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Pharmacokinetic and Pharmacodynamic Studies of the Histamine H_1-Receptor Antagonist Ebastine in Dogs

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Abstract—The pharmacokinetics and pharmacodynamics of ebastine at single oral doses of 10 and 20 mg were studied in six healthy beagle dogs. Plasma concentrations of the active metabolite of ebastine were measured at predetermined times after the dose. At these times an intradermal injection of 0·01 mL of a 0·2 mg mL^{-1} histamine diphosphate solution was given, and wheal areas were computed. The plasma elimination half-life of ebastine was 4·38 ± 1·01 h after 10 mg ebastine and 4·09 ± 0·74 h after 20 mg ebastine; the distribution volume was 3·99 ± 0·88 and 3·65 ± 0·75 L kg^{-1} after 10 and 20 mg of ebastine, respectively; the clearance after the 10 mg dose of ebastine was 0·67 ± 0·24 L h^{-1} kg^{-1} and after 20 mg ebastine was 0·63 ± 0·17 L h^{-1} kg^{-1}. The mean histamine-induced wheal areas were significantly suppressed from 1 to 25 h after the 10 mg dose ebastine and from 1 to 32 h after the 20 mg dose ebastine, compared with the mean predose wheal areas ($P < 0·001$). Maximum suppression of the wheals was 75 and 82% from 10 and 20 mg ebastine, respectively. A combined pharmacokinetic-pharmacodynamic model was used to analyse the relationship between inhibition of wheal skin reaction and changes in the active metabolite of plasma concentration after ebastine administration. A significant delay of 3–4 h was present between the maximum effect and the peak plasma concentration. Calculated from mean data, the rate constant for equilibration of the drug between plasma and effect site was 0·17 and 0·22 h^{-1} after 10 and 20 mg ebastine with a half-life of 4·13 and 3·56 h, respectively, and the steady-state plasma concentration resulting in 50% of maximal effect was 18·9 ± 4.8 ng mL^{-1} after 10 mg and 18·2 ± 5.7 ng mL^{-1} after 20 mg ebastine.

In the last decade, more histamine H_1 antagonists have been developed, which in contrast to the classical antihistamines, evoke less or no sedation, allowing them to be used at full potency (Simons & Simons 1991).

Ebastine (4-diphenylmethoxy-1-(3-(4-terbutylbenzoyl)-propyl) piperidine) has been selected after pharmacological studies had shown that ebastine is a selective H_1-receptor antagonist with no relevant anticholinergic, cardiovascular or central effects; it appears to be a potent long-acting drug in asthmatic airways and in allergic disorders (Roberts et al 1987; Vincent et al 1988b; Wood-Baker & Holgate 1990).

Ebastine seems to be extensively metabolized by a first-pass effect to an active carboxylic acid metabolite which appears to exert the majority of the pharmacological actions associated with ebastine (Vincent et al 1988a).

In the present study, the antihistaminic action of ebastine in the dog was evaluated at two different dose levels by pharmacokinetic-pharmacodynamic modelling, using the active carboxylic acid metabolite plasma concentrations related to inhibition of the wheal skin reaction induced by intradermal injection of 2 μg histamine.

Materials and Methods

A total of six mature male beagle dogs with mean weights of 13·3 ± 1·5 kg was studied. Each dog received a single 10 or 20 mg oral dose of ebastine as one or two commercial tablets of 10 mg (Ebastel). Blood samples were collected at predose, and 0·5, 1, 2, 3, 4, 6, 8, 10, 25, and 32 h after dosing. The plasma was separated and frozen at −20°C.

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Intradermal testing with 0·01 mL histamine phosphate (0·2 mg mL^{-1}) was carried out at the same time as blood samples were obtained. The shaven abdomen was used for each test and the sequence of the sites chosen was identical in all dogs. The reaction was checked by intravenous injection of Evans blue (60 mg mL^{-1} in saline, 0·1 mL kg^{-1}). Wheal circumference was traced at 10 min with a felt-tip pen and then transferred to transparent paper. All injections and tracings of wheal circumferences were made by the same investigator. Wheal areas were measured by a computerized planimetric system.

