BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **20**: 101–106 (1999)

Pharmacokinetic–Pharmacodynamic Analysis of the Arrhythmogenic Potency of a Novel Antiallergic Agent, Ebastine, in Rats

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ABSTRACT: Ebastine (EBS), a novel nonsedative antiallergic agent, is similar to terfenadine in its chemical structure. However, clinical arrhythmogenicity of EBS remains controversial. In this study, we evaluated the possible arrhythmogenic potency of EBS as assessed by QT prolongation from a pharmacokinetic–pharmacodynamic viewpoint in comparison with that of terfenadine. EBS was intravenously infused into anesthetized rats at a rate of 3.0 or 10 mg/kg/h for 60 min, and electrocardiographic effects were continuously monitored from lead II. The plasma concentrations of EBS and its major metabolite, carebastine, were also measured under the same conditions. When intravenously administered, EBS exhibited QT prolongation in an infusion rate-dependent manner, with a lag time. Pharmacokinetic–pharmacodynamic analysis of EBS based on the effect-compartment model revealed values of EC_{50}, E_{max} and $EC_{10\ ms}$ (where 10 ms of QT prolongation was evoked) of 0.73 μg/mL, 12.5 ms and 2.90 μg/mL, respectively. The $EC_{10\ ms}$ value of EBS was five times higher than that of terfenadine reported previously (Ohtani *et al*., *J. Pharm. Pharmacol*., **49**, 458–462 (1997)). In conclusion, EBS was suggested to be less arrhythmogenic than terfenadine. Copyright © 1999 John Wiley & Sons, Ltd.

Key words: ebastine; terfenadine; pharmacodynamics; ECG; arrhythmia

Introduction

It has become apparent that terfenadine can cause fatal cardiac arrhythmia [1–3] under certain conditions, and the adverse reaction is attributed to the arrhythmogenicity of the unchanged terfenadine, which acts as a potent inhibitor of a cardiac potassium channel [4]. When the first-pass metabolism forming the pharmacologically active acid metabolite was impaired by hepatic dysfunction or by co-administration of drugs that inhibit cytochrome P450 3A4, an increase in the bioavailability of unchanged terfenadine, resulting in its elevated plasma level, leads to fatal arrhythmia including torsades de pointes [4,5].

Ebastine (EBS), a novel nonsedative antiallergic agent, is similar to terfenadine in its chemical structure (Figure 1) and its pharmacokinetic characteristics. Orally administered EBS undergoes extensive first-pass biotransformation and it is almost completely converted to an acid metabolite [6,7], carebastine, which exerts the antiallergic action.

Therefore, EBS might also cause arrhythmia under certain conditions. Although there have been no clinical reports of QT prolongation nor of arrhythmia associated with EBS, this agent has been shown to inhibit cardiac potassium current *in vitro* [8]. Moreover, Hey *et al*. [9] reported in guinea pigs that intravenous injection of EBS evoked QT prolongation, suggesting its possible arrhythmogenicity, albeit less potent than terfenadine. However, there have been no reports describing the quantitative relationship between QT prolongation and plasma EBS concentration *in vivo*.

We reported [10] previously that for evaluation of the arrhythmogenic risk of drugs it is appropriate to analyse the time profile of QT prolongation with intravenous infusion of the drug in rats. In the last study we carried out a comparative pharmacokinetic–pharmacodynamic analysis for the arrhythmogenic risks of terfenadine and epinastine [11].

This study was performed to quantitatively investigate the arrhythmogenicity of EBS in rats in comparison with that of terfenadine.

