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

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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-methyldopa, 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 (C_{max}) averaged higher, and the peak time (t_{max}) 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 t_{max} and C_{max} 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-methyldopa, 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 nonsulfonylurea 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 transcellular 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). [^{3}H]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 benzylic position of the phenylalanine moiety, as determined by ^{3}H-NMR. Cell culture reagents were purchased from Gibco (Gaithersburg, MD). [^{14}C]D-mannitol (specific activity 55 mCi/mmol) was

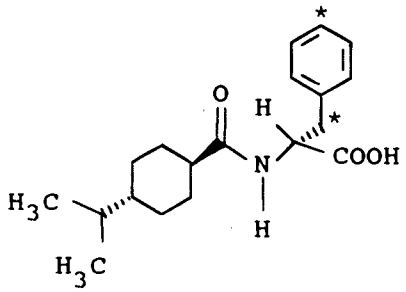

Fig. 1. The chemical structure of SDZ DJN 608. Asterisks (*) indicate positions of the tritium label.

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obtained from New England Nuclear (Boston, MA), whereas [^{3}H]D-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 5 min 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 tC_{18} 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 (C_{max}) of plasma DJN 608 and the time of peak (t_{max}) 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 (Vd_{ss}) was calculated using the following equation (9):

$$Vd_{ss} = \frac{\text{Dose} \cdot \text{AUMC}}{(\text{AUC})^2}$$

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, C_{max}, and t_{max}. 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

Table 1. Plasma Concentrations of SDZ DJN 608 Following a Single Oral (30 mg) or Intravenous (5 mg) Dose in the Dog

Time After Dose (h)	Plasma Concentration (ng/ml)[a]				
	Fasting		Postprandial		Fasting
	Tablet	Liquid capsule	Tablet	Liquid capsule	Intravenous
0.083	—[b]	—	—	—	2530 ± 270
0.25	152 ± 263	1050 ± 936	33.7 ± 29.3	3120 ± 5140	1560 ± 86.6
0.5	4070 ± 6700	4650 ± 3150	1820 ± 3090	3330 ± 4490	1250 ± 73.7
1	3940 ± 4830	8530 ± 3150	1930 ± 3260	6590 ± 4260	964 ± 107
2	3570 ± 2320	6750 ± 611	1620 ± 2160	4520 ± 1890	639 ± 30.4
3	4450 ± 344	4710 ± 490	2790 ± 2730	3560 ± 1280	458 ± 34.4
4	3610 ± 649	3500 ± 650	2180 ± 2260	2590 ± 575	362 ± 17.0
6	2550 ± 491	1910 ± 607	2130 ± 898	1330 ± 300	249 ± 13.9
8	1440 ± 232	1050 ± 249	1530 ± 606	962 ± 251	192 ± 52.5
24	156 ± 48.1	265 ± 276	117 ± 36.1	143 ± 29.6	49.1 ± 14.6

[a]Mean ± SD, N = 3.
[b]Not determined.

(Keystone, Bellefonte, PA) with an isocratic pump, using a mobile phase of 45% acetonitrile/55% monobasic sodium phosphate (50 mM, adjusted to pH 2.5 with concentrated phosphoric acid). The flow rate was 1.5 ml/min and detection was made by UV at 210 nm.

Transcellular Transport

Cell Culture

Caco-2 cells were obtained from Dr. Ken Audus (University of Kansas) at passage 41. The method of Hidalgo et al. (5), with minor modifications, was used to grow cell monolayers. Briefly, cells were grown in T-150 flasks at 37°C in Dulbecco's modified Eagle's medium (D-MEM), supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% nonessential amino acids and 0.1% gentamicin, in an atmosphere of 5% CO_2 and 90% humidity. The medium was changed every 2–5 days until the monolayers reached confluency. The cells were rinsed once with unsupplemented D-MEM and incubated with 0.25% trypsin in 1 mM EDTA for 10 min at 37°C. The cells were then diluted with medium and seeded onto Snapwell® (0.4 μm-pore polycarbonate membrane, Costar, Cambridge, MA) transwell inserts at 63,000 cells/cm^2. The medium was changed every 2–5 days. The degree of morphologic differentiation of the Caco-2 cells and the formation of monolayers were assessed at 14, 21, and 28 days postseeding by transmission electron microscopy. Cells at passages 62, 66, and 69 were fixed and processed as described by Hildago *et al.* (5). Silver cross sections through the cell monolayer were examined using a Zeiss EM9 transmission electron microscope at various magnifications (4,000×–140,000×). The integrity of the monolayer was confirmed by measuring the transepithelial electrical resistance (TEER) just before undertaking the transport studies (5) and also by including radiolabeled mannitol as a leakage marker in the donor buffer during each study.

