Effect of Food on the Bioavailability of SDZ DJN 608, an Oral Hypoglycemic Agent, from a Tablet and a Liquid-Filled Capsule in the Dog

Francis L. S. Tse,1,3 D. Labbadia,1 K. Habucky,1 A. Karara,1 and S. Au2

Received September 22, 1995; accepted November 21, 1995

Purpose. The effect of food on the bioavailability of SDZ DJN 608, a D-phenylalanine derivative, was investigated in three mature, male beagle dogs.

Methods. Each dog received, under fasting and postprandial conditions, a 30 mg oral dose as a tablet (T) and a liquid-filled capsule (LC). Additionally, a 5 mg intravenous dose was given in the fasting state. Doses in the same dog were separated by 1-week washout periods. Serial plasma samples were collected for 24 h postdose and analyzed for SDZ DJN 608 using HPLC. Model-independent pharmacokinetic parameters were compared between treatments by 3-way ANOVA. In vitro dissolution profiles of T and LC were generated using the USP paddle method. In addition, the transport of SDZ DJN 608 through a Caco-2 cell monolayer was examined at concentrations of 0.1 and 1 mM, in the absence and presence of an aromatic amino acid, L-α-methyl-dopa, the transport of which is mediated by the large neutral amino acid (LNAA) carrier.

Results. In the dog, SDZ DJN 608 was rapidly absorbed. The peak plasma concentration (Cmax) averaged higher, and the peak time (tmax) shorter, after LC than T, though the differences were not statistically significant. This finding is consistent with in vitro dissolution data showing that, at both pH 1.2 and pH 6.8, the dissolution rate of LC was faster than that of T. No significant difference in the area under curve (AUC) was observed between LC and T, the absolute bioavailability of both being complete in the fasting state. Whereas the presence of food showed little effect on the tmax and Cmax of either dosage form, it significantly reduced the AUC, the effect (ca. 20%) being not different between LC and T. In the Caco-2 model, the mucosal-to-serosal permeability of SDZ DJN 608 was independent of concentration and unaffected by L-α-methyl-dopa, suggesting passive diffusion of the former.

Conclusions. Food had little effect on the absorption rate but significantly reduced the bioavailability of SDZ DJN 608 regardless of the dosage form. This effect is unlikely to be caused by inhibition of the transepithelial transport of SDZ DJN 608 by amino acids in the diet.

KEY WORDS: hypoglycemic; bioavailability; food; dosage form; dissolution; Caco-2 cell model.

INTRODUCTION

Sandoz compound SDZ DJN 608, N-(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine, is a new nonsulfonyl-

urea oral hypoglycemic agent (1) currently being developed for the treatment of Type II diabetes (NIDDM, non-insulin dependent diabetes mellitus). Preliminary studies in rats and dogs have shown that the compound stimulates insulin secretion with a rapid onset and short duration of action, thus rendering it ideal for the rapid control of hyperglycemia following a meal without causing delayed hypoglycemia (2). Strict control of blood glucose levels may reduce the frequency and progression of diabetic complications (3).

Since SDZ DJN 608 is intended for use with meals, the potential effect of food on its bioavailability needs to be determined. Additionally, it would be useful to understand the mechanism of drug transport across the intestinal mucosa. If the transport is passive, it may be possible to modify any food effect by formulation changes. Conversely, if the transport of this phenylalanine derivative is active and mediated by the large neutral amino acid (LNAA) carrier, it may compete with amino acids in the diet for the carrier (4), in which case any food effect on the bioavailability of SDZ DJN 608 most likely will be independent of formulation factors. Using the dog as an animal model, the present study investigated the bioavailability of SDZ DJN 608 under fasting and postprandial conditions. Two formulations with different in vitro dissolution characteristics, a tablet (T) and a liquid-filled capsule (LC), were tested in the in vivo experiments. Furthermore, the mechanism of transport of SDZ DJN 608 across the intestinal epithelium was studied using Caco-2 cell monolayers as a model system (5,6). It has been shown that Caco-2 cells have a LNAA carrier system capable of mediating the transepithelial transport of amino acids (7).

MATERIALS AND METHODS

Chemicals

SDZ DJN 608 (figure 1) substance and the tablet (Lot #PRD-EC2-253, 30 mg), liquid-filled capsule (Lot #PRD-DR-3-043, 30 mg), and intravenous solution (Lot #T-125, 5 mg/ml) were supplied by Sandoz Research Institute (East Hanover, NJ). [3H]SDZ DJN 608 (Isotope Laboratory, Sandoz), specific activity 31.4 mCi/mmol and radiochemical purity $>98\%$, was synthesized with 77.5% of the tritium label at the para position of the phenyl ring and 22.5% of the label at the benzyl position of the phenylalanine moiety, as determined by 3H-NMR. Cell culture reagents were purchased from Gibco (Gaithersburg, MD). [14C]D-mannitol (specific activity 55 mCi/mmol) was

![Fig. 1. The chemical structure of SDZ DJN 608. Asterisks (*) indicate positions of the tritium label.](image-url)
Bioavailability of SDZ DJN 608 in the Dog

obtained from New England Nuclear (Boston, MA), whereas [3H]-mannitol (specific activity 26.4 Ci/mmol) and L-α-methyldopa were from Sigma (St. Louis, MO). Reagents used for transmission electron microscopy were of EM grade. All other chemicals were of reagent or HPLC grade.

