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SHORT COMMUNICATION

Effects of Omeprazole or Cola Beverage on the Pharmacokinetics of Oral DA-8159, a New Erectogenic, in Rats

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ABSTRACT: The changes in pharmacokinetics of DA-8159 by omeprazole with respect to inhibition of CYP3A1/2 in rats were evaluated. After oral administration of DA-8159 at dose of 30 mg/kg to rats pretreated with oral omeprazole at 30 mg/kg for 1 week, the total area under the plasma concentration–time curve from time zero to time infinity (*AUC*) of DA-8159 was significantly greater (37.5% increase) than that in control rats. This could be due to inhibition of metabolism of DA-8159 by inhibition of CYP3A1/2 by omeprazole. The $AUC_{\text{DA-8164 (a metabolite of DA-8159)}}/AUC_{\text{DA-8159}}$ ratio was also smaller (32.4% decrease) with omeprazole. After oral administration of DA-8159 at a dose of 30 mg/kg to rats without or with cola beverage, the pharmacokinetic parameters of DA-8159 and DA-8164 were not significantly different between two groups of rats. This suggested that cola beverage did not have any considerable effects on CYP3A1/2 in rats. Copyright © 2005 John Wiley & Sons, Ltd.

Key words: DA-8159; pharmacokinetics; omeprazole; cola beverage; rats

Introduction

The extent of the relative oral bioavailability (*F*) of itraconazole was significantly reduced in healthy volunteers who had fasted when the gastric pH was increased by pretreatment with ranitidine [1]. Coadministration of itraconazole with cola beverage, however, produced a sharp transient decrease in the gastric pH and increased the *F* to a level equivalent to that observed during normal gastric acid conditions [1]. The *F* of itraconazole was significantly reduced when omeprazole was coadministered in healthy volunteers [2]. This could be due to pH-dependent dissolution and absorption of itraconazole, a basic drug with a pK_a of 3.7.

A new inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type V (PDE V), DA-8159, 5-[2-prospyloxy-5-(1-methyl-2-pyrollidinylethylamidosulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo (4,3-d)pyrimidine-7-one, was synthesized (Research Laboratory of Dong-A Pharmaceutical Company, Yongin, Republic of Korea) for the treatment of male erectile dysfunction. The negative logarithms of acidic dissociation constants of DA-8159, pKa_1 and pKa_2, were approximately 6.5 and 12.5, respectively. The log partition coefficient (octanol/Sørensen phosphate buffer of pH 7) of DA-8159 was 1.85. DA-8159 (MW 516.6 Da and melting point 162°–164°C) is a weak basic drug. The solubilities of DA-8159 in buffer solutions having pHs 2, 5, 7 and 10, and in distilled water were 27.4, 12.5, 0.82, 0.033 and 0.019 mg/ml,

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respectively. DA-8159 was metabolized to DA-8164 (N-dealkylated DA-8159; 5-[2-propyloxy-5-(aminosulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo(4,3-d)pyrimidine -7-one) in mice, rats, rabbits, dogs and humans [3]. The mechanism [4] and erectogenic effects [5,6] of DA-8159 in animals were reported. DA-8159 is now being evaluated in phase III clinical trial for the treatment of male erectile dysfunction.

After oral administration of DA-8159 at a dose of 30 mg/kg to male Sprague–Dawley rats, the extent of absolute oral bioavailability was approximately 38.0%, the intestinal and hepatic first pass effects were approximately 58% and 9.6% of oral dose, respectively [7]. More than 99% of oral dose of DA-8159 was absorbed from rat gastrointestinal tract [7]. Hence, if the total area under the plasma concentration–time curve from time zero to time infinity (*AUC*) of DA-8159 is changed by omeprazole or cola beverage, the mechanism could be different from itraconazole; not due to changes in absorption of DA-8159. This could be related to inhibition of hepatic microsomal cytochrome P450 (CYP) isozyme by omeprazole since the CYP3A1/2 catalyzed metabolism of DA-8159 in rats [8] and omeprazole is a competitive inhibitor of the CYP3A family based on *in vitro* rat hepatic microsomes [9]. The purpose of this study is to report the greater *AUC* of DA-8159 by omeprazole with respect to inhibition of CYP3A1/2 by omeprazole in rats.

