JPP Journal of Pharmacy and Pharmacology

JPP 2005, 57: 1397–1405

Received March 25, 2005
Accepted June 20, 2005
DOI 10.1211/jpp.57.11.0004
ISSN 0022-3573

Pharmacokinetic interaction between 5-[2-propyloxy-5-(1-methyl-2-pyrollidinylethylamidosulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo (4,3-d) pyrimidine-7-one (DA-8159) and nitroglycerin in rats

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Abstract

The pharmacokinetic interaction between 5-[2-propyloxy-5-(1-methyl-2-pyrollidinylethylamidosulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo (4,3-d)pyrimidine-7-one (DA-8159), a new erectogenic, and nitroglycerin has been evaluated in rats. Male Sprague-Dawley rats received DA-8159 (30 mg kg^{-1}) as a single intravenous or oral dose with the simultaneous single intravenous administration of nitroglycerin (2.5 mg kg^{-1}). After simultaneous intravenous administration, the total area under the plasma concentration–time curve from time zero to time infinity (AUC_{inf}) of DA-8159 (746 vs 457 μg min mL^{-1}) was found to be significantly greater than with DA-8159 alone. Also, after simultaneous intravenous administration total body clearance (CL) (40.2 vs 65.6 mL min^{-1} kg^{-1}), renal clearance (CL_R) (1.65 vs 5.11 mL min^{-1} kg^{-1}), and nonrenal clearance (CL_{NR}) (38.3 vs 60.2 mL min^{-1} kg^{-1}) of DA-8159 were significantly slower compared with DA-8159 alone. The slower CL_{NR} of DA-8159 could have been due to the inhibition of the metabolism of DA-8159 by nitroglycerin, since DA-8159 is metabolized via CYP3A1/2 in rats and nitroglycerin inhibits CYP3A1/2 in rats. The slower CL_R of DA-8159 could have been due to the urine flow rate-dependent CL_R of DA-8159 in rats. After the simultaneous intravenous administration of nitroglycerin and DA-8159, the AUC_{inf} of nitroglycerin was significantly smaller (635 vs 960 μg min mL^{-1}), which could have been due to the cardiac output-dependent CL of nitroglycerin. However, after the oral administration of DA-8159, the pharmacokinetic parameters of DA-8159 with and without the intravenous administration of nitroglycerin became comparable. This was not due to the decrease in nitroglycerin's gastrointestinal absorption of DA-8159, but could have been due to changes in nitroglycerin's intestinal first-pass effect of DA-8159. Human studies are required to determine the administration time of DA-8159 when nitroglycerin is concomitantly taken.

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Funding: This study was supported in part by a grant from the Korea Ministry of Health & Welfare (02-PJ2-PG4-PT01-0024), 2004–2005.

Introduction

A new inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type V (PDE V), 5-[2-propyloxy-5-(1-methyl-2-pyrollidinylethylamido sulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo (4,3-d)pyrimidine-7-one (DA-8159), was synthesized (Research Laboratory of Dong-A Pharmaceutical Company, Yongin, Korea) for the treatment of male erectile dysfunction. Shim et al (2003) reported that the extent of the absolute oral bioavailability (F) of DA-8159 at a dose of 30 mg kg^{-1} was 38.0%. They reported that the unabsorbed fraction from the gastrointestinal tract was 0.673% of this dose, and that the intestinal and hepatic first-pass effects were approximately 58 and 9.6% of the oral dose, respectively, in rats. Based on the in-vitro metabolism of DA-8159 in microsomes containing the Baculovirus-expressed rat hepatic microsomal cytochrome P450 (CYP) isozyme, DA-8159 was metabolized to three metabolites; 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)), M1 (hydroxy DA-8159), and M2 (N-demethyl DA-8159) (Choi et al 2002). The formation of DA-8164 was not a main metabolic pathway for DA-8159 in rats: the intrinsic clearance (CL_{int}) values for the formation of M1,

M2, and DA-8164 were 43.0, 0.08, and 16.6 mL min^{-1} mg $protein^{-1}$, respectively (Choi et al 2002). Glucuronide- and sulfate-conjugations were not involved in the metabolism of DA-8159 (Choi et al 2002). The mechanism (Doh et al 2002) and erectogenic effects (Ahn et al 2003; Kang et al 2003, 2004) of DA-8159 were reported. For example, in-vitro experiments using a series of PDE isozymes (PDE I, II, III, V, and VI) indicated that DA-8159 was a highly selective and potent antagonist, PDE V from human and rabbit platelets producing IC50 values of 8.25 and 5.84 nM, respectively. In diabetic rabbits, DA-8159 was given orally (1–10 mg kg^{-1}), and the length of the uncovered penile shaft was measured in a time-course manner in the presence or absence of intravenous sodium nitroprusside. The results showed that DA-8159 induced a dose-dependent penile erection, which was potentiated by intravenous sodium nitroprusside. DA-8159 was given orally (0.3–10 mg kg^{-1}) to rabbits with acute spinal cord injury (ASCI) with a surgical transection of the spinal cord at the L2–L4 (lumbar vertebra) or ischaemic-reperfusion. The erection was evaluated in the same way as were the diabetic rabbits. DA-8159 also induced a dose-dependent penile erection in both models of ASCI rabbits. The efficacy of DA-8159 was potentiated and the effective doses were significantly decreased by an intravenous injection of sodium nitroprusside. In normal, conscious rabbits (the control group), DA-8159 showed a significant increase in penile erection. DA-8159 is now being evaluated in a phase III clinical trial in Korea for the treatment of male erectile dysfunction.