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Contract/grant sponsor: Tokyo Biochemical Research Foundation; Contract/grant number: B3-06

CCC 0142–2782/99/020101–06$17.50

Received 14 July 1998
Accepted 30 November 1998

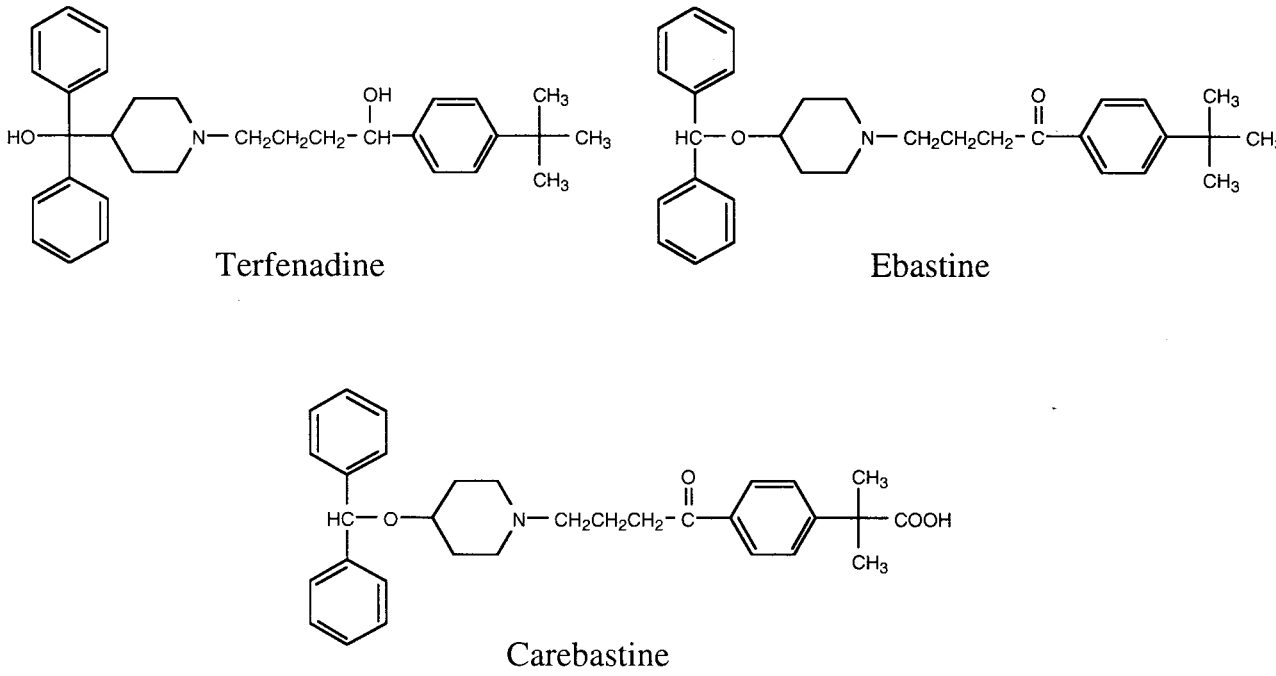

Figure 1. The chemical structures of ebastine, its major metabolite (carebastine) and terfenadine

Materials and Methods

Chemicals

EBS and carebastine were gifts from Dainippon Pharmaceutics Co. Ltd (Osaka, Japan). All other chemicals used were of reagent grade and obtained from commercial sources.

Pharmacodynamic Experiments

Male Sprague–Dawley rats weighing 300–450 g were purchased from Nippon Seibutsu Zairyou Center (Tokyo, Japan) and anesthetized by i.p. administration of urethane (1.2 g/kg) and α-chlorarose (30 mg/kg). The precordial and limb hair were removed with hair removing cream (Hair remover, Kanebo, Tokyo, Japan). With the animals restrained in the supine position, the trachea, right jugular vein and right carotid artery were cannulated with polyethylene tubing. The body temperature was maintained at 37.5 ± 0.5°C throughout the experiments by means of a hot-water circulating heat pad placed beneath the animals. Electrocardiography (ECG) was performed and the results were analysed by the method of Ohtani *et al*. [10]. The QT interval was defined as the time from the start of QRS complex to the end of the T wave, at which time the amplitude of the T wave declined to 10% of its maximal value.