Materials and Methods

Chemicals

DA-8159, DA-8164 and sildenafil (an internal standard of high-performance liquid chromatographic, HPLC, analysis) were supplied from Research Laboratory of Dong-A Pharmaceutical Company. Omeprazole was supplied from Yungjin Pharmaceutical Company (Seoul, Republic of Korea). Cola beverage was purchased from a convenience store. Other chemicals were of reagent grade or HPLC grade.

Animals

Male Sprague–Dawley rats (weighing 150–200 g) were purchased from Charles River Company Korea (Biogenomics, Seoul, Republic of Korea). Rats were randomly divided into four groups, rats pretreated with omeprazole and cola beverage, and their respective control rats. All rats were provided with food (Sam Yang Company, Seoul, Republic of Korea) and water *ad libitum*, and maintained in a light-controlled room (light: 0700–1900, dark: 1900–0700) kept at a temperature of $22^\circ \pm 2^\circ$C and a relative humidity of $55\% \pm 5\%$ (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, Republic of Korea). The protocol of this study was approved by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University.

Omeprazole study

The procedures for the pretreatment of rats including the cannulation of the carotid artery were as reported [7,8]. Rats were given either oral omeprazole (dissolved in 0.1 M carbonate buffer of pH 9.8 with a minimum amount of 10 N NaOH, and adjusted to a final pH of approximately 10 with HCl; 30 mg/kg per day) or 0.1 M carbonate buffer for 1 week (total oral volume of 5 ml/kg) [10]. On day 7 after fasting for 12 h with free access to tap water, DA-8159 (dissolved in 0.05 M citric acid) at a dose of 30 mg/kg was administered orally (total oral volume of 6 ml/kg) using a feeding tubing to control rats ($n = 8$) and rats pretreated with omeprazole ($n = 9$). Approximately 0.22 ml aliquots of blood samples were collected at 0 (to serve as a control), 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600 and 720 min after oral administration of DA-8159. An approximately 0.3 ml aliquot of the heparinized 0.9% NaCl-injectable solution (20 units/ml) was used to flush each cannula immediately after each blood sampling to prevent blood clotting. Blood samples were centrifuged immediately and a 100 µl aliquot of the plasma sample was stored in a -70°C freezer (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC) until HPLC analysis of DA-8159 and DA-8164 [11]. At the end of 24 h, the metabolic cage was rinsed twice with 20 ml of distilled water and the rinsings were combined with the 24 h urine. After measuring the exact volume of the combined urine sample, two 100 µl aliquots of the combined urine sample were

stored in a −70°C freezer until HPLC analysis of DA-8159 and DA-8164 [11]. At the same time (24 h), each rat was exsanguinated and killed by cervical dislocation. And then the entire gastrointestinal tract (including its contents and feces) was removed, transferred into a beaker containing 100 ml of methanol (to facilitate the extraction of DA-8159 and DA-8164), and cut into small pieces using scissors. After manual shaking and stirring with a glass rod, two 100 µl aliquots of the supernatant were collected and stored in a −70°C freezer until HPLC analysis of DA-8159 and DA-8164 [11].

Cola beverage study

After fasting for 12 h with free access to tap water, cola beverage was administered orally (5 ml/kg) 1 min before DA-8159 administration [12]. In the control group, rats were pretreated with the same volume of water. DA-8159 at a dose of 30 mg/kg was administered orally (the same solution as used in the omeprazole study) using a feeding tubing to control rats ($n = 7$) and rats pretreated with cola beverage ($n = 9$). Other procedures were similar to those in the omeprazole study.

HPLC analysis of DA-8159 and DA-8164

The concentrations of DA-8159 and DA-8164 in the above biological samples were analysed by a slight modification of the reported HPLC method developed from our laboratories [11]. To a 0.1 ml aliquot of biological sample, a 0.05 ml aliquot of 0.1 M Na_2CO_3 containing 3 µg/ml of sildenafil (an internal standard) and a 1 ml aliquot of ethylether were added. After vortex-centrifugation at 12,000 rpm for 2 min, the ether layer was collected and dried under a gentle stream of nitrogen gas. A 0.1 ml aliquot of the mobile phase was added to reconstitute the residue and a 0.05 ml aliquot was injected directly onto a reversed-phase HPLC column. The mobile phase, 20 mM KH_2PO_4 (pH = 4.7): acetonitrile (72:28; v/v), was run at a flow-rate of 1.5 ml/min, and the column effluent was monitored by a UV detector set at 292 nm at room temperature. The retention times of DA-8159, DA-8164 and sildenafil were approximately 9.7, 17.1 and 6.9 min, respectively. The detection limits of DA-8159 and DA-8164 in plasma and urine were all 20 ng/ml. The coefficients of variation (inter- and intra-day) were below 8.7%.

