Nasal Absorption Kinetic Behavior of Azetirelin and Its Enhancement by Acylcarnitines in Rats

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Purpose. The long-term stability and nasal absorption characteristics of a basic nasal formulation of azetirelin, a thyrotropin-releasing hormone analog and its absorption enhancement by incorporation of acylcarnitines in the formulation were investigated.

Methods. The long-term stability of basic nasal azetirelin formulations at 25 °C was predicted by calculation from the Arrhenius plot of the data on 6 months' storage at 40, 50 and 60 °C. Nasal azetirelin absorption characteristics were kinetically examined by intranasal administration to rats, determination of plasma azetirelin level by radioimmunoassay, and fitting the data to a two-compartment model including absorption rate.

Results. Basic nasal azetirelin formulations of pH 4.0 and pH 5.1 were predicted to be highly stable. Residual azetirelin after 2 years storage at 25 °C was greater than 95%. Nasal absorption characteristics of this formulation in the pH 4.0–6.3 range showed pH-dependency, with pH 4.0 showing the highest absolute bioavailability (Bioav) of 17.1%. This nasal Bioav was 21 times greater than that of oral administration (0.8%). Acylcarnitines with 12 or more carbon atoms in the acyl chain greatly enhanced nasal absorption of azetirelin: Bioav with lauroylcarnitine chloride (LCC) and palmitoylcarnitine chloride were 96.9% and 72.9%, respectively. This enhancement by LCC plateaued at the low concentration of 0.1%.

Conclusions. The basic nasal azetirelin formulation at pH 4.0 is stable and shows adequate absorption, with nasal absorption having greater Bioav than oral absorption. The 12-carbon acylate LCC was the strongest enhancer among acylcarnitines and provided near-total delivery of the administered dose to the blood.

KEY WORDS: azetirelin; thyrotropin-releasing hormone analog; stability; nasal absorption; acylcarnitines.

INTRODUCTION

Azetirelin is a novel analog of the tripeptide thyrotropin-releasing hormone (TRH) in which the pyroglutamyl moiety of TRH is substituted by an (oxo-azetidinyl) carbonyl moiety. The activating effects of azetirelin on the CNS, such as its inhibition of pentobarbital-induced sleep and reserpine-induced hypothermia, are about 10–100 times more potent and 8–36 times longer-acting than those of TRH in mice (1). Unlike TRH, which undergoes rapid enzymatic inactivation in the body, azetirelin is extremely stable in plasma and is degraded much more slowly than TRH in brain homogenates (2). This increased metabolic stability of azetirelin may confer greater pharmacological potency and efficacy over TRH. Although azetirelin has strong potential pharmacological properties when administered by intravenous injection, its estimated oral bioavailability is reported to be very low, around only 2% (3).

In recent years, the systemic delivery of peptides and proteins by nasal administration as a non-parenteral route has received growing attention. However, development of clinically useful peptides and proteins has met with limited success. Larger molecules in particular show little or no systemic absorption upon nasal administration and require the use of effective and safe enhancers. Among enhancers studied to date, however, polyoxyethylene 9-lauryl ether, deoxycholate, sodium tauro-24, 25-dihydrofusidate, and lysophosphatidylcholine have been recently shown to produce irreversible or severe side effects on mucocilliary transport rate, nasal morphology and ciliary beat frequency (4). In contrast, acylcarnitines are endogenous amino acid-like compounds which play a role in the cellular mitochondrial transport system by carrying fatty acids across the mitochondrial membrane (5). The local mucosal safety of palmitoylcarnitine has been recently demonstrated. This compound's enhancement of drug absorption (6) and mucosal trans-epithelial electrical resistance (7) were reversible, and no mucosal morphological change was reported (6,7).

The aim of the present study was to investigate the nasal absorption profile of azetirelin using a rat model. First, the long-term stability of basic nasal azetirelin formulations was tested at various pHs and storage temperatures. Second, the potential nasal azetirelin absorption in these solutions was assessed. Finally, the enhancing ability of acylcarnitines on nasal absorption of azetirelin was investigated using various acylcarnitines.

MATERIALS AND METHODS

Chemicals and Drug Solutions

Azetirelin (N^6-[(S)-4-oxo-2-azetidinyl] carbonyl]-l-histidyl-l-prolineamide dihydrate, MW=384.39, pKa=6.2) was synthesized in the Central Research Laboratories of Yamanouchi Pharmaceutical Co., Ltd., dl-Carnitine hydrochloride (CHC), dl-octanoylcarnitine chloride (OCC), dl-lauroylcarnitine chloride (LCC), palmitoyl-dl-carnitine chloride (PCC), sodium taurocholate (TRC), phenylephrine hydrochloride, and sodium 1-heptansulfonate were purchased from Sigma Chemical Co., Ltd. (USA). All other reagents used were of reagent grade.

The basic nasal formulation contained azetirelin (50 mg/mL) in 0.01 M isotonic citrate buffer, with benzalkonium chloride (0.01%) as a preservative. After incorporation of azetirelin and acylcarnitines, all solutions were adjusted for pH with NaOH or HCl. PCC was incorporated in the basic formulation before use.

Stability Studies

Three basic nasal solutions at pH 4.0, 5.1 and 6.3 were prepared. All sample solutions were sealed in 5-mL glass ampoules and stored in an oven at 40, 50 or 60 °C for 6 months. At specified storage times, samples were collected and stored in a freezer at −20 °C until HPLC analysis. pH values were also measured in each sample to confirm pH stability.
HPLC Analysis

HPLC analysis was carried out by the internal standard method. Samples were diluted 50 times and mixed with an equivalent volume of 0.1% phenylephrine hydrochloride internal standard. Ten milliliters of this mixture was injected into a Nucleosil 5C18 HPLC column (5 μm, 4 mm × 150 mm, Chemco Science Co., Ltd., Japan) for separation of analytes and detection at 220 nm. The mobile phase was a mixture of 2% sodium 1-heptansulfonate : acetonitrile : methanol (50:3:3), pumped at 1.5 mL/min at less than 40 °C. The concentration of azetrelin was determined by comparing the peak area ratio (drug/internal standard) of sample with that of standard solutions from the calibration curve.