After stabilization of the ECG and body temperature, physiological salt solution (PSS: NaCl, 135 mM; $NaHCO_3$, 11.9 mM; KCl, 5.4 mM; $CaCl_2$, 1.8 mM; $MgCl_2$, 1.0 mM) was infused into the jugular vein at a rate of 2.32 mL/h for 10 min with an infusion pump (MODEL 975, Harvard Apparatus, MA, USA). EBS (3.0 or 10 mg/kg/h) was then infused in the same manner. EBS was dissolved in PSS according to the method previously described by Webb [12]. Briefly, EBS was dissolved in a stoichiometrically equivalent amount of oleic acid and benzyl alcohol, and subsequently solubilized in PSS with the use of a surfactant (Tween 80; Polysorbate 80 USP XIX).

ECG was carried out prior to PSS administration, from 1 min before to 19 min after the start of infusion and at 20, 30, 40, 50 and 60 min postinfusion.

Pharmacokinetic Experiments

Pharmacokinetic studies were performed using another group of animals not used in the pharmacodynamic experiments. All conditions were identical to those of the pharmacodynamic experiments as described above, with the exception of blood sampling from the carotid artery at 2, 5, 15, 30 and 60 min after drug administration. Blood samples (approximately 250 μL) were centrifuged to collect 100 μL of plasma. The concentrations of EBS and its major metabolite, carebastine, in plasma were determined by high performance liquid chromatography (HPLC) as described below.

Determination of EBS and Carebastine in Rat Plasma by HPLC

Aliquots of 50 μL of plasma were mixed with 150 μL of drug-free plasma and with 400 μL of methanol and acetonitrile mixture (1:1, v/v). The

samples were diluted with 400 μL of water and spiked with 200 μL of 0.2 M acetate buffer (pH 4.0). After mixing, the sample was centrifuged at 1300 × *g* for 10 min, and the supernatant was applied to a separation column (Bond Elut C18 Column, Varian, CA, USA), which was then washed with water and methanol. The compounds were eluted with a mixture of methanol and 50 mM phosphate buffer (pH 2.5) (9:1, v/v). The eluate was spiked with an external standard (flunarizine hydrochloride) and dried *in vacuo* at 55°C. The residue was reconstituted with 100 μL of the mobile phase as described below and applied onto the HPLC. The column used for chromatographic separation was a Spherisorb S5CN (25 cm × 4.6 mm i.d.) which was kept at 40°C. The mobile phase consisted of acetonitrile, methanol and 12 mM ammonium acetate buffer (20:30:48, v/v), and was pumped at a rate of 1.5 mL/min. Absorbance was determined at 254 nm.

Model Analysis

The pharmacokinetic parameters, i.e. V_1, k_{21}, α and β, were distinctly derived for each infusion rate by fitting the plasma concentration of EBS (C_p) to a conventional 2-compartment model with zero-order infusion, using the nonlinear least-squares regression program (MULTI [13]), where V_1 is the distribution volume of the central compartment, k_{21} indicates the rate constant for transfer from the peripheral to the central compartment, and α and β represent the exponential rate constants for the 2-compartment model.

The effect compartment model introduced by Sheiner *et al*. [14] was applied for analysis of QT prolongation evoked by EBS because a delay in the effect against the plasma concentration was observed. The pharmacological effect (E) was related to the concentration of the effect-compartment (C_e) by Equation (1).

$$E = \frac{E_{max} \cdot C_e}{EC_{50} + C_e}, \quad (1)$$

where E_{max} and EC_{50} denote the maximum effect and concentration where the half-maximal effect was evoked, respectively. C_e was calculated with a conventional 2-compartment model with zero-order infusion [15] as follows:

$$C_e = \frac{I \cdot k_{e0}}{V_1}\left[\frac{(k_{21}-\alpha)(e^{\alpha \cdot t}-1)\, e^{-\alpha \cdot t}}{\alpha(\beta-\alpha)(k_{e0}-\alpha)} + \frac{(k_{21}-\beta)(e^{\beta \cdot t}-1)\, e^{-\beta \cdot t}}{\beta(\alpha-\beta)(k_{e0}-\beta)} + \frac{(k_{21}-k_{e0})(e^{k_{e0} \cdot t}-1)\, e^{-k_{e0} \cdot t}}{k_{e0}(\alpha-k_{e0})(\beta-k_{e0})}\right], \quad (2)$$

where k_{e0} indicates the elimination rate constant from the effect compartment and I represents the infusion rate of the drug.

