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General Pharmacological Properties of the New H^+/K^+ ATPase Inhibitor DBM-819

Woo-Kyu PARK*, Jae Yang KONG, Hyun-Jung KIM, Dong Ha LEE[1], Hong LIM[1], and Hyae Gyeong CHEON

Pharmaceutical Screening Research Laboratory, Korea Research Institute of Chemical Technology, Yusong-Gu, Daejon 305-343, Korea

[1]Dongbu Hannong Chemical Co. Ltd., Yusong-Gu, Daejon 305-380, Korea

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Abstract–The effects of a newly synthesized H^+/K^+ATPase inhibitor, 1-(2-methyl-4-methoxypheny)-4-[(3-hydroxypropyl)amino]-6-methyl-2,3-dihydropyrrolo[3,2-c]quinoline (DBM-819), on the central nervous system, isolated smooth muscle, cardiovascular and digestive systems and renal function were investigated in various experimental animals. Oral administration of DBM-819 had no effect on the central nervous system except body temperature of mice slightly decreased at doses of 15 and 50 mg/kg. DBM-819 produced a moderate analgesic effect in acetic acid-induced writhing test in mice at 50 mg/kg (*p.o.*). In conscious rats, DBM-819 (15 and 50 mg/kg, *p.o.*) showed a slight increase in blood pressure and a small decrease in heart rate. DBM-819 had an significant effect on agonist-induced contraction of guinea pig ileum at 1.5×10^{-5} g/ml. No significant effect of DBM-819 (5 and 15 mg/kg, i.p) on urinary volume or urinary excretion of Na^+, K^+ and Cl^- was observed in rats. DBM-819 had no significant effect on intestinal transport of a semisolid meal in mice at 15 and 50 mg/kg (*p.o.*). These findings suggest that DBM-819 exerts no significant pharmacological effects on the central nervous system and renal function at 15 mg/kg (*p.o.*), but produces some effects on the smooth muscle and circulatory system.

Key words □ DBM-819, H^+/K^+ ATPase, general pharmacology

Gastric H^+/K^+ ATPase inhibitors have been considered as effective remedies for acid-related diseases. Among such inhibitors, omeprazole and lansoprazole are the most widely used for the treatment of peptic ulcer. These compounds bind covalently to the H^+/K^+ ATPase and inactivate it irreversibly (Lorentzon *et al.*, 1985). The irreversible inactivation of the enzyme makes it difficult to predict and reproduce the pharmacokinetic and pharmacodynamic profiles of compounds such as omeprazole and lansoprazole. Recently, 1-(2-methyl-4-methoxypheny)-4-[(3-hydroxypropyl) amino]-6-methyl-2,3-dihydropyrrolo[3,2-c]quinoline (DBM-819, Fig 1), a potent and reversible inhibitor of the H^+/K^+ ATPase, has been developed. *In vitro* and animal studies have demonstrated that DBM-819 is a more potent inhibitor of H^+/K^+ ATPase than omeprazole (Cheon *et al.*, 2001a, 2001b). In addition, DBM-819 would overcome the side effects associated with the irreversibility of the effects of substituted benzimidazoles such as omeprazole and lansoprazole. DBM-819 is expected, therefore, to be effective for the treatment of acid-related diseases such as gastric ulcer and gastritis.

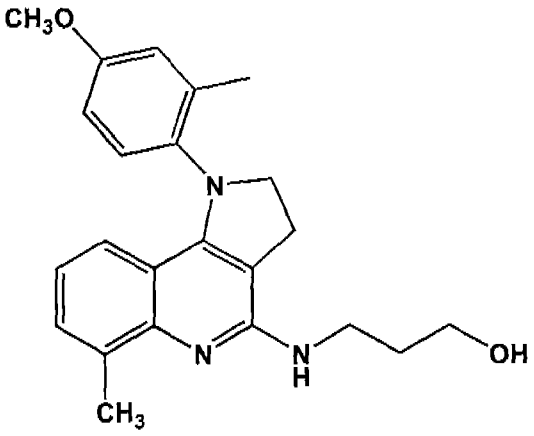

Fig. 1. Chemical structure of DBM-819.