Pharmacokinetics analysis

Standard methods [13] were used for the calculation of the following pharmacokinetic parameters; the *AUC*, terminal half-life and time-averaged renal clearance (Cl_r). The maximum plasma concentration (C_{max}) and time to reach a C_{max} (T_{max}) were directly read from the experimental data.

The harmonic mean method was used to calculate the mean value of Cl_r [14] and terminal half-life [15].

Statistical analysis

A value of $p < 0.05$ was considered to be statistically significant using the *t*-test between the two means for the unpaired data. All data are expressed as mean ± standard deviation.

Results

After oral administration of DA-8159 with omeprazole, the plasma concentrations of DA-8159 were higher and C_{max} of DA-8159 was significantly higher (132% increase) than those in control rats (Figure 1). This resulted in a significantly greater *AUC* (37.5% increase) in rats with omeprazole (Table 1). The T_{max} was significantly shorter (56.1% decrease) with omeprazole. Other pharmacokinetic parameters of DA-8159 listed in Table 1 were not significantly different between without and with omeprazole (Table 1). The pharmacokinetic parameters of DA-8159 listed in Table 1 were not significantly different between without and with cola beverage.

After oral administration of DA-8159 without or with omeprazole or cola beverage, the formation of DA-8164 was rapid; DA-8164 was detected in plasma from the first blood sampling time, 15 min (Figure 1 and Table 1). The pharmacokinetic parameters of DA-8164 after oral administration of DA-8159 with omeprazole were not significantly different except significantly faster