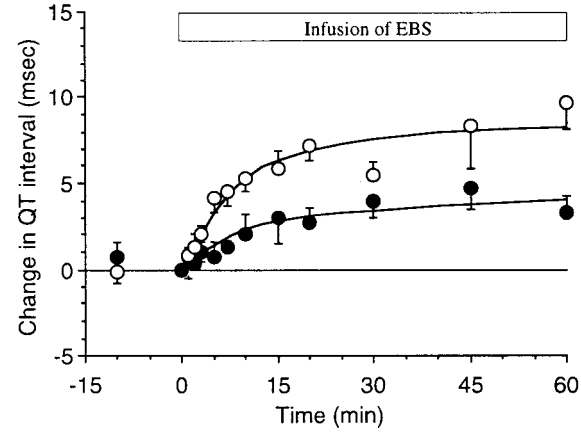

Figure 2. Effects of ebastine on QT interval in rats. The curves represent the simulation profiles of QT prolongation derived from Equations (1) and (2) (●, 3.0 mg/kg/h; ○, 10 mg/kg/h; mean ± S.E.M. from three to five rats)

The pharmacodynamic parameters, E_{max}, EC_{50} and k_{e0}, were derived by simultaneous fitting of the ECG effects (E) at all infusion rates to Equations (1) and (2) using the nonlinear least-squares regression program (MULTI [13]).

Results

Effects of EBS on ECG

Figure 2 shows time profiles of QT interval during constant intravenous infusion of EBS. Although EBS evoked QT prolongation in an infusion rate-dependent manner, the maximal prolongation was relatively slight (9.7 ms) even at higher infusion rates (Figure 2 and Table 1). EBS also evoked bradycardia and PR prolongation in an infusion rate-dependent manner in the current dosage range (data not shown).

Pharmacokinetics of EBS and Carebastine

Figure 3 shows pharmacokinetic profiles of EBS during constant intravenous infusion at the two infusion rates. Pharmacokinetic parameters are presented in the legend to Figure 3. While EBS apparently reached the steady-state concentration of

Table 1. Pharmacodynamic parameters of ebastine for QT prolongation, compared with those of terfenadine

Parameters	Ebastine	Terfenadine[a]
k_{e0} (min^{-1})	0.132 ± 0.043*	0.0604 ± 0.0206*
E_{max} (ms)	12.5 ± 1.8*	30.3 ± 7.1*
EC_{50} (μg/mL)	0.731 ± 0.255*	1.04 ± 0.47*
E_{max}/EC_{50} (ms · mL/μg)	17.1	29.2
$EC_{10\ ms}$ (μg/mL)[b]	2.90	0.512

[a] From Reference [11]; [b] concentration required to evoke QT prolongation by 10 ms.
* Estimates ± S.D. obtained by curve-fitting with MULTI [13].

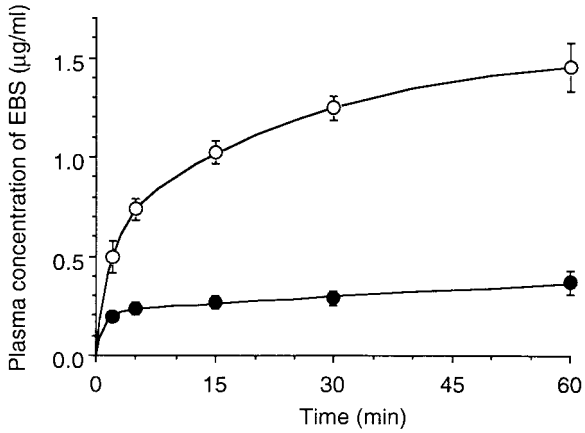

Figure 3. The pharmacokinetic profiles of ebastine during its constant intravenous infusion into rats. The curves represent fitting lines based on a 2-compartment open model with a zero-order infusion process. The pharmacokinetic parameters of V_1, k_{21}, α and β obtained by curve-fitting with MULTI [13] were 226 mL/kg, 0.0138, 0.974 and 0.00245 min^{-1} for the infusion rate of 3.0 mg/kg/h, and 411 mL/kg, 0.0914, 0.614 and 0.0390 min^{-1} for infusion at a rate of 10 mg/kg/h, respectively (●, 3.0 mg/kg/h; ○, 10 mg/kg/h; mean ± S.E.M. from five rats)