*To whom correspondence should be addressed.

In the present study, as a part of preclinical evaluation of DBM-819, the general pharmacological effects of DBM-819 on central nervous system, smooth muscles, cardiovascular and respiratory systems and renal function were investigated.

MATERIALS AND METHODS

Animals

The animals used were male ICR mice, male Sprague-Dawley rats (Bio Genomics Inc. Seoul, Korea), and male Hartley albino guinea pigs (Hanil Laboratory Animal Inc., Jeonju, Korea). Animals were housed in an acrylfiber cage in a controlled room (temperature 22±2°C, relative humidity 50±5%), were maintained on a 12-hr light/dark cycle and were given solid diet and tap water *ad libitum*. All experimental procedures were consistent with the guidelines issued by the U.S. national institutes of health and institutional animal care and use committee of our institute.

Drugs

Acetic acid, charcoal activated, acetylcholine chloride, histamine dihydrochloride and hydroxypropyl-β-cyclodextrin were purchased from Sigma (St. Louis, MO, USA). Pentobarbital sodium was obtained from Hanlim Pharm. Ind. Co. (Seoul, Korea). DBM-819 was synthesized in Dongbu Hannong Chemical Co., Ltd. (Daejon, Korea). For oral administration, DBM-819 was suspended in 40%-hydroxypropyl-β-cyclodextrin (40% HBC) solution.

Effects on general behavior

The methods used were based on that described by Irwin (1968). Mice (22-25 g, 5 mice per group) were administered orally with DBM-819 (15 and 50 mg/kg), and were observed at 0.5, 1, 1.5, 2, 4 and 24 hrs after the drug administration. Control group of the mice were injected (*p.o.*, 0.2 ml/20 g) with 40% HBC solution.

Effect on central nervous system

Spontaneous locomotor activity

Mice (20-25 g, 10 mice per group) were orally administered with DBM-819 or vehicle (40% HBC). Each mouse was then placed in an activity cage with an automatic recording device (AM1052, Benwick Electronics, Benwick, UK), and activity counts for 5 min were recorded 30, 60, 90, 120 and 240 min after the drug administration, respectively.

Pentobarbital-induced sleeping time

Mice (22-27 g, 10 mice per group) were orally administered with DBM-819 or vehicle, and 1 hr later, pentobarbital (32 mg/kg) was injected intraperitoneally into the mice. The time of onset of sleep and the duration of sleeping time of each mouse were recorded.

Rotarod test

Mice (20-25 g, 10 mice per group) were orally administered with DBM-819 or vehicle and were subjected to the rotarod tests. One hour after the drug administration, the mouse was placed on a 1 inch diameter knurled plastic rod rotating at 6 rpm (Ugo-Basile, Italy), and the rotarod deficit (%) was obtained by counting the number of animals fallen from the rotating rod within 1 minute (Dunham *et al.*, 1957).

Maximal electroshock (MES)-induced convulsion

Maximal seizures were elicited by a 60 Hz alternating current of 50 mA delivered for 0.2 sec via corneal electrodes (Krall *et al.*, 1978). Groups of 10 mice (20-22 g) received DBM-819 or vehicle orally were subjected to electric shock. The incidence of tonic convulsion was determined.

Pentylenetetrazol (PTZ)-induced convulsion

PTZ (85 mg/kg) was injected subcutaneously, and the mice were then observed for 30 min after injection and the first generalized clonic seizure with loss of righting reflexes was used as endpoint (Krall *et al.*, 1978). DBM-819 was administered orally 1 hr before the injection of PTZ.