Kim at al (2005) reported that DA-8159 was metabolized and DA-8164 was formed mainly via CYP3A1/2 in rats. For example, the total area under the plasma concentration–time curve from time zero to time infinity (AUC_{inf}) values of DA-8159 and DA-8164 were significantly smaller (18.9% decrease) and greater (22.8% increase), respectively, in rats pretreated with dexamethasone (a main inducer of CYP3A1/2 in rats). Reversed results (17.6 and 54.5% increase and decrease, respectively) were obtained in rats pretreated with troleandomycin (a main inhibitor of CYP3A1/2 in rats). However, the AUC_{inf} values of DA-8159 did not change significantly in rats pretreated with 3-methylcholanthrene, phenobarbital, isoniazid, or quinine (main inducers of CYP1A1/2, 2B1/2, and 2E1, and a main inhibitor of 2D1 in rats, respectively). Moreover, the AUC_{inf} values of DA-8164 were not significantly greater or smaller with the above mentioned enzyme inducers and inhibitors. It was reported (Vuppugalla & Mehvar 2004a) that nitric oxide (nitroglycerin is a donor of nitric oxide) rapidly and concentration-dependently decreased CYP isozymes except for CYP2D1 in isolated and perfused rat livers. It was reported also (Vuppugalla & Mehvar 2004b) that nitric oxide inhibited CYP3A2 in the isolated and perfused rat livers. Therefore, a pharmacokinetic interaction between DA-8159 and nitroglycerin could be expected in rats.

Other PDE V inhibitors, such as sildenafil and tadalafil, interact with organic nitrate, resulting in a synergistic drop in blood pressure in man (Webb et al 1999, 2000; Dishy et al 2001; Kloner et al 2003) and rats (Ishizuka et al 2000). Established guidelines recommend that nitrate should not be given until 24 h (six half-lives of sildenafil) after sildenafil has been taken (Cheitlin et al 1999). The aim of this study was to report the pharmacokinetic interaction between DA-8159 and nitroglycerin after the simultaneous administration of a single intravenous or oral dose of DA-8159 (30 mg kg^{-1}) and a single intravenous dose of nitroglycerin (2.5 mg kg^{-1}) to male Sprague-Dawley rats.

Materials and Methods

Chemicals

DA-8159, DA-8164, and sildenafil (an internal standard of high-performance liquid chromatographic, HPLC, assay) were supplied by the Research Laboratory of Dong-A Pharmaceutical Company. Nitroglycerin was supplied by Abbott Laboratories (North Chicago, IL, USA). Other chemicals were of reagent or HPLC grade.

Animals

Male Sprague-Dawley rats (seven-weeks old, 265–305 g) were purchased from Charles River Company Korea (Orient, Seoul, Korea). The rats were maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, Korea) at a temperature of between 20 and 23°C with a 12-h light/dark cycle, lights on 0700 h and lights off 1900 h, and a relative humidity of 50%. The rats were housed in metabolic cages (Tecniplast, Varese, Italy) with a supply of filtered pathogen-free air. Food (Samyang Company, Seoul, Korea), and water were freely available. The Animal Care and Use Committee of the College of Pharmacy of Seoul National University approved the animal study protocol.

Pretreatment of rats

In the early morning, the rats were put under light ether anaesthesia. The jugular vein and carotid artery of each animal were cannulated with polyethylene tubing (Clay Adams, Parsippany, NJ) (Kim et al 1993). Both cannulas were exteriorized to the dorsal side of the neck, where each cannula was terminated with a long Silastic tubing (Dow Corning, Midland, MI, USA). Both Silastic tubings were inserted into a wire coil to allow free movement of the rat. Each rat was housed individually in a rat-metabolic cage (Daejong Scientific Company, Seoul, Korea) and allowed to recover from anaesthesia for 4–5 h before the study commenced. Animals were not restrained during the study. A heparinized 0.9% NaCl-injectable solution (20 U mL^{-1}; 0.3 mL) was used to flush each cannula to prevent blood clotting. Food and water were freely available during the experiment.

Intravenous study

DA-8159 (dissolved in 0.05 M citric acid to produce a 15 mg mL^{-1} solution) at a dose of 30 mg kg^{-1} (n = 8), nitroglycerin (the nitroglycerin-injectable solution,

5 mg mL^{-1}, was diluted in 0.05 M citric acid to produce a 1.25 mg mL^{-1} solution) at a dose of 2.5 mg kg^{-1} (n = 8), or the two drugs administered simultaneously (n = 10) were infused for 1 min via the jugular vein of the rats. An approximately 0.22-mL blood sample (0.12 mL for DA-8159 alone and nitroglycerin alone) was collected via the carotid artery at 0 (to serve as a control), 1 (at the end of the infusion), 5, 15, 30, 60, 120, 180, 240, 360, 480, and 600 min for DA-8159 alone and simultaneously administered drugs. For nitroglycerin alone the samples were taken at 0, 1, 5, 15, 30, 60, 90, 120, 180, 240, and 360 min after the beginning of the infusion. The blood samples were immediately centrifuged and a 50-μL plasma sample (two 50-μL samples for the simultaneously administered drugs) was collected in a 1.5-mL PE tube that contained 10 μL 0.1 M $AgNO_3$ to prevent the degradation of nitroglycerin by plasma albumin for nitroglycerin assay (Dicarlo & Melgar 1969). Two 50-μL plasma samples (one 50-μL sample for DA-8159 alone and for nitroglycerin alone) were stored in a −70°C freezer (Revco ULT 1490 D-N-S; Western Mednics, Ashville, NC, USA) until the HPLC analysis of DA-8159 and DA-8164 (Shim et al 2002), and the gas chromatographic (GC) analysis of nitroglycerin (Janssens et al 1989). At the end of the 24-h experiment, each metabolic cage was rinsed with 15 mL distilled water, and the rinsing water was combined with the 24-h urine. After the exact volume of the 24-h urine and the combined urine were measured, a 50-μL portion of the combined urine sample was stored in a −70°C freezer until the HPLC analysis of DA-8159 and DA-8164 (Shim et al 2002). At the same time (i.e. after 24 h), each rat was exsanguinated and killed through cervical dislocation. The abdomen was opened for DA-8159 alone and for both simultaneously administered drugs. The entire gastrointestinal tract (including its contents and faeces) was removed, transferred into a beaker containing 100 mL methanol (to facilitate the extraction of DA-8159 and DA-8164), and cut into small pieces with scissors. After it was stirred with a glass rod, a 0.05-mL sample of the supernatant was collected from each beaker and stored in a −70°C freezer until the HPLC analysis of DA-8159 and DA-8164 (Shim et al 2002).