Transport Studies

Caco-2 cells at 28–32 days postseeding were washed free of medium and incubated at 37°C in Krebs bicarbonate buffer, pH 7.4, for one hour. After the TEER of each monolayer and support was measured, the transwell inserts were mounted in diffusion chambers (Costar). Six milliliters of a donor buffer were placed on the apical side of a cell insert, whereas 6 ml of a receiver buffer were added to the basolateral side. The

Table 2. Pharmacokinetic Parameters of SDZ DJN 608 Following a Single Oral (30 mg) or Intravenous (5 mg) Dose in the Dog

Parameter[a]	Fasting		Postprandial		Fasting
	Tablet	Liquid capsule	Tablet	Liquid capsule	Intravenous
C_{max} (ng/ml)	6940 ± 4220	8930 ± 2470	4700 ± 1520	7600 ± 3940	2530 ± 270
t_{max} (h)	2.2 ± 1.4	1.3 ± 0.6	3.3 ± 2.5	1.4 ± 1.4	0.083 ± 0
AUC (ng·h/ml)	37300 ± 5770	40500 ± 6440	28800 ± 2350*	31400 ± 4450*	6200 ± 689
f	1.02 ± 0.22	1.09 ± 0.12	0.78 ± 0.03	0.84 ± 0.03	1.00
AUMC (ng·h^2/ml)	—[b]	—	—	—	32100 ± 4250
Vd_{ss} (l/kg)	—	—	—	—	0.33 ± 0.02
CL (l/h/kg)	—	—	—	—	0.064 ± 0.002
Half-life (h)	—	—	—	—	7.4 ± 1.5

[a]Parameters are defined in text. Values represent mean ± SD, N = 3.
[b]Not determined.
*Different from fasting condition, $p < 0.01$.

donor buffer was Krebs buffer containing 40 mM radiolabeled mannitol and 0.1 or 1 mM SDZ DJN 608 or [^{3}H]SDZ DJN 608, with or without 10 mM L-α-methyldopa. The receiver buffer was 40 mM D-glucose in Krebs buffer. Mixing was accomplished with 5% CO_2 in O_2. Samples (0.2 ml) were collected from the receiver side immediately before and at 30, 60, 90, and 120 min after initiation of experiment, and replaced each time with fresh receiver buffer. The samples were analyzed for either SDZ DJN 608 by HPLC or [^{3}H]SDZ DJN 608 by liquid scintillation counting (Model 2500TR, Packard, Downers Grove, IL). Additionally, the serosal to mucosal transport of SDZ DJN 608 was studied by repeating the above procedure, but placing the donor buffer without L-α-methyldopa on the basolateral side and the receiver buffer on the apical side of the Caco-2 monolayer.

Data Analysis

The effective permeability coefficient, P_{eff}, was calculated using the following equation (6):

$$P_{eff} = \frac{V_R}{A \cdot C_o} \cdot dC/dt$$

where V_R is the volume of the receiver chamber, A is the exposed surface area, 1.13 cm^2, C_o is the initial donor concentration of SDZ DJN 608, and dC/dt is the initial slope of a plot of the cumulative receiver concentration versus time. Comparison between two means was performed using Student's *t* test. Statistical significance was defined as $p < 0.05$.

RESULTS

Dog Study

The plasma concentrations and pharmacokinetic parameters of SDZ DJN 608 are summarized in tables 1 and 2, respectively. SDZ DJN 608 was rapidly absorbed in the dog. The C_{max} averaged higher, and the t_{max} shorter, after LC than T, though the differences were not statistically significant ($p > 0.1$). There was no significant effect of food on C_{max} or t_{max}. With respect to AUC, no significant difference was observed between LC and T, the absolute bioavailability of both being complete in the fasting state. However, the presence of food significantly reduced the AUC values, the effect being not different between LC (−22 ± 7%) and T (−21 ± 16%).

Based on the results of the intravenous study, the half-life of DJN 608 was 7.4 ± 1.5 h. The steady-state volume of distribution averaged 0.33 l/kg, and the mean total body clearance was 0.064 l/h/kg.

In Vitro Dissolution

The dissolution profiles of SDZ DJN 608 tablets and liquid-filled capsules in simulated gastric and intestinal fluids are shown in figure 2. The dissolution rates of both dosage forms appeared to be independent of the pH of the dissolution medium. At either pH 1.2 or pH 6.8, the dissolution of SDZ DJN 608 was more rapid from LC than from T.

Transcellular Transport

In this study, the TEER values of the Caco-2 monolayers averaged 729 ± 50 (SD) Ω cm^2 (N = 18), similar to that reported by other investigators (11). The mannitol leakage was 0.2 ± 0.1% per hour, further confirming the integrity of the monolayers. As shown in table 3, the permeability of SDZ DJN 608 from mucosal to serosal side of the Caco-2 monolayer was similar between concentrations of 0.1 and 1 mM, and was not affected by the presence of 10 mM L-α-methyldopa. The P_{eff} values for serosal-to-mucosal transport averaged significantly greater than that for mucosal-to-serosal transport, but only at 0.1 mM. The serosal-to-mucosal permeability was concentration-dependent, the mean P_{eff} at 1 mM (6.94×10^{-6} cm/sec) being significantly smaller than that at 0.1 mM (8.30×10^{-6} cm/sec).