Analgesic activity

DBM-819 or vehicle was administered orally, and 1 hr later, each mouse was injected with 1 % acetic acid (0.1 ml/10 g. i.p.) and was placed immediately in an observation cage, and the numbers of writhing episodes during the subsequent 10-min period were counted (Collier *et al.*, 1968). Percent inhibition was calculated from the average writhing scores in the drug-treated group and vehicle-treated control animals.

Body temperature

Body temperature was measured rectally using an electrothermometer (Thermalert TH-5, Physitemp, USA). Male mice (22-25 g) with stable rectal temperature were used in groups of 8 animals per dose level. DBM-819 or vehicle was administered orally and rectal temperatures were measured 30, 60, 90, 120 and 240 min after the drug administration.

Effect on smooth muscle

Agonist-induced contractions in isolated guinea pig ileum

Segments of myenteric plexus-longitudinal muscle, about

2-2.5 cm long obtained from male Hartley guinea pig (350-450 g) ilcum were used (Rang, 1964). Isolated preparations were mounted vertically in organ baths containing 20 ml of Krebs-Henscleit bicarbonate solution (mmol/L: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1, NaH_2PO_4 0.4, $NaHCO_3$ 11.9, D-glucose 5.6) at 37°C, and aerated with 95 % O_2/5% CO_2. The preparations were equilibrated for about 60 min before each experiment. The tension of the preparation was isotonically recorded with a force displacement transducer (K30, Hugo Sachs Electronic, Germany) and displayed on a chart recorder (Multicorder MC 6625, Hugo Sachs Electronic, Germany). After 10 min exposure of the preparations to test drug, agonists (acetylcholine and histamine) were added and the effects of the test drug on the contraction induced by each agonist were studied.

Effect on cardiovascular system

Mean blood pressure and heart rate in conscious rats

Male Sprague-Dawley rats (350-450 g) were anesthetized with ketamine hydrochloride (125 mg/kg, i.p.), and the polyethylene (PE-50) catheter connected to PE-10 catheter filled with heparinized saline solution (20 IU/ml) was inserted into the femoral artery for recording arterial blood pressure and heart rate. The animals were allowed 1 day to recover and stabilize in individual cages. On the day of experiment, rats were kept moving free in individual cages in a quiet room, and the arterial catheter was connected to a Isotec pressure transducer (Healthdyne, Marietta, GA, USA) coupled to a Graphtec Linearcorder (model 3310, Graphtec Corp., Tokyo, Japan). Arterial blood pressure and heart rate were monitored for 24 hrs after single oral administration of DBM-819.

Effect on gastrointestinal system

Charcoal meal propulsion

The mice fasted overnight were administered intraperitoneally with DBM-819 or vehicle. Thirty minutes after the injection of test drug, each mouse received orally 0.2 ml of 5 % w/v suspension of charcoal in distilled water. Twenty minutes after the administration of charcoal meal, the mice were sacrificed and the distance traversed by the charcoal meal along the small intestine from the pyloric sphincter was measured (Takemori, 1969). This distance was calculated as a percentage of the total length of the gut.

Effects on urine and electrolytes excretion

Male Sprague-Dawley rats (150-250 g) were used for each treatment group. After intraperitoneal injection of test drug, 2.5 ml/100 g saline was administered orally and the volume of urine was measured 5 hrs thereafter. Na^+, K^+ and Cl^- were measured with Na/K/Cl analyzer (Ciba-Corning, USA).

Statistics.

The data were expressed as a mean±SEM. The statistical significances between groups were assessed by one-way analysis of variance (ANOVA) followed by Dunnett's test or Chi-square test. Differences at p values of less than 0.05 were considered to be statistically significant.

RESULTS

Effect on general behavior

Oral administration of DBM-819 at doses of 15 and 50 mg/kg showed no observable changes in behavioral, neurological and autonomic profiles in mice during 24 hrs period (data not shown).