approximately 0.3 μg/mL within 10 min after commencement of infusion at a rate of 3.0 mg/kg/h, the EBS concentration increased rapidly at 10 mg/kg/h and then gradually increased to approximately 1.5 μg/mL at 60 min, indicating a nonlinear relationship between EBS concentration and infusion rate. Carebastine was not detected until 15 min after the start of infusion except in one of five animals infused at the higher rate. Plasma average concentrations of carebastine at 60 min after the start of infusion were 0.14 and 0.21 μg/mL at rates of 3.0 and 10 mg/kg/h, respectively, which were lower than EBS concentrations.

Pharmacokinetic–Pharmacodynamic Relationship of EBS

A delay in QT prolongation against the plasma EBS concentration was observed. Moreover, the difference in maximal QT prolongation between the high and low infusion rates was less than 2-fold, whereas the ratio of maximal EBS concentration was more than 4-fold, indicating a saturable relationship between plasma concentration of EBS and QT prolongation. Therefore, the relationship between the plasma EBS concentration and QT prolongation was analysed using an E_{max} model including an effect compartment. Table 1 shows the pharmacodynamic parameters for EBS, along with those of terfenadine reported previously [11].

Discussion

In this study, we investigated the arrhythmogenicity of ebastine. Although no clinical cases of EBS-induced QT prolongation or torsades de pointes have been reported, unchanged EBS was demonstrated to possess arrhythmogenic potency to some extent *in vitro* and *in vivo* in experimental animals [9]. However, no studies have been conducted to elucidate the quantitative relationship between plasma EBS concentration and QT prolongation. Therefore, it would be clinically worthwhile to analyse this relationship for assessment of the clinical arrhythmogenic risk of EBS in comparison with that of terfenadine.

In the present study, intravenously administered EBS was found to evoke QT prolongation in rats which is consistent with previous findings in guinea pigs [9]. However, both E_{max} and E_{max}/EC_{50} values for EBS were smaller than terfenadine. Therefore, EBS is considered to be less potent for QT prolongation than those for terfenadine. The $EC_{10\ ms}$ value (where 10 ms of QT prolongation is theoretically evoked) of EBS was 5-fold higher than that of terfenadine (Table 1). It is interesting to note that the E_{max} value of EBS for QT prolongation was as small as 12.5 ms, while that of terfenadine was reported to be 30.3 ms [11] under the same experimental conditions. This finding is consistent with the report of Ko *et al*. [8] that the maximum blockade with EBS of *HERG*-induced rapidly delayed rectifier potassium current (I_{Kr}) was 46% and that with terfenadine was up to 80%. This less potent inhibitory feature of EBS on I_{Kr} corresponded well with our result regarding the reduced risk of QT prolongation by EBS in rats. Along with the QT prolongation, EBS also evoked bradycardia and PR prolongation without II° or III° atrioventricular block (data not shown). These results are also consistent with a previous report by Hey *et al*. [9].

Carebastine was reported [9] to be impotent for QT prolongation in guinea pigs after intravenous administration even at a high dose of 50 mg/kg, which far exceeded the theoretical amount of carebastine biologically transformed from EBS in the present study even at the higher infusion rate of 10 mg/kg/h. Thus, the effect of carebastine on QT interval was considered to be negligible under the current experimental protocol.

After the start of infusion, a delay of QT prolongation was observed against the immediate increase in plasma EBS concentration (Figures 2 and 3). Similar results were obtained with terfenadine [11]. Therefore, analysis of the relationship between QT prolongation and plasma EBS concentration was carried out with a kinetic model incorporating an 'effect' compartment. Although EBS, along with terfenadine and quinidine, is considered to evoke QT prolongation via blockade of cardiac potassium current [4,8], only quinidine has no lag time [10] in contrast to EBS and terfenadine, suggesting that the delay observed with EBS may be attributable to its pharmacokinetic process such as drug transfer from blood to its effect site (i.e. the ventricles).