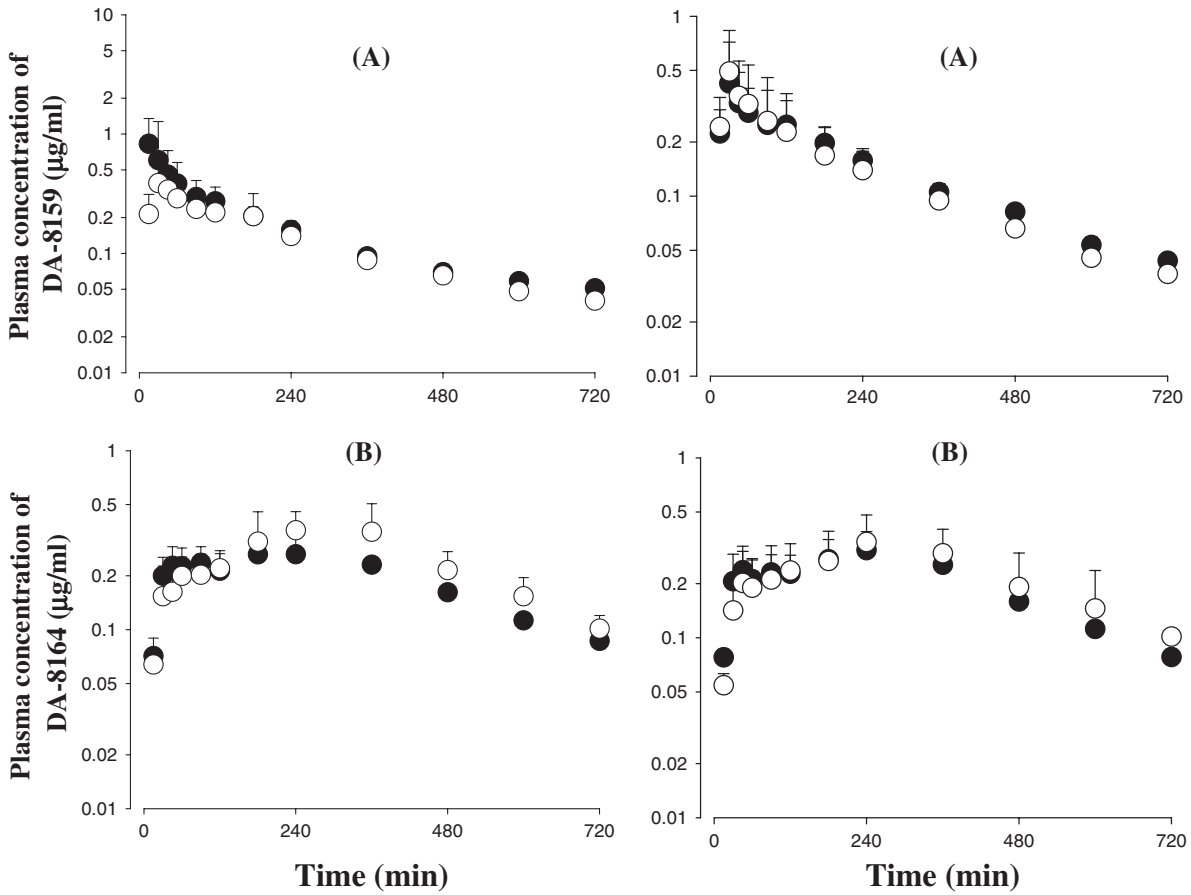

Figure 1. Mean arterial plasma concentration–time profiles of DA-8159 (A) and DA-8164 (B) after oral administration of DA-8159 at a dose of 30 mg/kg to rats without (○; $n = 8$) and with (●; $n = 9$) oral omeprazole (left panel), or without (○; $n = 7$) and with (●; $n = 9$) oral cola beverage (right panel). Bars represent standard deviation

T_{max} (35.7% decrease) with omeprazole (Table 1). The pharmacokinetic parameters of DA-8164 were also not significantly different between without and with cola beverage (Table 1).

Discussion

Shim *et al*. [7] reported that the pharmacokinetic parameters of DA-8159 were dose-independent after intravenous administration at doses of 5–30 mg/kg and oral administration at doses of 20–30 mg/kg to rats. Hence, the oral dose of 30 mg/kg was arbitrarily chosen in this study.

After oral administration of DA-8159 without or with omeprazole or cola beverage, absorption of DA-8159 was rapid for all rats studied; DA-8159 was detected in plasma from the first blood sampling time, 15 min, and reached C_{max} early; the T_{max} values were 46.9, 20.6, 38.6 and 50.0 min for without or with omeprazole, and without or with cola beverage, respectively (Figure 1 and Table 1). After oral administration of DA-8159, absorption of DA-8159 was almost complete for all rats studied; the total amounts of unchanged DA-8159 recovered from the whole gastrointestinal tract at 24 h ($GI_{24\,h}$) were 2.13%, 1.63%, 2.11% and 2.21% of oral dose of DA-8159 for without or with omeprazole, and without or with cola beverage, respectively (Figure 1 and Table 1). Note that the small values of $GI_{24\,h}$ of DA-8159 were not due to degradation of DA-8159; DA-

Table 1. Mean (± standard deviation) pharmacokinetic parameters of DA-8159 and DA-8164 after oral administration of DA-8159 at a dose of 30 mg/kg to rats without (OMC) and with (OMT) oral omeprazole, or without (CBC) and with (CBT) oral cola beverage