Effect on spontaneous locomotor activity

As shown in Table I, DBM-819 (15 and 50 mg/kg) did not show any significant change in spontaneous locomotor activ-

Table II. Effect of DBM-819 on pentobarbital-induced sleeping time in mice

Drugs	Dose (mg/kg, *p.o.*)	Sleeping time (min)	
		Onset	Duration
Control	–	5.0±0.5	43.9±6.5
DBM-819	15	4.7±0.5	55.6±8.0
	50	4.6±0.3	50.7±5.4

Each value represents the mean±SEM (n=9~10).

Table I. Effect of DBM-819 on spontaneous locomotor activity in mice

Drugs	Dosc (mg/kg, *p.o.*)	Activity (counts/5 min)					
		Before	Time after administration (min)				
			30	60	90	120	240
Control	–	395.8±38.0	394.3±51.4	379.1±52.1	286.2±30.5	256.3±36.7	188.4±55.6
DBM-819	15	451.7±52.6	392.9±54.6	319.9±21.3	261.1±32.2	293.1±32.3	210.7±36.1
	50	349.6±42.4	304.0±48.9	279.9±42.8	311.0±36.8	295.4±25.7	238.3±61.4

Each value represents the mean±SEM (n=9~10).

ity for 4 hrs, when compared to the vehicle-treated control group.

Effect on pentobarbital-induced sleeping time

Orally administered DBM-819 (15 and 50 mg/kg) slightly increased the pentobarbital-induced sleeping time in mice. However, there were no statistical significances, when compared with the control group (Table II).

Effect on rotarod test

DBM-819 (15 and 50 mg/kg) had no effect on skeletal muscle coordination in mice (Table III).

Effects on MES- and PTZ-induced convulsions

DBM-819 (15 and 50 mg/kg) did not prevent the tonic extensor seizures induced by a MES. In addition, subcutaneous PTZ-induced clonic convulsion was not blocked by DBM-819 at all (Table IV).

Table III. Effect of DBM-819 on rotarod activity in mice

Drugs	Dose (mg/kg, *p.o.*)	% Rotarod deficit*				
		30 min	60 min	90 min	120 min	180 min
Control	–	0	0	0	0	0
		0	0	0	0	0
DBM-819	15	0	0	0	0	0
	50	0	0	0	0	0

* % of the mice fallen from the rotating rod (n=10).

Table IV. Effect of DBM-819 on maximal electroshock (MES)- and pentylenetetrazol- induced convulsions in mice

Drugs	Dose (mg/kg, *p.o.*)	% Convulsion*	
		MES	Pentylenetetrazol
		Tonic	Clonic
Control	–	100	100
		100	100
DBM-819	15	100	100
	50	100	100

*count the number of mice convulsing (n=10).

Table V. Effect of DBM-819 on acetic acid-induced writhing test in mice

Drugs	Dose (mg/kg, *p.o.*)	No. of writhings observed (%-Analgesia)
Control	–	25.3±2.8
DBM-819	15	18.8±2.9 (26)
	50	11.9±1.7 (53)*

Each value represents the mean±SEM (n=10). *p<0.05, when compared to the control group.

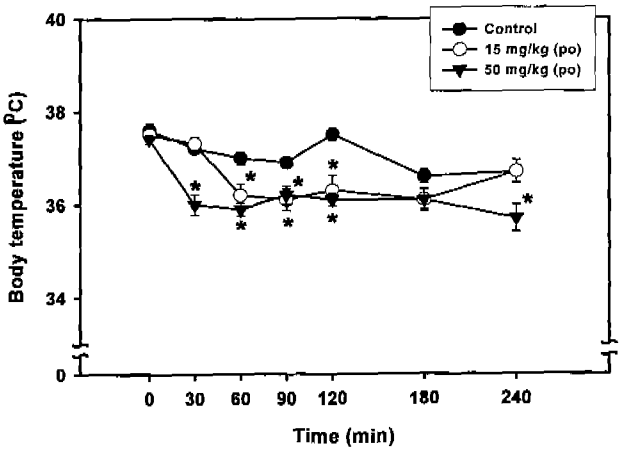

Fig. 2. Effect of DBM-819 on body temperature in mice. Eacl value represents the mean±SEM (n=10). *p<0.05, when com pared to the vehicle control group.