Plasma protein binding of DA-8159 and nitroglycerin in rats

Protein binding of DA-8159 and nitroglycerin to the plasma of rats (n = 5 for each rat) was determined 90 min after the intravenous administration of DA-8159, nitroglycerin, or both drugs using the ultrafiltration method. An approximately 0.8-mL blood sample was collected via the carotid artery 90 min after the beginning of the infusion. The blood samples were immediately centrifuged and a 300-μL portion of the plasma sample was collected into Ultrafree-MC (10000 NMWL Filter Unit; Millipore Corporation, Bedford, MA, USA). The plasma samples were centrifuged at 9000 *g* for 30 min and a 50-μL portion of the upper and lower layers were stored in a −70°C freezer until the HPLC analysis of DA-8159 and DA-8164 (Shim et al 2002), and the GC analysis of nitroglycerin (Janssens et al 1989).

Oral study

DA-8159 (the same solution that was used for the intravenous study) at a dose of 30 mg kg^{-1} was orally administered to rats using a feeding tube with (n = 8) or without (n = 9) the simultaneous intravenous administration of nitroglycerin (the same solution that was used in the intravenous study) at a dose of 2.5 mg kg^{-1}. Blood samples were collected at 0, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, and 720 min after the oral administration of DA-8159. Other procedures were similar to those in the intravenous study.

HPLC analysis of DA-8159 and DA-8164

Concentrations of DA-8159 and DA-8164 in the above samples were analysed using a slight modification of the reported HPLC method (Shim et al 2002). To a 0.05-mL biological sample was added 0.1 mL 0.1 M Na_2CO_3 containing 3 μg mL^{-1} sildenafil (an internal standard), and 1 mL ethylether. After vortex-centrifugation at 16 000 *g* for 8 min, the ether layer was collected and dried under a gentle stream of nitrogen gas. A 0.1-mL sample of the mobile phase was added to reconstitute the residue and a 0.05-mL sample was directly injected onto a reversed-phase column. The mobile phase, 20 mM KH_2PO_4:acetonitrile (72:28, v/v), was run at a flow rate of 1.5 mL min^{-1}, and the column effluent was monitored with 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 0.02 μg mL^{-1}. The coefficients of variation of the assay (within-day and between-days) were below 10.1% for the plasma and 9.91% for the urine. This HPLC method was validated according to international guidelines (International Conference on Harmonization 1995).

GC analysis of nitroglycerin

Concentration of nitroglycerin in the above samples was analysed through a slight modification of the reported GC method (Janssens et al 1989). To deproteinize a 0.05-mL biological sample 0.1 mL acetonitrile was added. After vortex-mixing and centrifugation at 16 000 *g* for 4 min, the supernatant was collected and dried under a gentle stream of nitrogen gas. Ethylacetate 10 μL was added to reconstitute the residue and a 1-μL sample was directly injected onto a capillary column. The gas chromatograph was equipped with a ^{63}Ni-electron capture detector and a splitter equipped with a salinized direct injection insert. The split flow rate was 19 mL min^{-1}. Nitrogen gas was used as a make-up gas at a flow rate of 60 mL min^{-1}. The injector, oven, and detector temperatures were maintained at 150, 100, and 300°C, respectively. The retention time of

nitroglycerin was approximately 5.2 min. The detection limit of nitroglycerin in the plasma was 0.05 $\mu g\,mL^{-1}$.

Pharmacokinetic analysis

The AUC_{inf} was calculated using the trapezoidal rule extrapolation method. This method employed the logarithmic trapezoidal rule for the calculation of the area during the declining plasma level phase (Chiou 1978), and the linear trapezoidal rule for the rising plasma level phase. The area from the last datum point to time infinity was estimated by dividing the last measured plasma concentration by the terminal rate constant.

Standard methods (Gibaldi & Perrier 1982) were used to calculate the time-averaged total body (CL), renal (CL_R), and nonrenal (CL_{NR}) clearances, terminal half-life, total area under the first moment of the plasma concentration–time curve from time zero to time infinity (AUMC), mean residence time (MRT), and apparent volume of distribution at a steady state (Vd_{ss}) (Kim et al 1993). The maximum plasma concentration (C_{max}) and time to reach a C_{max} (T_{max}) were directly read from the experimental data. The F was calculated by dividing the AUC_{inf} after the oral administration by the AUC_{inf} following the intravenous administration. The harmonic mean method was used to calculate the mean values of Vd_{ss} (Chiou 1979), terminal half-life (Eatman et al 1977), and each clearance (Chiou 1980).

Statistical analysis

The nonparametric Mann–Whitney Rank Sum Test was performed after the test for normality (Kolmogorov–Smirnov) and equal variance (Levene Median) for each parameter using the SigmaStat program (Systat Software Inc., Richmond, CA, USA). A P value of less than 0.05 was considered to be statistically significant. All results are expressed as mean ± s.d.

Results

Pharmacokinetics of DA-8159 and DA-8164 after intravenous administration of DA-8159 with or without simultaneous intravenous administration of nitroglycerin in rats

After the intravenous administration of DA-8159, the mean arterial plasma concentrations of DA-8159 declined in a polyexponential manner in both groups of rats with higher levels after the simultaneous administration than those after DA-8159 alone (Figure 1A). This resulted in a significantly greater AUC_{inf} of DA-8159 (63.2% increase) in the rats (Table 1). After the simultaneous administration, the CL (38.7% decrease), CL_R (67.7% decrease), and CL_{NR} (36.4% decrease) of DA-8159 became significantly slower, and Vd_{ss} of DA-8159 (42.1% decrease), percentages of the intravenous dose of DA-8159 excreted in 24-h urine as the unchanged drug (45.6% decrease; $Ae_{0-24\,h}$), and 24-h urine output (50.2% decrease) became significantly smaller than those after DA-8159 alone (Table 1). However, the terminal half-life, MRT, and percentage of the intravenous dose of DA-8159 recovered from the entire gastrointestinal tract at 24 h as the unchanged drug ($GI_{24\,h}$) for the two groups of rats was not significantly different (Table 1).