HPLC analysis

For extraction of the ebastine acid metabolite, 0·5 mL plasma was pipetted into 100 × 16 mm test-tubes and 60 μL internal standard (10 μg mL^{-1}) and 1 mL sodium acetate buffer (0·2 M, pH = 4·5) were added. The internal standard used was the compound LAS Z-120 (*p*-(3-(4-(-α-hydroxy-diphenyl-methyl)-1-piperydyl)propyloxy)-α,α-dimethyl-phenylacetic acid, Lab. Almirall). The samples were mixed by vortex mixer for 15 s and centrifuged at 1500 *g* for 15 min. The supernatant layer was removed and submitted to solid-phase extraction using C2 cassettes for an automated sample processor AASP (Varian Associates). The HPLC system consisted of an AASP automatic injector, 6000A high-pressure pump, 490 variable wavelength absorbance detector set at 257 nm (Waters Associates, Milford, MA, USA) and an Access Chrom data System (Perkin-Elmer Nelson Systems, Inc) in a VAX 3400 (Digital Corporation). A Lichrospher 100 RP-18, 5 μm column (150 × 4 mm, Merck) was used with a mobile phase of 40% acetonitrile and 60% ammonium acetate buffer (0·2 M, pH = 4·5) containing 0·1% triethylamine. At a flow rate of 1 mL min^{-1}, the retention

times for the metabolite and internal standard were 9 and 6 min, respectively. Calibration curves were constructed by plotting the peak area ratios (ebastine acid metabolite/LAS Z-120) vs ebastine acid metabolite concentration and were linear over the concentration range 5–500 ng mL^{-1}. The coefficient of variation at the minimum quantification limit was 18% (six replicates).

Pharmacokinetic analysis

The plasma concentrations from each individual dog were plotted against time. Pharmacokinetic parameters were calculated by standard equations (Gibaldi & Perrier 1982). The terminal elimination rate constants (k_e) were estimated by linear least-squares regression of the terminal phase of the log concentration–time profiles using a Siphar programme (Simed). The elimination half-life was calculated by the equation:

$$t_{\frac{1}{2}} = \frac{0{\cdot}693}{k_e} \tag{1}$$

The linear trapezoidal rule was used to calculate the area under the plasma concentration vs time curve from zero to the last point in time measured (t_n), the remaining area to infinity was determined from the last concentration measured (C_n) and k_e by the following equation:

$$AUC = \frac{C_n}{k_e} \tag{2}$$

Clearance (CL/F) was calculated by equation:

$$CL/F = \frac{Dose}{AUC} \tag{3}$$

where F is the fraction of the active metabolite available to the biophase (bioavailability).

The apparent volume of distribution (Vd/F) was calculated by the equation:

$$Vd/F = \frac{Dose}{AUC \cdot k_e} \tag{4}$$

Pharmacokinetic-pharmacodynamic model

To determine dynamics, the plasma active metabolite plasma concentration–time curves were fitted with a one- and two-compartment, first-order kinetic model by nonlinear least-squares regression. The Akaike Information Criterion (AIC) test (Yamaoka et al 1978) was calculated in order to evaluate the best fit. The values for wheal inhibition were then fitted to the estimated kinetic parameters by an effect-compartment dynamic model, based on the original work of Sheiner et al (1979).

The hypothetical effect compartment, linked to the central compartment of the kinetic model by a first-order rate constant, receives a negligible quantity of drug and therefore does not alter overall kinetics:

$$\frac{dC_e}{dt} = k_{1e}C_1 - k_{eo}C_e \tag{5}$$

where C_e and C_1 are the drug concentration in the effect and central compartments, k_{1e} is the rate constant for the minute amounts of drug transferred from the central to the effect compartment and k_{eo} is the rate constant for drug removal from the effect compartment, resulting in a pharmacodynamic effect. The sigmoidal relationship between drug concentration and effect can be expressed by the general form of the Hill equation (Wagner 1968). This equation permits estimation of the steady-state plasma concentration corresponding to 50% of maximum response (C_{ss}), the half-life for equilibration between the central and effect compartments ($t_{\frac{1}{2}eo}$), and the exponent describing the steepness of the concentration–effect curve (δ).

Also, the biological half-life ($t_{\frac{1}{2}B}$) was calculated by the wheal suppression values vs time by the equation:

$$t_{\frac{1}{2}} = \frac{0{\cdot}693}{k_B} \tag{6}$$

where k_B is the diminution of the effect rate constant.

