AUC_{0\text{-}8h}$ was used in place of $AUC_{0\text{-}\infty}$ (AUC from 0 to infinity) because the plasma levels at 8 h after oral administration of cinnarizine were nearly zero in almost cases. The bioavailability of cinnarizine after oral administration of commercial capsules was strongly affected by the gastric acidity of the human subjects (Ogata et al., 1986), but there seems to be no distinct difference in plasma levels of cinnarizine in beagle dogs between the high and low gastric acidity groups (Fig. 3). The correlations between gastric pH and pharmacokinetic parameters, $AUC_{0\text{-}8h}$, C_{max} and T_{max}, were very poor, $r = 0.236$, 0.173 and 0.084, respectively. Furthermore, we found no significant differences in pharmacokinetic parameters such as plasma levels at each sampling time, C_{max} and $AUC_{0\text{-}8h}$ between the high and low gastric acidity groups of beagle dogs. This result is in marked contrast to the finding with metronidazole, which showed gastric acidity-dependent bioavailability from sugar-coated tablets in both human subjects and beagle dogs (Ogata et al., 1985b). Table 1 therefore shows the mean values of pharmacokinetic parameters, C_{max}, T_{max} and $AUC_{0\text{-}8h}$, irrespective of gastric acidity. There was no significant difference between capsules A and B in any pharmacokinetic parameter.

TABLE 1

MEAN PHARMACOKINETIC PARAMETERS AFTER ORAL ADMINISTRATION OF CINNARIZINE AS CAPSULES TO BEAGLE DOGS

Parameter	Mean (S.E.)		ANOVA (Formulation)
	Capsule A	Capsule B	
C_{max} (ng/ml)	70.7 (13.2)	52.3 (14.5)	N.S. [a]
$AUC_{0\text{-}8h}$ (ng·h/ml)	200.3 (47.4)	130.8 (40.9)	N.S.
T_{max} (h)	1.2 (0.2) [b]	1.4 (0.3) [c]	– [d]

[a] Not significant ($P > 0.05$).
[b] n = 11.
[c] n = 9.
[d] Analysis could not be performed because 1 and 3 dogs did not show any plasma level of cinnarizine during 8 h after dosing of capsules A and B, respectively.

Comparison of T_{max} between beagle dogs and humans

Fig. 4 shows individual T_{max} values observed after oral administration of capsules A and B to beagle dogs and humans (Ogata et al., 1986). The T_{max} values in beagle dogs for both formulations were smaller than those in humans.

Discussion

The pharmacokinetics of cinnarizine after an oral dose (as a solution) showed a non-linear-like disposition (Fig. 2). Although some pharmacokinetic studies on cinnarizine disposition have been reported in humans (Morrison et al., 1979; Hundt et al., 1980) and animals (Soudijn and van Wijngaarden, 1968; Allewijn, 1968; Akada et al., 1976; Dell and Fiedler, 1977), no clear evidence for non-linear kinetics of cinnarizine has been presented. Thus, although apparently non-linear behavior of cinnarizine was observed here at high dose, 50 mg (3.45–4.76 mg/kg; mean 4.06 mg/kg), in beagle dogs, it is considered that the disposition of the drug can be regarded as a linear function of dose at doses up to 25 mg of cinnarizine.

Gastric pH did not affect the bioavailability of cinnarizine from two commercial capsules in beagle dogs, although bioavailability in human subjects showed a distinct dependence on gastric acidity with the same preparations. The gastric pH values did not also correlate well with the pharmacokinetic parameters. As previously described (Ogata et al., 1986), the bioavailability of cinnarizine is mainly determined by the amount dissolved in the stomach. Then, the discrepancy between the results for cinnarizine bioavailability in humans and beagle dogs may be ascribable to some physiological differences of the gastrointestine.

The main factor determining the amount dissolved in the stomach should be the gastric pH. However, the wide inter- and intra-subject variation of gastric acidity was observed in beagle dogs (Fig. 1), which may help to mask the gastric acidity effect. The pH values were determined 90 min after the drug administration, not immediately before or after the drug administration, in order to avoid the disturbance of drug absorption. However, the pH value may not distinctly represent the pH value at which the preparation is disintegrated and dissolved since the gastric pH of beagle dogs was shown to vary with time after intake of water (Ogata et al., 1982b).

The T_{max} values in beagle dogs ranged from 0.5 to 2 h compared with values of 2–8 h in humans. Such a narrow range and small value of T_{max} with no relationship to the dissolution rates of the drugs from the preparations tested was frequently observed in beagle dog studies (Ogata et al., 1982b; Aoyagi et al., 1982; Aoyagi et al., 1984; Ogata et al., 1984; Aoyagi et al., 1985), although human subjects show wide variations of T_{max} in proportion to the dissolution rate when the same preparations are administered. The narrow range and small value of T_{max} may also eliminate the gastric pH effect.

From the results obtained in this study, we conclude that beagle dogs cannot be used as an animal model for predicting the bioavailability of cinnarizine from capsules in humans, although some problems for evaluating the gastric pH of beagle dogs could be pointed out in this study.

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