After the intravenous administration of DA-8159, the formation of DA-8164 became rapid. DA-8164 was detected in plasma from the second blood sampling time, 5 min, for both groups of rats (Figure 1B). The T_{max} values of DA-8164 turned out to be 22.5 and 25.0 min for without and with nitroglycerin, respectively (Table 1). After the simultaneous administration, the mean arterial plasma concentrations of DA-8164 became higher and C_{max} became significantly higher (31.8% increase) than those after DA-8159 alone (Figure 1B). This resulted in a significantly greater AUC_{inf} of DA-8164 (48.6% increase) in the rats (Table 1). Other pharmacokinetic

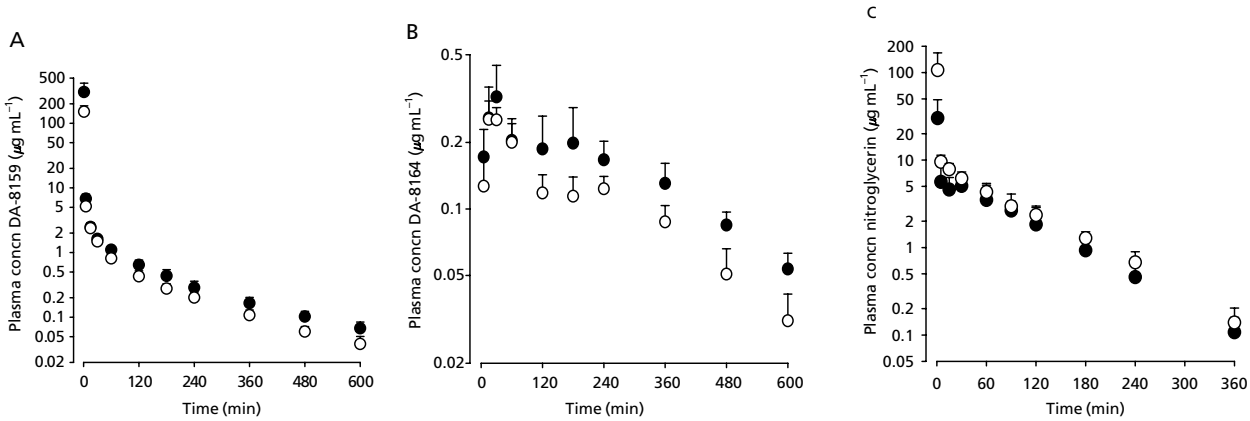

Figure 1 Mean arterial plasma concentration–time profiles of DA-8159 (A) and DA-8164 (B) after intravenous administration of DA-8159 30 mg kg^{-1} with (n = 10; ●) or without (n = 8; ○) simultaneous intravenous administration of nitroglycerin 2.5 mg kg^{-1} to rats, and nitroglycerin (C) after intravenous administration of nitroglycerin 2.5 mg kg^{-1} with (n = 10; ●) or without (n = 8; ○) simultaneous intravenous administration of DA-8159 30 mg kg^{-1} to rats. Vertical bars represent s.d.

Table 1 Pharmacokinetic parameters of DA-8159 and DA-8164 after intravenous administration of 30 mg kg^{-1} DA-8159 without or with intravenous administration of 2.5 mg kg^{-1} nitroglycerin, and nitroglycerin after intravenous administration of 2.5 mg kg^{-1} nitroglycerin without or with intravenous administration of 30 mg kg^{-1} DA-8159

Parameter	DA-8159		Parameter	Nitroglycerin	
	Without nitroglycerin (n = 8)	With nitroglycerin (n = 10)		Without DA-8159 (n = 8)	With DA-8159 (n = 10)
Body weight (g)	280 ± 12.0	270 ± 4.08[a]	Body weight (g)	297 ± 23.9	270 ± 4.08[b]
Urine output (mL 24 h^{-1})	22.1 ± 9.09	11.0 ± 3.80[a]			
DA-8159			Nitroglycerin		
AUC_{inf} (μg min mL^{-1})	457 ± 62.2	746 ± 139[c]	AUC_{inf} (μg min mL^{-1})	960 ± 180	635 ± 274[a]
Terminal half-life (min)	151 ± 30.3	171 ± 33.6	Terminal half-life (min)	56.2 ± 6.99	57.3 ± 8.82
MRT (min)	69.3 ± 14.6	71.9 ± 21.2	MRT (min)	73.4 ± 10.6	83.9 ± 14.3
Vd_{ss} (mL kg^{-1})	4390 ± 1470	2540 ± 1470[a]	Vd_{ss} (mL kg^{-1})	188 ± 46.5	310 ± 214[b]
CL (mL min^{-1} kg^{-1})	65.6 ± 9.52	40.2 ± 7.54[c]	CL (mL min^{-1} kg^{-1})	2.60 ± 0.549	3.71 ± 2.49[a]
CL_R (mL min^{-1}kg^{-1})	5.11 ± 1.90	1.65 ± 0.701[c]			
CL_{NR} (mL min^{-1} kg^{-1})	60.2 ± 8.37	38.3 ± 7.22[c]			
$Ae_{0-24\,h}$ (% of DA-8159 dose)	8.34 ± 2.04	4.54 ± 1.46[c]			
$GI_{24\,h}$ (% of DA-8159 dose)	0.458 ± 0.202	0.486 ± 0.240			
Plasma protein binding (%)	85.1 ± 6.27	90.8 ± 3.29	Plasma protein binding (%)	53.4 ± 24.1	50.4 ± 30.0
DA-8164					
AUC_{inf} (μg min mL^{-1})	69.3 ± 10.6	103 ± 18.2[c]			
Terminal half-life (min)	160 ± 32.6	196 ± 52.7			
C_{max} (μg mL^{-1})	0.267 ± 0.0464	0.352 ± 0.102[a]			
T_{max} (min)	22.5 ± 8.02	25.0 ± 7.50			
$Ae_{0-24\,h}$ (% of DA-8159 dose)	0.0714 ± 0.0392	0.188 ± 0.165			
$GI_{24\,h}$ (% of DA-8159 dose)	0.0561 ± 0.00786	0.0994 ± 0.0536			

Values are mean ± s.d. [a]$P < 0.05$, significantly different from without DA-8159 or nitroglycerin. [b]$P < 0.01$, significantly different from without DA-8159. [c]$P < 0.001$, significantly different from without nitroglycerin.

parameters of DA-8164, listed in Table 1, were not significantly different between the two groups of rats.