In this study, we employed a k_{e0} value independent of infusion rate. Although another model including two k_{e0} values dependent on infusion rate is also possible, introduction of separate k_{e0} values did not yield a significant improvement in the fit when assessed by F-statistics [16].

A nonlinear relationship between the plasma EBS kinetics and infusion rates was observed. Nonlinear metabolism of EBS is the most plausible explanation for this observation. In fact, average plasma concentrations of the major metabolite, carebastine, 60 min after the start of infusion were 0.14 and 0.21 μg/mL at infusion rates of 3.0 and 10 mg/kg/h, respectively, supporting the saturation hypothesis.

The plasma concentration of EBS in the present experimental protocol (0.2–1.5 μg/mL) was much higher than the maximum EBS concentration after oral administration of regular doses in humans (< 6 ng/mL) [6]. On the other hand, when administered orally, EBS undergoes extensive first-pass metabolism and it is almost completely converted to the active metabolite. Therefore, under conditions of impaired first-pass metabolism in disease states or due to drug interactions, plasma EBS concentration could be markedly elevated. In fact, plasma concentration of EBS was reported [17] to be elevated to 20.3 and 55.7 ng/mL in patients receiving ketoconazole (400 mg/day) or erythromycin (500 mg, q6h), respectively, even lower than the plasma EBS concentration range in the present study. However, the EBS concentration range in the present study may be clinically feasible if EBS exceeding its ordinary dose is administered to patients with impaired first-pass metabolism.

EBS was found to be less potent for QT prolongation than terfenadine. Concerning the clinical arrhythmogenic risks of these agents, however, it is an essential requisite to evaluate their kinetic susceptibility to metabolic inhibitors and their plasma concentrations after adminstration of ordinary dosages of these drugs. EBS was not detected in plasma after repeated administration of twice the ordinary dosage (20 mg/day) for 7 days [6] (detection limit: 6 ng/mL). Plasma concentration of unmetabolized terfenadine after repeated administration (60 mg, twice/day) was estimated to be 1–2 ng/mL [18]. Thus, the concentrations of unmetabolized EBS and terfenadine after administration of ordinary doses are considered to be comparable. On the other hand, only limited studies have been conducted to compare the pharmacokinetic susceptibilities of EBS and terfenadine. The plasma concentration of terfenadine was reported to reach up to 57 ng/mL in a patient receiving terfenadine with ketoconazole, who demonstrated torsades de pointes [2]. On the other hand, when EBS was concomitantly administered with erythromycin or itraconazole, plasma EBS concentration should reach up to 20.3 and 55.7 ng/mL, respectively, although there is no apparent correlation between plasma EBS concentration and QTc prolongation [15]. In addition, Hashizume *et al*. [19] reported that EBS is metabolized not only via the CYP3A-mediated pathway but also via another hydroxylating pathway independent of CYP3A, which has not been reported for terfenadine. These observations suggested that the pharmacokinetic susceptibilities of EBS and terfenadine are also comparable.

The present study quantitatively demonstrated in rats that intravenously administered EBS itself exerts an arrhythmogenic effect exhibited by QT-prolongation, although the arrhythmogenicity of EBS was less potent than that of terfenadine. Even taking into consideration clinical factors such as ordinary plasma concentrations and metabolic susceptibility, clinical arrhythmogenic risk of EBS is likely to be lower than that of terfenadine. However, if EBS exceeding its ordinary dose is administered to patients with impaired first-pass metabolism due to hepatic dysfunction or co-administered with high doses of drugs that inhibit metabolism of EBS, arrhythmogenic risk may be increased. Further studies of the kinetics of EBS and terfenadine in a clinical setting are necessary to confirm the above hypothesis.

Acknowledgements

This study was supported in part by a Grant-in-Aid from the Tokyo Biochemical Research Foundation (B3-06). We acknowledge Miss M. Hirota for her technical assistance. We also acknowledge Dainippon Pharmaceutical Co. Ltd for their assistance in measuring plasma concentrations of EBS and carebastine.

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