Results

Pharmacokinetics

The mean metabolite plasma concentrations vs time plot is shown in Fig. 1. After 10 mg ebastine, the mean peak plasma concentration (C_{max}) was $142{\cdot}8 \pm 39{\cdot}8$ ng mL^{-1} and occurred at $3{\cdot}6 \pm 1{\cdot}8$ h (t_{max}). After 20 mg ebastine, C_{max} was $244{\cdot}4 \pm 47{\cdot}1$ ng mL^{-1} and t_{max} $4{\cdot}6 \pm 0{\cdot}7$ h.

Main pharmacokinetic parameters of ebastine active metabolite from six animals at the doses of 10 and 20 mg ebastine are listed in Table 1.

Pharmacodynamics

The histamine-induced wheal areas were analysed as absolute values and as percent reduction of predrug control values by two-way analysis of variance. Before beginning the study with ebastine, all the animals included in the study had intradermal histamine injections at 1, 2, 4, 6, 8 and 12 h in the absence of the drug. The wheal areas obtained were not significantly different between the various times within the same dog. When the animals received ebastine, the wheal area values obtained at any given time were significantly different. Flare reaction cannot be seen in the dog and results are therefore not given in this study.

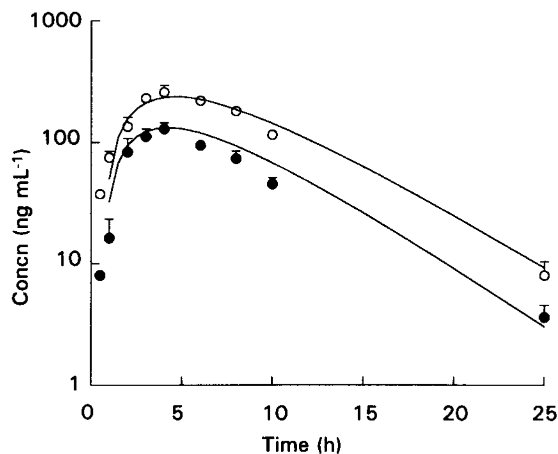

FIG. 1. Mean plasma concentration–time curves of the carboxylic acid metabolite after oral administration of ebastine (● 10, ○ 20 mg).

Table 1. Pharmacokinetic parameters of the ebastine active metabolite in beagle dogs after a single 10 or 20 mg oral dose of ebastine.

Animal	AUC (ng h mL^{-1})	$t_{\frac{1}{2}}$ (h)	CL/F (L h^{-1} kg^{-1})	Vd/F (L kg^{-1})
10 mg ebastine				
A	1659·4	4·31	0·51	3·18
B	1299·5	3·82	0·55	3·02
C	1966·5	5·60	0·45	3·63
D	994·6	3·20	0·97	4·49
E	1380·2	5·60	0·53	4·29
F	730·0	3·77	0·98	5·32
Mean ± s.d.	1338·4 ± 444·8	4·38 ± 1·01	0·67 ± 0·24	3·99 ± 0·88
20 mg ebastine				
A	3447·4	3·57	0·49	2·54
B	1669·9	3·88	0·84	4·73
C	3093·6	4·36	0·57	3·62
D	2885·6	3·51	0·82	4·15
E	2405·7	3·76	0·61	3·31
F	3177·2	5·48	0·45	3·52
Mean ± s.d.	2779·9 ± 645·8	4·09 ± 0·74	0·63 ± 0·17	3·65 ± 0·75

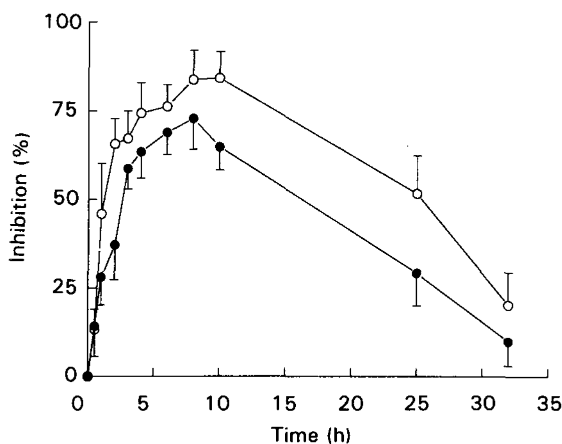

FIG. 2. Percentage change (mean ± s.e.m., n = 6) in histamine-induced wheal area by ebastine (● 10, ○ 20 mg).

Table 2. Dynamic model parameters for the effect of ebastine on wheal skin reaction induced from histamine.