Pharmacokinetics of nitroglycerin after intravenous administration of nitroglycerin with or without simultaneous intravenous administration of DA-8159 in rats

After the intravenous administration of nitroglycerin, the mean arterial plasma concentrations of nitroglycerin declined in a polyexponential manner in both groups of rats, with lower levels after the simultaneous administration than those after nitroglycerin alone (Figure 1C). After simultaneous administration, the AUC_{inf}, CL, and Vd_{ss} of nitroglycerin became significantly smaller (33.9% decrease), faster (42.7% increase), and larger (64.9% increase), respectively, than those after nitroglycerin alone (Table 1). However, the terminal half-life and MRT values of nitroglycerin did not turn out to be significantly different between the two groups of rats (Table 1).

Pharmacokinetics of DA-8159 and DA-8164 after oral administration of DA-8159 with or without simultaneous intravenous administration of nitroglycerin in rats

The mean arterial plasma concentration–time profiles of DA-8159 and DA-8164 after the oral administration of DA-8159 with or without the simultaneous intravenous administration of nitroglycerin are shown in Figure 2. Some relevant pharmacokinetic parameters are listed in Table 2. Interestingly, the pharmacokinetic parameters of

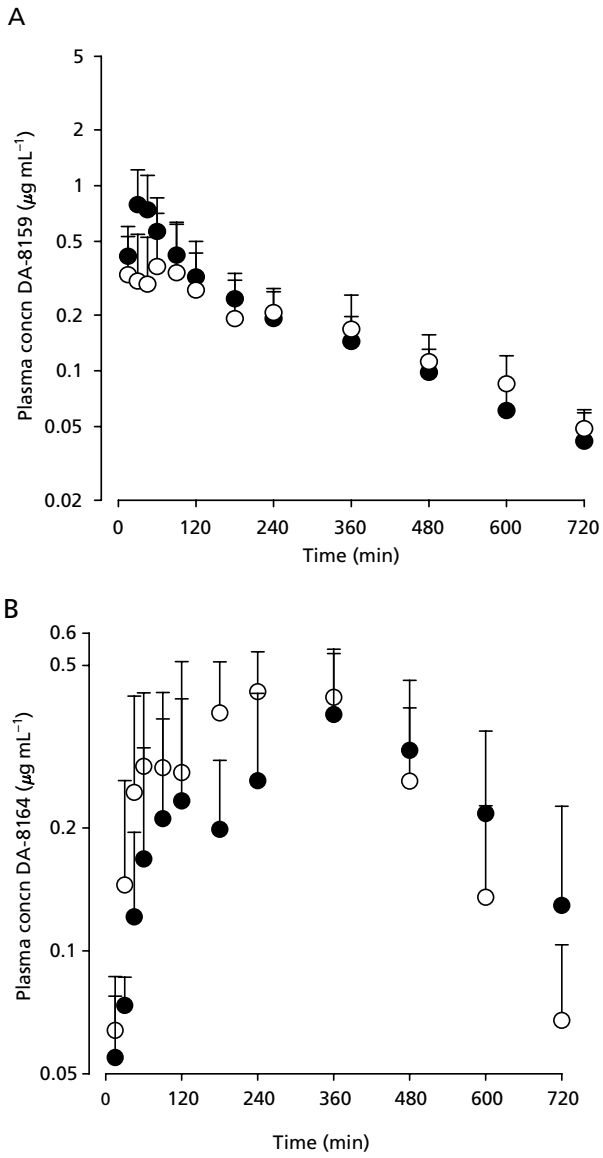

Figure 2 Mean arterial plasma concentration–time profiles of DA-8159 (A) and DA-8164 (B) after oral administration of DA-8159 30 mg kg^{-1} without (n = 9; ○) or with simultaneous (n = 8; ●) intravenous administration of nitroglycerin 2.5 mg kg^{-1} to rats. Vertical bars represent s.d.

DA-8159 and DA-8164 in the two groups of rats were not significantly different.

Plasma protein binding of DA-8159 and nitroglycerin in rats

The plasma protein binding values of DA-8159 were 85.1 and 90.8% without or with nitroglycerin, respectively; they were not significantly different (Table 1). The values of nitroglycerin were 53.4 and 50.4% without or with DA-8159, respectively. Again, these values were not significantly different (Table 1).

Table 2 Pharmacokinetic parameters of DA-8159 and DA-8164 after oral administration of DA-8159 30 mg kg^{-1} with or without simultaneous intravenous administration of nitroglycerin 2.5 mg kg^{-1} to rats

Parameter	Without nitroglycerin (n = 8)	With nitroglycerin (n = 10)
Body weight (g)	277 ± 3.63	272 ± 12.2
DA-8159		
AUC_{inf} (μg min mL^{-1})	158 ± 40.3	141 ± 46.4
Terminal half-life (min)	180 ± 40.1	217 ± 212
CL_R (mL min^{-1} kg^{-1})	4.80 ± 3.78	6.57 ± 2.32
C_{max} (μg mL^{-1})	0.844 ± 0.436	0.506 ± 0.301
T_{max} (min)	40.0 ± 10.6	50.6 ± 39.2
$Ae_{0-24\,h}$ (% of DA-8159 dose)	3.19 ± 1.94	3.38 ± 1.27
$GI_{24\,h}$ (% of DA-8159 dose)	2.02 ± 1.35	2.96 ± 3.23
F (%)	34.6	18.9
DA-8164		
AUC_{inf} (μg min mL^{-1})	211 ± 45.1	223 ± 124
Terminal half-life (min)	141 ± 86.2	178 ± 109
CL_R (mL min^{-1} kg^{-1})	0.0302 ± 0.0741	0.0747 ± 0.0657
C_{max} (μg mL^{-1})	0.490 ± 0.106	0.452 ± 0.229
T_{max} (min)	280 ± 99.5	315 ± 110
$Ae_{0-24\,h}$ (% of DA-8159 dose)	0.0584 ± 0.0505	0.0636 ± 0.0278
$GI_{24\,h}$ (% of DA-8159 dose)	0.178 ± 0.0872	0.182 ± 0.114

Values are mean ± s.d.

