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Acute Toxicity of the BK_{Ca} Channel Opener LDD175

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Abstract – LDD175 (4-choloro-7-trifluoromethyl-10*H*-benzo[4,5]furo[3,2-b]indole-1- carboxylic acid) is one of benzofuroindole derivatives that act as a potent BK_{Ca} channel openers. In the process of testing LDD175 as a new drug candidate, an acute toxicity study was carried out in mice. The mice were administered LDD175 intraperitoneally at dose of 0.2, 1, 10, 50, 100, 200, 400, 800 mg/kg and orally at dose of 10, 100, 400, 800 mg/kg body weight. After administering LDD175, the vital organs such as the liver, kidney and spleen were carefully observed for any significant pathological features or differences from the norm over a 14-day period. LDD175 did not induce any short-term toxicity at doses less than 100 mg/kg. A LD_{50} of LDD175 was 2493 mg/kg in male mice and 4908 mg/kg in female mice. Weight reduction was observed at a dose of 800 mg/kg in male, and 400 and 800 mg/kg in female. The kidney weight decreased in females after an intraperitoneal injection of LDD 175 high dose (> 400 mg/kg, i.p.), and the spleen weight increased in the male (800 mg/kg, i.p.) and female (400mg/kg, i.p.) mice. Inspite of the change in organ weights, there were neither histopathological changes nor any gross morphological abnormalities detected in any organ. LDD175 did not produce significant changes in the general behavior at doses of < 200 mg/kg, but decreased locomotor activity was observed at an intraperitoneal dose of 400 mg/kg. Its effects on the locomotor activity and activity on the rotarod were tested and compared with the effects of diazepam 5 mg/kg. The decrement in the locomotor activity and the activity on the rotarod induced by LDD175 was less serious than it by diazepam.

Keywords □ LDD175, acute toxicity, locomotor activity, BK_{Ca} channel opener

INTRODUCTION

LDD175 is 4-choloro-7-trifluoromethyl-10*H*-benzo[4,5]furo [3,2-b]indole-1- carboxylic acid. LDD175 is one of benzofuroindole derivatives. benzofuroindole analogues act as potent BK_{Ca} channel openers.(Gormemis *et al.*, 2005) The BK_{Ca} channels are distributed widely and play important roles in various cell functions. The activation of the BK_{Ca} channel in neurons is very important in repolarization and after-hyperpolarization of the cell membrane during the action-potential in the early period after releasing potassium as a result of calcium entry(Sah, 1996; Adams *et al.*, 1982; Storm, 1987). Therefore, the BK_{Ca} channels are important in determining the neuro-excitability. The BK_{Ca} channels have been suggested to be very important relaxation factors for the smooth muscle tone (Toro *et al.*, 1998). benzofuroindole analogues were shown to relax the smooth muscles of the bladder possibly through the activation of BK_{Ca} channels (Butera *et al.*, 2001). The aim of this study was to evaluate the toxicity of an effective benzofuroindole analogue, LDD175, acting on BK_{Ca} channel (Ha *et al.*, 2006; Gormemis *et al.*, 2005). This study examined the acute toxicity of the LDD175 in ICR mice to provide information on the safety of the LDD175 prior to evaluating its therapeutic efficacy in humans.

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MATERIALS AND METHODS

Experimental animals and materials

Male (ICR) mice, weighing 20-25 g were obtained from the Hanlim Laboratory Animals Co. (Hwasung, Korea). Mice were

acclimated to laboratory conditions at Uimyung research institute for neuroscience for at least one week. The mice were housed 10 per cage under a 12 hr light/dark cycle in a temperature (22±2°C) and humidity (55±5%) controlled animal room. The housing conditions were maintained for 7days, with all the animals in the same room. LDD175 was supplied from the Gwangju Institute of Science and Technology (Gwangju, Korea).

Acute toxicity

The acute toxicity of LDD175 was investigated after administration of the compound either intraperitoneally (i.p.) or orally (p.o.). The compound was suspended in 10 % tween80/water, and administered at doses of 0.2, 1, 10, 50, 100, 200, 400 and 800 mg/kg LDD175. The dose of 800 mg/kg was the maximum due to solubility of the compound. Ten males and females were used for each dosage level. The control group received an equal volume of the 10% Tween 80 vehicle. Prior to the experiments, the animals were fasted overnight with access to water *ad libitum*.