Dog Study

The study adhered to the “Principles of Laboratory Animal Care” (NIH publication #85-23, revised 1985), and was approved by the Sandoz Animal Care and Use Committee. Three mature male beagle dogs each weighing 12.7 kg were used. The dogs were housed individually in metabolism cages in a room with controlled temperature (22 ± 2°C) and humidity (50 ± 20%). They were fed once daily and had free access to water. For one week prior to the study, the dogs were trained to eat immediately when presented with food (Purina Certified Dog Chow, ≥9.0% fat).

The tablet and liquid-filled capsule were tested under both fasting and postprandial conditions, whereas an intravenous reference dose was administered only in the fasting condition. The oral dose was 30 mg per dog. For the fasting condition, the animals were fasted overnight before dosing and for 4 h postdose. They had free access to water except for 4 h postdose. For the postprandial condition, the dogs were fed 2 h before dosing. The intravenous dose (5 mg) was administered as a bolus injection (1 ml) via a cephalic vein. Not all dogs received the same treatment on all study days. Doses in the same animal were separated by 1-week washout periods.

Venous blood (~3 ml) was collected from each dog in a heparinized syringe immediately before and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 24 h after dosing. An additional blood sample was obtained at 5 min following the intravenous dose. Plasma was separated by centrifuging the blood and stored in polypropylene tubes (Sarstedt, Newton, NC) at −20°C until analysis.

Analysis of SDZ DJN 608

Plasma concentrations of SDZ DJN 608 were determined using a modification of the high pressure liquid chromatographic (HPLC) method of Sato et al. (8). The method employed a solid phase extraction (SPE) procedure for sample clean-up using a trifunctional tC18 sorbent (Sep-Pak®, Waters, Milford, MA). After thawing, plasma samples (0.5 ml) were spiked with the internal standard (A-4263, the tertiarybutyl derivative of SDZ DJN 608) and mixed with 2 ml of pH 6.6 phosphate buffer. The SPE cartridge was washed and preconditioned by passing through it methanol (2 ml), followed by water (3 ml) and pH 6.6 phosphate buffer (2 ml). The plasma samples were loaded on the SPE cartridge and washed with 3 ml of water, followed by gentle drying of the cartridge. SDZ DJN 608 and the internal standard were eluted with 5 ml of methanol which was evaporated and the residue was reconstituted in mobile phase. An aliquot of the reconstituted sample was injected onto a LC-18 Supelcosil® 2 cm × 4.6mm guard column and a LC-ABZ Supelcosil® 25 cm × 4.6 mm reversed phase HPLC column (Supelco, Inc., Bellefonte, PA). The mobile phase was 0.05 M sodium phosphate buffer (pH 6.3):acetonitrile (64:36, v/v) at a flow rate of 1 ml/min. A guard column wash step with acetonitrile:0.05 M phosphate buffer (1:1, v/v) was essential to eliminate late-eluting endogenous peaks. This was achieved during a 4 min wash cycle using a switching valve. Detection and quantitation were accomplished by monitoring the ultraviolet (UV) absorbance at 210 nm.

The daily plasma calibration standards were linear in the 20–10,000 ng/ml range. The coefficient of variation associated with the mean relative response factor of the daily standards was ±10% and the coefficient of determination was greater than 0.99 for all analytical runs. The lower limit of quantification was 20 ng/ml and the mean accuracy of quality control samples was greater than 88% for all analysis days. The results demonstrate that the method performed consistently, both within and across analysis days.

Pharmacokinetic Analysis

The observed peak concentration (Cmax) of plasma DJN 608 and the time of peak (tmax) were recorded, and the area under the concentration-time curve (AUC) was calculated from 0 to 24 h by the trapezoidal rule. The absolute bioavailability (f) of each oral dose was determined by the oral:intravenous ratio of the respective, dose-normalized AUC values. Using the intravenous dose data, the half-life of DJN 608 was estimated by linear regression analysis of the terminal phase of the plasma concentration profile. The apparent volume of distribution at steady-state (Vdss) was calculated using the following equation (9):

\[ V_{dss} = \frac{\text{Dose} \cdot \text{AUMC}}{\text{AUC}^2} \]

where AUMC is the area under the first moment of the plasma concentration-time curve obtained by the trapezoidal rule. The total body clearance (CL) was estimated as follows:

\[ CL = \frac{\text{Dose}}{\text{AUC}} \]

The effect of formulation and food on the bioavailability of DJN 608 in the dog was evaluated by applying 3-way ANOVA (10) to the parameters AUC, Cmax, and tmax. Differences were considered statistically significant at p < 0.05.

In Vitro Dissolution

The dissolution of SDZ DJN 608 from tablets and liquid-filled capsules was studied in simulated gastric fluid and intestinal fluid (both without enzyme) according to the USP apparatus 2 (paddle) method. The simulated gastric fluid (pH 1.2) was prepared by dissolving 2 g of sodium chloride and placing 7 ml of concentrated hydrochloric acid in water to make 1000 ml. The solubility of SDZ DJN 608 in this medium is 0.09 mg/ml. Five grams of polysorbate 80 was added to ensure sink condition. The simulated intestinal fluid (pH 6.8) was made by adding 118 ml of 0.2 N sodium hydroxide and 250 ml of 0.2 M monobasic potassium phosphate to water to make 1000 ml. The solubility of SDZ DJN 608 in this vehicle is 1.6 mg/ml. The paddle speed was set at 50 rpm and the water bath was maintained at 37°C. Five hundred milliliters of the dissolution medium were used for each tablet or capsule, and the samples collected at various time intervals were filtered through 0.45 μm microfiber filters (Whatman Uniprep®, Hillsboro, OR) before injected into an HPLC for analysis of SDZ DJN 608. The HPLC system consisted of a Deltabond® ODS reversed phase column.