Discussion

An internal report has found that DA-8164 is a main metabolite of DA-8159 in man and that the pharmacological effect of DA-8164 in terms of its PDE V inhibitory activity is half that of DA-8159. Hence, the pharmacokinetics of DA-8164 have been evaluated in this study. Shim et al (2003) reported that the AUC_{inf} values of DA-8159 were dose-proportional after its intravenous administration at doses of 5–30 mg kg^{-1} and oral administration at doses of 20–30 mg kg^{-1} to rats. Hence, DA-8159 30 mg kg^{-1} was arbitrarily chosen in this study. Nitroglycerin at a dose of 2.5 mg kg^{-1}, the greatest dose in all rat studies (Delaforge et al 1993), was chosen to obtain a significant drug interaction with DA-8159. Nitroglycerin is usually administered sublingually, but this route of administration is not easy in rats. After the sublingual administration of nitroglycerin, its absorption becomes rapid and bypasses the gastrointestine and liver. Hence, the plasma concentration–time profiles of nitroglycerin might be similar for sublingual and intravenous administration. Therefore, nitroglycerin was administered intravenously in rats.

After the intravenous administration of DA-8159, the contribution of the CL_R to CL of DA-8159 did not turn out to be considerable: the $Ae_{0-24\,h}$ values of the intravenous dose of DA-8159 without and with nitroglycerin were 8.34 and 4.54%, respectively (Table 1). This suggested that most of the intravenously administered DA-8159 was eliminated via the nonrenal route (CL_{NR}). The contribution of the gastrointestinal (including biliary) excretion of unchanged DA-8159 to

the CL_{NR} of DA-8159 seemed almost negligible. The $GI_{24\,h}$ values of DA-8159 were 0.458 and 0.486% of the intravenous dose of DA-8159 without and with nitroglycerin, respectively (Table 1). Moreover, the percentages of DA-8159 at an oral dose of 10 mg kg^{-1} excreted in 24-h bile as an unchanged drug were less than 0.1% in four rats after cannulation of the bile duct (Shim et al 2003). Note that the almost negligible values of $GI_{24\,h}$ (Table 1) were not due to the chemical and/or enzymatic degradation of DA-8159 in the gastrointestinal tract of the rat. DA-8159 was stable in various pH solutions and human gastric juices (Shim et al 2003). The above data suggested that the CL_{NR} values of DA-8159 (Table 1) could represent metabolic clearances of DA-8159. Thus, the changes in the CL_{NR} of DA-8159 (Table 1) could represent metabolic changes of DA-8159 in rats.

After the simultaneous intravenous administration of DA-8159 and nitroglycerin, the AUC_{inf} of DA-8159 was significantly greater than that in DA-8159 alone, which could have been due to the significantly slower CL of DA-8159 (Table 1). The slower CL of DA-8159 could have been due to the significantly slower CL_R and CL_{NR} of DA-8159 (Table 1). The slower CL_{NR} of DA-8159 could have been due to the inhibition of the metabolism of DA-8159 by nitroglycerin. DA-8159 is metabolized mainly via CYP3A1/2 in rats (Kim et al 2005), and nitroglycerin is also metabolized via CYP3A1/2 in rats. For example, the formation of glyceryl dinitrates and glyceryl mononitrates from glyceryl trinitrates (via denitration) was studied (Delaforge et al 1993); the highest microsomal activity was obtained from rats treated with dexamethasone, a main inducer of CYP3A1/2 in rats (Halpert 1988) and the reduced form of nicotinamide adenine dinucleotide phosphate. Hence, the significantly slower CL_{NR} of DA-8159 with nitroglycerin could have been due to the inhibition of CYP3A1/2 by nitroglycerin (Vuppugalla & Mehvar 2004a, b). The slower CL_R of DA-8159 could have been due to the significantly smaller $Ae_{0-24\,h}$ and the significantly greater AUC_{inf} of DA-8159 (Table 1). The smaller $Ae_{0-24\,h}$ could have been due to the urine flow rate-dependent CL_R of DA-8159 in rats (Kim et al 2004). The $Ae_{0-24\,h}$ of DA-8159 decreased with decreasing urine output (Kim et al 2004). The 24-h urine output became significantly smaller after the simultaneous administration of nitroglycerin compared with DA-8159 alone (Table 1). This was not due to a decrease in renal blood flow rate because of nitroglycerin-induced vasodilation. Nitroglycerin increased the renal blood flow rate in conscious rats (Phillips et al 1991).

The Vd_{ss} of DA-8159 after the intravenous dose of DA-8159 alone, 4390 mL kg^{-1}, was considerably large (Table 1), suggesting that DA-8159 had a lipophilic property. The log partition coefficients of the octanol/butanol solutions of DA-8159 were 0.76, 0.75, 0.81, and 1.85 for the buffer solutions with pH values of 1, 3, 5, and 7, respectively. This could be supported by the following results. The tissue-to-plasma ratios of DA-8159 were considerably greater-than-unity after both its intravenous and oral administration at a dose of 30 mg kg^{-1} to rats (Shim et al 2003). After its simultaneous administration, the Vd_{ss} of DA-8159 became significantly smaller (Table 1). However, this could not have been due to the significantly smaller free (unbound in plasma proteins) fractions of DA-8159. The plasma protein binding values of DA-8159 were similar in the two groups of rats (Table 1).

After simultaneous intravenous administration, the AUC_{inf} of DA-8164 became significantly greater (Table 1). This could have been due to the greater exposure of the parent drug, i.e. the greater AUC_{inf} of DA-8159 (Table 1). The $AUC_{inf,\ DA\text{-}8164}/AUC_{inf,\ DA\text{-}8159}$ ratios were similar between without or with nitroglycerin; the values were 15.2 and 13.8% for without and with nitroglycerin, respectively (Table 1).

After simultaneous intravenous administration, the CL of nitroglycerin became significantly faster (Table 1). This could have been due to the cardiac output-dependent CL of nitroglycerin in rats (Fung et al 1988). Bhatia et al (2003) and Mikhail et al (2004) reported that sildenafil, another PDE V inhibitor, increased cardiac output in man. After simultaneous intravenous administration, the Vd_{ss} of nitroglycerin was significantly greater (Table 1). However, this also was not due to the significantly greater free fractions of nitroglycerin in plasma; the plasma protein binding values of nitroglycerin were comparable between without and with DA-8159 (Table 1).