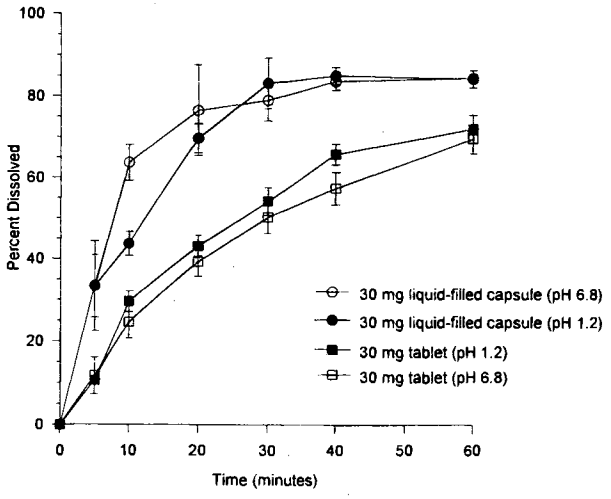

Fig. 2. Dissolution of SDZ DJN 608 tablets and liquid-filled capsules in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8) according to the USP paddle method at 50 rpm and 37°C. Each data point represents the mean ± SD of three determinations.

DISCUSSION

Previous studies by Hidalgo and Borchardt (7) have shown that the transcellular transport of L-phenylalanine in Caco-2 cell monolayers is mediated by a carrier system. The carrier appears to be shared by large neutral amino acids (LNAA) and cationic amino acids, and exhibits stereospecificity toward L-isomers. Based on these findings, the transport of SDZ DJN 608, a D-phenylalanine derivative, would not be expected to be governed by the LNAA carrier. Results in the present study confirmed the passive transport of SDZ DJN 608 from the mucosal to the serosal side of the Caco-2 monolayer, the P_{eff}

Table 3. Effective Permeability Coefficients of SDZ DJN 608 at 37°C in Caco-2 Cell Monolayers

Concentration of SDZ DJN 608 (mM)	P_{eff} (cm/sec × 10^6), mean ± SD, N = 3		
	I. Mucosal to serosal	II. Mucosal to serosal, with 10 mM L-α-methyldopa	III. Serosal to mucosal
0.1	7.04 ± 0.55*	6.99 ± 0.52*	8.30 ± 0.22
1	6.82 ± 0.75	8.46 ± 0.91	6.94 ± 0.19**

*Different from III, $p < 0.05$.
**Different from 0.1 mM, $p < 0.01$.

value being independent of drug concentration and unaffected by the presence of L-α-methyldopa, an aromatic amino acid which competes with L-phenylalanine for a common carrier system (7,12). In contrast, it is interesting to note that, at a concentration of 0.1 mM, the mean P_{eff} for serosal-to-mucosal transport of SDZ DJN 608 was significantly greater than that for mucosal-to-serosal transport. Additionally, the serosal-to-mucosal P_{eff} decreased significantly when the concentration increased to 1mM, thus suggesting that SDZ DJN 608 was transported from the serosal to the mucosal side of the Caco-2 monolayer by a carrier-mediated (capacity-limited) process as well as by passive diffusion. However, due to the often large concentration gradient across the intestinal epithelium during drug absorption, the potential impact of this exsorption process is of questionable clinical significance.

In the dog, SDZ DJN 608 was rapidly absorbed. The C_{max} averaged higher, and the t_{max} shorter, after LC than T, though the differences were not statistically significant. The observed trend 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. The dissolution profiles in simulated gastric and intestinal fluids were similar, thus suggesting no absorption window for SDZ DJN 608. Accordingly, no significant difference in AUC was observed between LC and T, the absolute bioavailability of both being complete in the fasting state. The presence of food appeared to have little effect on the rate of absorption of SDZ DJN 608 from either dosage form. This is a favorable characteristic of SDZ DJN 608 for which prompt drug action is required. On the other hand, food significantly reduced the extent of absorption of this compound. The magnitude of the food effect was not different between LC ($-22 \pm 7\%$) and T ($-21 \pm 16\%$), indicating that the mechanism of the drug-food interaction was not associated with the rate of release of SDZ DJN 608 from the dosage form. Since inhibition of the mucosal-to-serosal transport of SDZ DJN 608 by amino acids in the diet was demonstrated to be unlikely, it is possible that SDZ DJN 608 interacts directly with food components or food-induced gastrointestinal secretions (e.g bile salts) by complex formation, resulting in reduced bioavailability of the drug when administered in the postprandial state. Similar drug-food interaction has been reported for the antimicrobial agents kanamycin, polymyxin, tetracyclines, and many penicillins (13,14).

CONCLUSIONS

The absorption of SDZ DJN 608 from a 30 mg dose as T or LC in the dog was rapid, with or without concomitant food administration. In the fasting state, both dosage forms yielded complete bioavailability of the drug. The presence of food had little effect on the absorption rate, but significantly reduced the bioavailability of SDZ DJN 608, the effect being not different between LC and T. Studies using Caco-2 cell monolayers showed that the mucosal-to-serosal transport of SDZ DJN 608 is a passive process. Therefore, the observed food effect is unlikely to be caused by inhibition of the transepithelial transport of SDZ DJN 608 by amino acids in the diet.

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