The body weight, signs of toxicity and mortality were observed for 14 days after administration. The visual inspection was conducted everyday to evaluate changes in the skin, fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system as well as somatomotor activity and behavioral pattern.

At the time of sacrifice, mice were injected thiopental sodium and the internal organs such as livers, kidneys and spleen were excised and weighed.

Locomotor activity

The locomotor activities were tested for abnormal behavioral patterns. The observation apparatus consisted of nine plastic boxes (47×47 cm), and its field was bordered by 42cm high sidewalls. The total distance moved, the total movement time, and turn angles were monitored for 10 min after drug administration. (Kim *et al.*, 2003; Noldus *et al.* 2001)

Rotarod test

The rotarod test was carried out immediately to ensure motor coordination or balancing ability. The rotarod is a 5-lane device consisting of a rotating spindle (diameter 7.3 cm) with varying rotational speed (0-60 rpm) and individual compartments (lanes) for each mouse. The speed of rotarod was increased up to 60 rpm. The body of the mouse was placed perpendicular to the rotating axis, and the head against the direction of the rotation. The animal moves forward in order to stay on the rod. The total running time (sec) on the rota rod as well as the falling frequency over a 10 min period were recorded (Lee *et al.*, 2005; Farkas *et al.*, 2005).

Statistical analysis

The parameter estimates were generated using Prism 4 (Graphpad, San Diego, CA). The results are expressed as mean ± standard error of the mean (S.E.M.) or standard deviation (S.D.). The statistical significance was determined using one-way analysis of variance (ANOVA). A p value < 0.05 was considered significant.

RESULTS

The mortality was assessed for 14 days after administering LDD175. Among the 10 animals in each group, the number of dead animals after LDD175 administration was 4 (2 of each gender) at 400 mg/kg i.p., 5 (3 males and 2 females) at the 800 mg i.p., and 1 (female) in the 400 and 800 mg p.o.. Therefore, the LD_{50} by i.p. injection of LDD175 was 2493 mg/kg in the male mice and 4908 mg/kg in the female mice (Table I). The administration of LDD175 produced neither clinical signs nor adverse effects except a decrease in the movement at a high dose. The body weight increased in all groups (animal treated or control). However, the body weight gain of the control group was higher than that of high dose groups (> 400 mg/kg, p.o. and i.p.) (Table II). This effect was more pronounced in female.

Variations in the body weights in the male, from the first day to the 15th day, were 49.3 % and 48% for control group i.p. and p.o. On the other hand, the values were 33.8 % and 33.6 % for those treated with 800mg/kg i.p. and p.o., respectively. In the females the body weight gain values of the control group were 42.6 and 40.4 %. In contrast, the body weight gain of the 400 mg/kg i.p., 800 mg i.p., 400 mg/kg p.o., and 800 mg p.o. groups were 29.6, 27.2, 28.5 and 28 %, respectively. There was no significant difference in water and food intake of all groups.

For the organ weights after i.p. injection, the spleen weight of high dose groups (> 400 mg/kg, i.p.) increased significantly and kidney decreased compared with the control group (Table III). The organ weight ratio for spleen at 800mg/kg (i.p.) in male mice was 0.49 compared with 0.40 of the control group. The ratios at 400 and 800 mg/kg (i.p.) in the female were 0.51 and 0.50 compared with 0.41 of the control group. The ratios for kidney at 400 and 800 mg/kg (i.p.) in the female were 1.13 and 1.12 compared with 1.30 of the control group. In spite of

Table I. Lethal dose of LDD175 in the ICR mice.

		Male		Female		LD_{50} of LDD175 (mg/kg body weight)			
		i.p. (n=10)	p.o. (n=10)	i.p. (n=10)	p.o. (n=10)	Male.		Female	
Control	(10% Tween 80)	0	0	0	0	i.p.	p.o.	i.p.	p.o.
LDD175	10 (mg/kg)	0	0	0	0	2493	ND	4908	>4908
	100 (mg/kg)	0	0	0	0				
	400 (mg/kg)	2	0	2	1				
	800 (mg/kg)	3	0	2	1				

LD50 is the median lethal dose that kills 50% of treated animals.
LD_{50} of LDD175 was calculated by Litchfield & Wilcoxn's method.