Effect on analgesic activity

As shown in Table V, 15 mg/kg of DBM-819 did not reduce the frequency of writhes, whereas 50 mg/kg DBM-819 produced a marked and statistically significant inhibition of writhing responses showing 53% of analgesia in mice (p<0.05).

Effect on body temperature

DBM-819 (15 and 50 mg/kg) had a significant effect on body temperature in mice. Low dose, 15 mg/kg, of DBM-819 produced a statistically significant decrease in body temperature of mice at 60 min after the drug administration, and this hypothermic effect disappeared 3 hrs after dosing. However, the hypothermic effect induced by 50 mg/kg DBM-819 was prolonged up to 4 hrs after the drug administration (Fig. 2).

Table VI. Effect of DBM-819 on acetylcholine- and histamine-induced contractions in isolated guinea-pig ileum

Drug	Concentration (μg/ml)	Contraction response (% of maximal contraction)	
		Acetylcholine (10^{-5}M)	Histamine (10^{-5}M)
Control	–	94.8±3.2	100
DBM-819	5	61.1±7.5*	84.0±3.4
	15	33.2±2.8*	43.2±4.8*
	50	21.8±2.5*	22.4±5.8*

Each value represents the mean±SEM (n=3~5). *p<0.05, when compared to the control group.

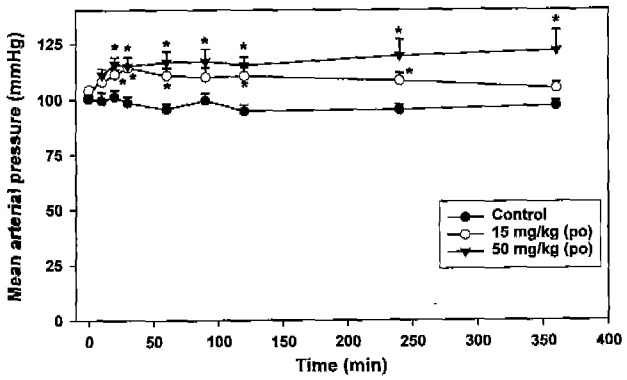

Fig. 3. Effect of DBM-819 on mean arterial blood pressure in conscious rats. Each value represents the mean±SEM (n=3~6). *p<0.05, when compared to the control group.

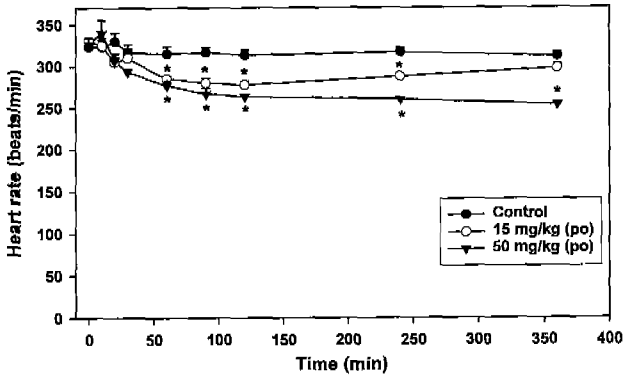

Fig. 4. Effect of DBM-819 on heart rate in conscious rats. Each value represents the mean±SEM (n=3~6). *p<0.05, when compared to the control group.

Effects on agonist-induced contractions

The additions of 15 and 50 μg/ml of DBM-819 significantly influence the contraction induced by acetylcholine (1.0×10^{-5} M) and histamine (1.0×10^{-5} M) in the isolated ileum from the guinea pig ($p<0.05$, Table VI).