Animal Experiments

All experiments were conducted in adherence to the “Principles of the Animal Ethical Committee of Yamanouchi Pharmaceutical Co., Ltd.” Male Fischer rats (135-182 g, 8 weeks) were fasted for 20 h before administration. Anesthesia was induced by intraperitoneal injection of sodium pentobarbital (Nembutal®; Abbott Laboratories, USA) at 50 mg/kg 10 min before administration and maintained with additional injections at 40 mg/kg. No surgical operation was conducted.

For intranasal administration, the nasopalatine was closed and a cymacrylate adhesive agent (Aron Alpha A®, Sankyo Co., Japan). Polyethylene tubing (PE10) connected to a 5-μL micro-syringe was then inserted about 4 mm into the right nasal cavity. A buffered azetrelin solution sample (1 mg/20 μL/kg) was administered within 1 min. For oral administration, a buffered azetrelin solution sample (10 mg/4 mL/kg) in saline (0.9% NaCl) was injected into the jugular vein. At each time point, a rat was sacrificed and blood was collected from the inferior vena cava with a heparinized syringe. Plasma was separated by centrifugation at 3000 rpm for 10 min at 4 °C and stored at −20 °C until assay. After extraction of a 0.5 mL plasma sample with methanol, the plasma concentrations of azetrelin were determined by radioimmunoassay (8).

Pharmacokinetic Analysis

For pharmacokinetic analysis of the overall absorption behavior of azetrelin in rats, a two compartment analysis model including a nasal (or oral) compartment was used. Equations 1 and 2 were derived by integration:

\[ C_1 = A e^{-\alpha x t} + B e^{-\beta x t} \]

\[ C_2 = D_1 \left[ \frac{(K_{21} - \alpha)}{V_c} \right] e^{-\alpha x t} + \left[ \frac{(K_{21} - \beta)}{(\alpha - \beta)} \right] e^{-\beta x t} \]

where \( V_c \) is the volume of the central compartment; \( \alpha \) and \( \beta \) are the first-order macro-rate constants describing the disposition of the drug; \( K_{21} \) and \( K_{21} \) are the first-order rate constants for the transfer of drug between central and peripheral compartments; \( C_1 \) and \( C_2 \) are the concentrations of drug administered intravenously and intranasally (or orally), respectively; \( D_1 \) and \( D_2 \) are the amounts of drug administered; and \( \text{LagT} \), \( F_a \), and \( K_e \) are the lag time, fraction absorbed, and first-order absorption rate constant of nasal (or oral) absorption, respectively. Absolute bioavailability (Bioav) was calculated as \( F_a \times 100 \). Absorption-time duration time, the time to absorption of 95% of total possible azetrelin absorption (T95%), was calculated as \( \text{LagT} - \ln(0.05/K_e) \).

Intravenous pharmacokinetic parameters were estimated by fitting Eq. 1 to the average data of rats. Using intravenous parameters of \( V_c \), \( K_{21} \), \( K_{21} \), \( \alpha \) and \( \beta \), intranasal (or oral) absorption pharmacokinetic parameters (LagT, F_a, and K_e) were estimated by fitting Eq. 2 to the average data sets. Computation was carried out using the nonlinear least-squares regression analysis program NONLIN® (Statistical Consultants, USA) on a VAX6210 digital computer (Digital Equipment Corp., USA). Hartley and Levenberg’s modification of the Gauss-Newton method was used.

RESULTS AND DISCUSSION

Degradation Kinetics of Basic Nasal Azetrelin Formulation

In order to optimize a basic nasal formulation, the stability of buffered azetrelin solutions was tested. Preliminary studies showed that azetrelin was not sufficiently stable at pHs greater than 7.0. Therefore, the pH range of the buffer was set between 4 and 6. Figure 1 (inset) shows representative stability profile sets for azetrelin in pH 4.0 citrate buffer solution containing a preservative at different temperatures (40, 50 and 60 °C) for 6 months. The linear relationship between logarithmic percentage remaining and storage time indicates pseudo-first-order degradation kinetics. The degradation rate constant was calculated from the slope of the graph by linear regression analysis. Regression lines were linear for all pH values studied, with a correlation coefficient (r) > 0.957.

The Arrhenius plot was constructed from these observed first-order degradation rate constants at three temperatures (Fig. 1). This plot shows a good linear relationship between logarithmic degradation rate (K_log) and the reciprocal of absolute temperature (T): \( r = 1.000 \) for pH 4.0, 0.999 for pH 5.1, and 0.997 for pH 6.3 (Table 1). Activation energy (E_a) was evaluated according to Arrhenius equation: In K_log = In A - (E_a/RT), where A is the frequency factor and K is the gas constant. From these Arrhenius parameters, the percent remaining (R_a) of azetrelin at specified temperature and storage time (t) conditions can be predicted by the equation \( R_a = 100 \times e^{-\frac{1}{A} \times \text{exp}(-E_a/RT) \times t} \). Long-term stability of basic nasal azetrelin formulations was predicted at 25 °C which is average room temperature. Therefore it is of greatest importance to assure product stability at this temperature. It was found that R_a was pH-dependent, greater than 95% residual azetrelin was predicted after 2 years’ storage for the pH 4.0 (95.1%) and 5.1 (97.6%) formulations, but less than 90% for the pH 6.3 (89.1%).