Table II. Body weights (g) of the mice (n=7~10) treated with LDD175.

			Body weight (male)			Body weight (female)		
			Initial	final (2 wks)	increased (%)	initial	final (2 wks)	increased (%)
i.p.	Control	(10% Tween80)	22.6±0.37	33.7±0.60	49.3±2.93	23.5±0.37	33.5±0.60	42.6±3.33
	LDD175	10(mg/kg)	23.1±0.48	32.9±0.54	42.7±2.15	22.5±0.33	31.7±0.67	40.9±2.85
		100(mg/kg)	24.0±0.23	35.1±0.55	46.2±01.07	23.2±0.36	30.7±0.76	32.3±2.21
		400(mg/kg)	22.1±0.35	34.4±0.43	53.6±01.84	22.8±0.16	29.4±0.43	29.6±1.91**
		800(mg/kg)	22.8±0.49	30.4±0.84	33.8±4.05*	23.9±0.46	30.4±0.75	27.2±2.87**
p.o.	Control	(10% Tween80)	22.5±0.43	33.3±0.91	48.0±2.72	22.3±0.43	31.3±0.81	40.4±3.61
	LDD175	10(mg/kg)	22.8±0.39	34.4±0.85	50.9±2.82	22.4±0.42	31.4±0.61	40.2±2.55
		100(mg/kg)	24.0±0.39	34.6±0.78	44.2±2.21	22.9±0.55	29.6±0.88	29.3±3.86
		400(mg/kg)	22.0±0.30	33.0±0.39	50.2±2.50	22.4±0.32	28.4±0.78	28.5±3.75*
		800(mg/kg)	26.13±0.23	34.7±0.61	33.6±2.76*	23.2±0.42	29.7±0.68	28.0±2.95*

Data are expressed as mean±S.D.. LDD175 treated groups versus control group, * $p<0.05$; ** $p<0.01$.

Table III. Organ weights and ratios to body weight of the mice (n=7~10) treated with LDD175.

		Male					female				
		Control	LDD175				Control	LDD175			
		(10% Tween80)	10 (mg/kg)	100 (mg/kg)	400 (mg/kg)	800 (mg/kg)	(10% Tween80)	10 (mg/kg)	100 (mg/kg)	400 (mg/kg)	800 (mg/kg)
i.p.	Liver (g)	1.91±0.06	1.84±0.08	1.97±0.05	1.97±0.09	1.69±0.13	1.71±0.05	1.61±0.06	1.56±0.05	1.51±0.04	1.51±0.09
	Spleen (g)	0.14±0.01	0.13±0.01	0.12±0.01	0.15±0.01	0.15±0.02	0.14±0.02	0.13±0.02	0.13±0.01	0.15±0.01	0.15±0.02
	Kidney (g)	0.54±0.01	0.54±0.02	0.60±0.04	0.52±0.04	0.44±0.02	0.44±0.02	0.40±0.02	0.35±0.02	0.33±0.02	0.34±0.02
	Liver (%)	5.65±0.14	5.59±0.22	5.61±0.08	5.73±0.29	5.56±0.35	5.06±0.14	5.07±0.18	5.08±0.15	5.13±0.09	4.95±0.28
	Spleen (%)	0.40±0.03	0.38±0.03	0.35±0.01	0.43±0.02	0.50±0.06*	0.41±0.05	0.42±0.05	0.47±0.03	0.51±0.04*	0.50±0.07
	Kidney (%)	1.59±0.04	1.57±0.03	1.69±0.08	1.51±0.10	1.43±0.07	1.30±0.05	1.25±0.07	1.14±0.05	1.13±0.07*	1.12±0.08*
p.o.	Liver (g)	1.89±0.09	1.86±0.07	1.93±0.06	1.81±0.06	1.84±0.04	1.59±0.09	1.57±0.08	1.38±0.07	1.43±0.10	1.56±0.11
	Spleen (g)	0.12±0.01	0.13±0.01	0.13±0.01	0.12±0.01	0.11±0.01	0.12±0.02	0.13±0.02	0.13±0.01	0.13±0.01	0.13±0.02
	Kidney (g)	0.56±0.02	0.54±0.03	0.54±0.02	0.54±0.02	0.48±0.02	0.46±0.02	0.45±0.03	0.39±0.02	0.36±0.03	0.37±0.03
	Liver (%)	5.66±0.17	5.40±0.02	5.59±0.16	5.48±0.13	5.30±0.04	5.08±0.27	5.00±0.24	4.99±0.16	5.00±0.21	5.27±0.38
	Spleen (%)	0.34±0.02	0.36±0.04	0.37±0.01	0.37±0.02	0.31±0.02	0.40±0.06	0.41±0.07	0.47±0.03	0.45±0.04	0.44±0.06
	Kidney (%)	1.68±0.03	1.56±0.09	1.57±0.04	1.63±0.05	1.38±0.05	1.47±0.07	1.4±0.10	1.38±0.06	1.28±0.10	1.25±0.08