After oral administration of DA-8159 with the simultaneous intravenous administration of nitroglycerin, the AUC_{inf} values of DA-8159 were comparable in the two groups of rats (Table 2), although the AUC_{inf} values of the intravenous DA-8159 were significantly greater after simultaneous intravenous administration of nitroglycerin (Table 1). This was not due to the decrease in the gastrointestinal absorption of DA-8159 after the simultaneous administration of nitroglycerin. It was possible that the $GI_{24\,h}$ values of DA-8159 after its oral administration without (2.02%) and with (2.96%) nitroglycerin (Table 2) might have been partly due to the gastrointestinal (including the biliary) excretion of the absorbed drug. Based on the linear pharmacokinetics of DA-8159 (Shim et al 2003), the mean 'true' fractions of oral dose unabsorbed (F_{unabs}) from the gastrointestinal tract in this study could be estimated using the following reported equations (Lee & Chiou 1983):

$$0.0202 = F_{unabs} + (0.346 \times 0.00458) \quad (1)$$

for without nitroglycerin, and

$$0.0296 = F_{unabs} + (0.186 \times 0.00486) \quad (2)$$

for with nitroglycerin, in which 0.346 (0.186) and 0.00458 (0.00486) were the F (Table 2) and $GI_{24\,h}$ after the intravenous administration without (with) nitroglycerin (Table 1), respectively. The calculated F_{unabs} values were 1.86 and 2.87% for DA-8159 alone and with nitroglycerin, respectively. Thus, more than 97% of the oral dose of DA-8159 was absorbed in both groups of rats. Although the exact reason for this was not clear, the comparable AUC_{inf} values of DA-8159 could be due to changes in intestinal first-pass effect of DA-8159 with nitroglycerin. The intestinal first-pass effect of DA-8159 was approximately 58% of the oral dose at 30 mg kg^{-1} in rats (Shim et al 2003). The $AUC_{inf,\ DA\text{-}8164}/AUC_{inf,\ DA\text{-}8159}$ ratios after oral administration, 134 and 158% for without or with

nitroglycerin, respectively, were considerably greater than those after the intravenous administration, which were 15.2 and 13.5%, respectively, as mentioned earlier. This supported a considerable first-pass effect for the formation of DA-8164 in rats. The F value of DA-8159 with nitroglycerin was considerably smaller (45.4% decrease) than without nitroglycerin (Table 2). This could have been due to significantly greater AUC_{inf} of DA-8159 after the simultaneous intravenous administration of nitroglycerin (Table 1).

Conclusions

After simultaneous intravenous administration of DA-8159 and nitroglycerin to rats, the AUC_{inf} values of DA-8159 and nitroglycerin were significantly greater and smaller, respectively, than those after each drug alone. The greater AUC_{inf} of DA-8159 was due to significantly slower CL_{NR} (due to nitroglycerin's inhibition of CYP3A1/2) and CL_R (due to significantly smaller $Ae_{0-24\,h}$ of DA-8159 because of significantly smaller 24-h urine output) of DA-8159. The smaller AUC_{inf} of nitroglycerin was due to significantly faster CL of nitroglycerin, possibly due to the increase in cardiac output by DA-8159. After the simultaneous oral administration of DA-8159 and intravenous administration of nitroglycerin, the AUC_{inf} of DA-8159 was comparable with that of DA-8159 alone. This could have been due to changes in the intestinal first-pass effects of DA-8159, and not to the decrease in gastrointestinal absorption of DA-8159. DA-8159 was developed as an oral agent. Although the AUC_{inf} values of DA-8159 and DA-8164 after the oral administration of DA-8159 were comparable without and with nitroglycerin, studies are required to determine the administration time of oral DA-8159 when volunteers are concomitantly taking nitroglycerin, while changes in their blood pressure are measured.

References

Ahn, B. O., Kang, K. K., Ahn, G. J., Kwon, J. W., Kim, W. B., Kang, K. S., Lee, Y. S. (2003) Efficacy of DA-8159, a new PDE5 inhibitor, for inducing penile erection in rabbits with acute spinal cord injury. *Int. J. Impot. Res.* **15**: 405–411

Bhatia, S., Frantz, R. P., Severson, C. J., Durst, L. A., McGoon, M. D. (2003) Immediate and long-term hemodynamic and clinical effects of sildenafil in patients with pulmonary arterial hypertension receiving vasodilator therapy. *Mayo Clin. Proc.* **78**: 1207–1213

Cheitlin, M. D., Hutter, A. M. Jr., Brindis, R. G., Ganz, P., Kaul, S., Russell, R. O. Jr., Zusman, R. M. (1999) ACC/AHA expert consensus document. Use of sildenafil (Viagra) in patients with cardiovascular disease. *J. Am. Coll. Cardiol.* **33**: 273–282

Chiou, W. L. (1978) Critical evaluation of potential error in pharmacokinetic studies using the linear trapezoidal rule method for the calculation of the area under the plasma level–time curve. *J. Pharmacokinet. Biopharm.* **6**: 539–549

Chiou, W. L. (1979) New calculation method for mean apparent drug volume of distribution and application to rational dosage regimen. *J. Pharm. Sci.* **68**: 1067–1069

Chiou, W. L. (1980) New calculation method of mean total body clearance of drugs and its application to rational regimens. *J. Pharm. Sci.* **69**: 90–91

Choi, S. J., Ji, H. Y., Lee, H.–Y., Kim, D. S., Kim, W. B., Lee, H. S. (2002) *In vitro* metabolism of a novel phosphodiesterase-5 inhibitor DA-8519 in rat liver preparations using liquid chromatography/electrospray mass spectrometry. *Biomed. Chromatogr.* **16**: 395–399

Delaforge, M., Servent, D., Wirsta, P., Ducrocq, C., Mansuy, D., Lenfant, M. (1993) Particular ability of cytochrome P-450 CYP3A to reduce glyceryl trinitrate in rat liver microsomes: Subsequent formation of nitric oxide. *Chem. Biol. Interact.* **86**: 103–117

Dicarlo, F. J., Melgar, M. D. (1969) Binding and metabolism of nitroglycerin by rat blood plasma. *Proc. Soc. Exp. Biol. Med.* **131**: 406–408

Dishy, V., Sofowora, G., Harris, P. A., Kandcer, M., Zhan, F., Wood, A. J., Stein, C. M. (2001) The effect of sildenafil on nitric oxide-mediated vasodilation in healthy men. *Clin. Pharmacol. Ther.* **70**: 270–279