Parameter	OMC ($n = 8$)	OMT ($n = 9$)	CBC ($n = 7$)	CBT ($n = 9$)
Initial body weight (g)	257 ± 5.00	253 ± 9.69	281 ± 14.1	281 ± 10.1
Final body weight (g)	282 ± 10.3	278 ± 8.84		
DA-8159				
AUC (μg min/ml)	104 ± 19.9	143 ± 20.7[b]	102 ± 29.5	116 ± 35.3
Terminal half-life (min)	255 ± 57.1	357 ± 161	215 ± 151	282 ± 111
Cl_r (ml/min/kg)	4.51 ± 1.41	3.53 ± 1.85	7.01 ± 2.12	5.21 ± 2.29
C_{max} (μg/ml)	0.407 ± 0.121	0.944 ± 0.216[b]	0.522 ± 0.211	0.498 ± 0.263
T_{max} (min)	46.9 ± 30.5	20.6 ± 11.2[c]	38.6 ± 11.8	50.0 ± 32.7
$A_{eDA\text{-}8159,\ 0\text{–}24\,h}$[a]	1.65 ± 0.418	1.98 ± 0.838	2.40 ± 0.844	1.98 ± 0.494
$GI_{DA\text{-}8159,\ 24\,h}$[a]	2.13 ± 1.30	1.63 ± 1.25	2.11 ± 0.61	2.21 ± 1.95
DA-8164				
AUC (μg min/ml)	196 ± 38.0	182 ± 36.1	216 ± 106	172 ± 37.2
Terminal half-life (min)	188 ± 88.7	278 ± 167	292 ± 155	196 ± 194
C_{max} (μg/ml)	0.387 ± 0.118	0.328 ± 0.0710	0.354 ± 0.138	0.356 ± 0.103
T_{max} (min)	308 ± 98.5	198 ± 97.7[c]	249 ± 54.0	218 ± 83.2
$A_{eDA\text{-}8164,\ 0\text{–}24\,h}$[a]	0.0426 ± 0.0140	0.0456 ± 0.0155	0.0987 ± 0.0857	0.0826 ± 0.0270
$GI_{DA\text{-}8164,\ 24\,h}$[a]	0.459 ± 0.230	0.435 ± 0.280	0.207 ± 0.0725	0.221 ± 0.0275

[a] Expressed in terms of % of oral dose of DA-8159.
[b] Significantly different ($p < 0.001$) from OMC group.
[c] Significantly different ($p < 0.05$) from OMC group.

8159 was relatively stable in various pH solutions for up to 48 h incubation in a water-bath shaker kept at 37°C and at a rate of 50 oscillations per min [16]. The total amounts of unchanged DA-8159 excreted in 24 h urine ($Ae_{0-24\,h}$) were almost negligible; the values were 1.65%, 1.98%, 2.40% and 1.98% of oral dose of DA-8159 for without or with omeprazole, and without or with cola beverage, respectively (Table 1). The above data suggested that almost all of the orally administered DA-8159 is metabolized in rats.

Shim *et al*. [3] reported that the metabolism of DA-8159 was mediated mainly via CYP3A1/2 in male Sprague–Dawley rats. For example, in rats pretreated with dexamethasone (a main inducer of CYP3A1/2 in rats) and troleandomycin (a main inhibitor of CYP3A1/2 in rats), the *AUC* values of DA-8159 were significantly smaller (18.9% decrease) and greater (17.6% increase), respectively, than that in control rats. However, the *AUC* values of DA-8159 were not significantly different by pretreatment with phenobarbital, isoniazid, 3-methylcholanthrene and quinine (main inducers of CYP2B1/2, 2E1 and 1A1/2, and a main inhibitor of 2D1, respectively, in rats).

Soons *et al*. [17] reported that omeprazole seemed to inhibit CYP3A4 in healthy male subjects; the *AUC* of nifedipine (the oxidation of nifedipine is mainly catalysed by CYP3A4 based on *in vitro* human liver microsomes [18]) was increased by 26% by short-term (20 mg/day for 8 days) omeprazole treatment. It was also reported [19] that omeprazole has a low potential to inhibit CYP3A based on human liver microsomes. Human CYP3A4 and rat CYP3A1(23) proteins have 73% homology [20]. Hence, the significantly greater *AUC* of DA-8159 with omeprazole could be due to inhibition of metabolism of DA-8159 by inhibition of CYP3A1/2 by omeprazole. As mentioned earlier, this could not be due to increased oral absorption of DA-8159 in rats with omeprazole since approximately 99% of the oral dose of DA-8159 was absorbed in rats [3]. The *AUC* values of DA-8159 were not significantly different between without or with cola beverage, suggesting that cola beverage does not have any considerable effect on CYP3A1/2 in rats. The $AUC_{DA\text{-}8164}/AUC_{DA\text{-}8159}$ ratio was smaller (32.4% decrease) with omeprazole; the ratios were 1.88 and 1.27 for without and

with omeprazole, respectively (Table 1). This could also support inhibition of CYP3A1/2 by omeprazole.

In summary, after oral administration of DA-8159 with oral omeprazole in rats, the *AUC* of DA-8159 was significantly greater (Table 1) due to inhibition of CYP3A1/2 by omeprazole. However, the *AUC* of DA-8159 was not changed by cola beverage suggesting that cola beverage does not have considerable effect on CYP3A1/2 in rats.

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