Dose (mg)	k_{eo} (t h^{-1})	$t_{\frac{1}{2}eo}$ (h)	δ	C_{ss} (ng mL^{-1})
10	0·17 ± 0·05	4·13 ± 1·19	1·84 ± 0·80	18·9 ± 4·8
20	0·22 ± 0·07	3·56 ± 1·49	1·89 ± 0·30	18·2 ± 5·7

The mean wheal area after intradermal injection of 0·01 mL of a 0·2 mg mL^{-1} dilution of histamine diphosphate was significantly suppressed from 1 to 25 h after a single 10 or 20 mg dose of ebastine, compared with the mean predose wheal area ($P < 0·001$). The inhibition (%) from 10 or 20 mg doses of ebastine vs time is shown in Fig. 2.

Parameters for the dynamic model are listed in Table 2. The dynamic model for effect chosen was a sigmoid E_{max} model.

The biological half-lives ($t_{\frac{1}{2}B}$) were 10·1 and 12·2 h for the 10 and 20 mg doses, respectively.

Discussion

Second-generation, relatively nonsedating histamine H_1-receptor antagonists are extensively used for the symptomatic treatment of allergic rhinoconjunctivitis and chronic urticaria in man. Information on the pharmacokinetics and pharmacodynamics of these drugs, would allow the optimization of the therapeutic approach (Simons & Simons 1991).

The present study provides a pharmacokinetic and pharmacodynamic model for the investigation of histamine H_1-receptor antagonists in the dog. Ebastine, like terfenadine (Garteiz et al 1982) or loratadine (Radwanski et al 1987), undergoes extensive first-pass metabolism to an active metabolite which exerts most of the histamine H_1-antagonist action (Vincent et al 1988a; Martinez-Tobed et al 1992).

The mean peak concentrations were proportional to the doses (10 or 20 mg), indicating that there was no metabolic saturation. The results obtained for C_{max} and t_{max} are of the same order of magnitude as the values observed in man at doses of 10 or 50 mg (Vincent et al 1988a, b). In contrast, the terminal half-life is shorter in the dog (4·09–4·38 h) than in man (10·3–12·5 h). The mean apparent distribution volume and the mean total clearance were not significantly different when the dose was 10 or 20 mg.

The inhibition of histamine skin wheal is used as an index to evaluate the efficacy and the duration of the antihistamine H_1 effect. In the present study, the mean histamine-induced wheal areas were significantly suppressed from 1 to 25 or 32 h by the 10 or 20 mg dose, respectively, as compared with the mean predose wheal areas. The maximum wheal area was suppressed by over 75 and 85% after 10 or 20 mg, respectively (Fig. 2).

The mean peak plasma concentration of the active metabolite preceded the peak suppressive effect of the wheal by 3–4 h, as has previously been reported for other histamine H_1-receptor antagonists such as chlorpheniramine (Simons et al 1982a), brompheniramine (Simons et al 1982b), hydroxyzine (Simons et al 1984) or triprolidine (Simons et al 1986). This is probably due to the mechanism of action of the histamine H_1-receptor antagonists.

The half-life obtained from the effect compartment ($t_{\frac{1}{2}eo}$), measures the rate of equilibration in the site of action. In our study the value for that parameter was about 4 h, which could partially explain the delay in pharmacological effects with respect to the drug plasma concentration.

The values of C_{ss} are 18·9 ± 4.8 and 18·2 ± 5.7 ng mL^{-1} for the 10 and 20 mg doses, respectively. The difference observed in some results is probably due to the changes in the E_{max} which has been calculated from our experimental data (and not previously fixed). The E_{max} fit is then responsible for these variations. Nevertheless, the results indicate that ebastine is a very active drug.

Wheal suppression after a 10 or 20 mg dose ebastine appears to be excellent in the dog. Twenty four hours after the administration of a 20 mg dose ebastine (1·5 mg kg^{-1}), over 50% histamine wheal inhibition can be seen, and significant suppression of the wheal was found even when metabolite plasma concentrations were low. The biological half-life is more prolonged than would be predicted from the terminal plasma half-life for the active metabolite. In these cases, although the plasma half-life does not support it, probably 20 mg doses ebastine once or twice daily would be appropriate for satisfactory relief of itchiness and for promoting faster healing of skin lesions in dogs with atopic dermatitis. At present, there are no published pharmacokinetic or pharmacodynamic data on ebastine in allergic dogs, and an effort to study this further would be of interest.

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