Doh, H., Shin, C. Y., Son, M., Ko, J., Yoo, M., Kim, S. H., Kim, W. B. (2002) Mechanism of erectogenic effect of the selective phosphodiesterase type 5 inhibitor, DA-8159. *Arch. Pharm. Res.* **25**: 873–878

Eatman, F. B., Colburn, W. A., Boxenbaum, H. G., Posmanter, H. N., Weinfeld, R. E., Ronfeld, R., Weissman, L., Moore, J. D., Gibaldi, M., Kaplan, S. A. (1977) Pharmacokinetics of diazepam following multiple dose oral administration to healthy human subjects. *J. Pharmacokinet. Biopharm.* **5**: 481–494

Fung, H. L., Blei, A., Chong, S. (1988) Interpretation of nitrate plasma concentrations. Effect of cardiac output on nitroglycerin pharmacokinetics in experimental animals. *Eur. Heart J.* **9** (Suppl. A): 39–43

Gibaldi, M., Perrier, D. (1982) *Pharmacokinetics*. 2nd edn, Marcel–Dekker, New York

Halpert, J. R. (1988) Multiplicity of steroid-inducible cytochrome P-450 in rat liver microsomes. *Arch. Biochem. Biophys.* **263**: 59–68

International Conference in Harmonization (ICH Q2A, Q2B) (1995) Guidelines on Validation of Analytical Procedures.

Ishizuka, N., Saito, K., Akima, M., Matsubara, S., Saito, M. (2000) Hypotensive interaction of sildenafil and nicorandil in rats through the cGMP pathway but not by K (ATP) channel activation. *Jpn. J. Pharmacol.* **84**: 316–324

Janssens, J. J., Selala, M. I., Daelemans, F. F., Andries, S. W., Schepens, P. J. (1989) Quantitative determination of nitroglycerin by capillary gas chromatography-electron capture detection. *J. Pharm. Biomed. Anal.* **7**: 1631–1634

Kang, K. K., Ahn, G. J., Ahn, B. O., Yoo, M., Kim, W. B. (2003) DA-8159, a new PDE5 inhibitor, induces penile erection in conscious and acute spinal cord injured rabbits. *Eur. Urol.* **43**: 689–695

Kang, K. K., Choi, S. M., Ahn, G. J., Kwon, J. W., Kim, W. B. (2004) The effect of DA-8159 on corpus carvernosal smooth muscle relaxation and penile erection in diabetic rabbits. *Urol. Res.* **32**: 107–111

Kim, S. H., Choi, Y. M., Lee, M. G. (1993) Pharmacokinetics and pharmacodynamics of furosemide in protein–calorie malnutrition. *J. Pharmacokinet. Biopharm.* **21**: 1–17

Kim, Y. C., Kwon, J. W., Kim, W. B., Lee, I., Lee, M. G. (2004) Pharmacokinetic changes of DA-8159, a new erectogenic, after intravenous and oral administration to rats with diabetes mellitus induced by streptozotocin. *J. Pharm. Sci.* **93**: 2374–2387

Kim, Y. C., Shim, H. J., Lee, J. H., Kim, S. H., Kwon, J. W., Kim, W. B., Lee, M. G. (2005) Effects of enzyme inducers and inhibitors on the pharmacokinetics of intravenous DA-8159, a new erectogenic, in rats. *Biopharm. Drug Dispos.* **26**: 233–241

Kloner, R. A., Hutter, A. M., Emmick, J. T., Mitchell, M. I., Denne, J., Jackson, G. (2003) Time course of the interaction between tadalafil and nitrates. *J. Am. Coll. Cardiol.* **42**: 1855–1860

Lee, M. G., Chiou, W. L. (1983) Evaluation of potential causes for the incomplete bioavailability of furosemide: gastric first-pass metabolism. *J. Pharmacokinet. Biopharm.* **11**: 623–640

Mikhail, G. W., Prasad, S. K., Li, W., Rogers, P., Chester, A. H., Bayne, S., Stephens, D., Khan, M., Gibbs, J. S., Evans, T. W., Mitchell, A., Yacoub, M. H., Gatzoulis, M. A. (2004) Clinical and haemodynamic effects of sildenafil in pulmonary hypertension: Acute and mid-term effects. *Eur. Heart J.* **25**: 431–436

Phillips, K., Gardiner, S. M., Kemp, P. A., Bennett, T. (1991) Factors affecting the regional haemodynamic responses to glyceryl trinitrate and molsidomine in conscious rats. *Br. J. Pharmacol.* **104**: 151–158

Shim, H. J., Lee, E. J., Jung, Y. H., Kim, S. H., Kim, S. H., You, M., Kwon, J. W., Kim, W. B., Lee, M. G. (2002) Determination of a new phosphodiesterase V inhibitor, DA-8159, in plasma and urine by high-performance liquid chromatography. *J. Pharm. Biomed. Anal.* **30**: 527–533

Shim, H. J., Kim, Y. C., Park, K. J., Kim, D. S., Kwon, J. W., Kim, W. B., Lee, M. G. (2003) Pharmacokinetics of DA-8159, a new erectogenic, after intravenous and oral administration to rats: Hepatic and intestinal first-pass effects. *J. Pharm. Sci.* **92**: 2185–2195

Vuppugalla, R., Mehvar, R. (2004a) Hepatic disposition and effects of nitric oxide donors: rapid and concentration-dependent reduction in the cytochrome P450-mediated drug metabolism in isolated perfused rat livers. *J. Pharmacol. Exp. Ther.* **310**: 718–727

Vuppugalla, R., Mehvar, R. (2004b) Short-term inhibitory effects of nitric oxide on cytochrome P450-mediated drug metabolism: time dependency and reversibility profiles in isolated perfused rat livers. *Drug Metab. Dispos.* **32**: 1446–1454

Webb, D. J., Freestone, S., Allen, M. J., Muirhead, G. J. (1999) Sildenafil citrate and blood-pressure-lowering drugs: results of drug interaction studies with an organic nitrate and a calcium antagonist. *Am. J. Cardiol.* **83**: 21C–28C

Webb, D. J., Muirhead, G. J., Wulff, M., Sutton, J. A., Levi, R., Dinsmore, W. W. (2000) Sildenafil citrate potentiates the hypotensive effects of nitric oxide donor drugs in male patients with stable angina. *J. Am. Coll. Cardiol.* **36**: 25–31