Effects on mean blood pressure and heart rate

As shown in Fig. 3 & 4, orally administered DBM-819 (15 and 50 mg/kg) produced a slight but significant increase in the blood pressure and a significant decrease in the heart rate up to 4-6 hrs after the drug injection ($p<0.05$).

Table VII. Effect of DBM-819 on gatroinestinal motility in mice

Drugs	Dose (mg/kg, i.p.)	Transfer rate (%)
Control	–	46.3±3.3
DBM-819	15	59.3±4.2
	50	42.8±5.3

Each value represents the mean±SEM (n=9~11).

Effect on gastrointestinal system

As shown in Table VII, DBM-819 had no effect on gastrointestinal motility in mice at 15 and 50 mg/kg (i.p.).

Effects on urine and electrolytes excretion

Intraperitoneal (i.p.) administration of DBM-819 (5 and 15 mg/kg) did not show any effects in the urinary volume and urinary excretions of Na^+, K^+ and Cl^- (Table VIII).

DISCUSSION

It has been reported that DBM-819 showed potent anti-secretory and anti-ulcer activities in animal models. Gastric acid secretion was markedly inhibited by intraduodenal administration of DBM-819 (ED_{50}; 3.5 mg/kg). In addition, indomethacin-induced ulcer was also strongly blocked by DBM-819, and its ED_{50} value is 3.1 mg/kg, *p.o.* (Cheon *et al.*, 2001a). The highest oral dose of 50 mg/kg, therefore, used in the present experiments in mice and rats corresponds to more than 15 times of ED_{50} values of the DBM-819 in basal acid secretion or drug-induced ulcers.

In the present study, the effects of DBM-819 on central nervous system, smooth muscle, circulatory system and renal function were investigated. DBM-819 did not affect spontaneous locomotor activity, pentobarbital-induced sleeping time, skeletal muscle coordination and tonic-clonic convulsion even at 50 mg/kg (*p.o.*). DBM-819, however, produced a

Table VIII. Effect of DBM-819 on urine volume and electrolytes collected over 5 hrs in hydrated rats

Drugs	Dose (mg/kg, i.p.)	Parameter			
		Urine vol (ml/100g/5h)	Na^+ (μEq/100g/5h)	K^+ (μEq/100g/5h)	Cl^- (μEq/100g/5h)
Control	–	1.52±0.14	45.9±7.19	119±13.3	113±18.9
DBM-819	15	1.53±0.09	73.4±7.27	105±14.1	135±13.1
	50	2.04±0.24	60.6±7.83	145±8.44	125±12.4

Each value represents the mean±SEM (n=4~5).

slight hypothermic effect at 15 and 50 mg/kg, and showed an analgesic effect in acetic acid-induced writhing test at 50 mg/kg. These findings suggest that DBM-819 does not produce any effect on the central nervous system at low dose of 15 mg/kg.

In smooth muscle, DBM-819 produced an inhibitory effect on the contractions induced by acetylcholine and histamine in the isolated ileum of the guinea pig at concentrations of 15 and 50 μg/ml, suggesting the pharmacological action of DBM-819 on autonomic nervous system.

In circulatory system, a slight increase in the blood pressure and a decrease in heart rate were observed in conscious rats at 15 and 50 mg/kg (*p.o.*) DBM-819, indicating that the cardiovascular system may be influenced by DBM-819.

DBM-819 at 15 and 50 mg/kg (i.p.) had no significant effect on intestinal transport of a semisolid meal in mice.

As regard urine output and electrolytes metabolism, there were no significant effects on urinary volume or urinary excretions of Na^+, K^+ and Cl^- at 5 and 15 mg/kg (i.p.).

In addition, the vehicle (40% HBC) solution used in the present study did not show any observable effect in normal mice or rats.

In summary, the present results show that 15 and 50 mg/kg DBM-819 did not produce any effects on the general behavior, central nervous system and renal function, but circulatory and autonomic systems were slightly influenced by DBM-819.

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