Data are expressed as mean±S.D. of organ weights or ratio of organ weight/body weight.
LDD175 treated groups versus control group, * $p<0.05$.

the change in organ weights, there were neither histopathological changes nor any gross morphological abnormalities detected in any organ.

The effects of LDD175 on the locomotor activity and activity on the rotarod were tested and compared with the effects of diazepam 5mg/kg. The behavioral changes examined were less or vigorous activity, balancing ability and stereotype behavior. There was the significant decrease in the locomotor activity and activity on the rotarod (Figure 1, 2 and 3). LDD175 did not produce any significant changes in the general behavior at doses

< 100 mg/kg. The administration of LDD175 400 mg/kg i.p. significantly decreased the rearing frequency, movement time and distance compared with the control group. Especially, reduction of locomotor activities in center area was predominant. The administration of LDD175 400 mg/kg i.p. also significantly decreased the endurance time on the rotarod and increased the falling frequency from the rotarod for 10 minutes. The efficacy on the locomotor activity and rotarod test was less than that of diazepam.

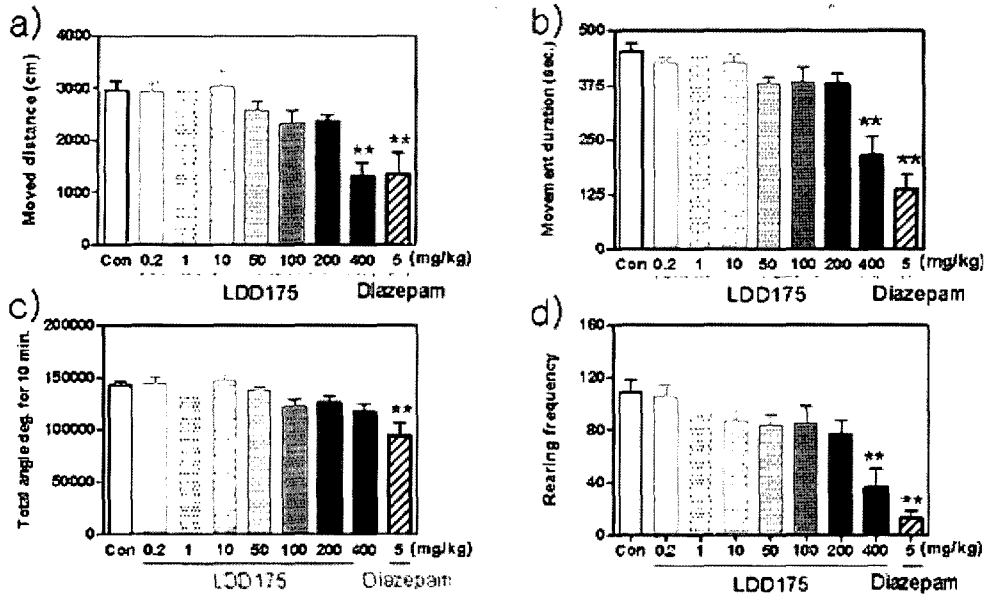

Fig. 1. Effects of LDD175(i.p.) on the locomotor activity in mice (n=9~10). a) the distance moved for 10 min. b) the movement duration for 10 min. c) the number of turn angles for 10 min. d) the rearing frequency for 10 min. Data are expressed as the mean± S.E.M. (* p <0.05, ** p<0.01 compared to control group).

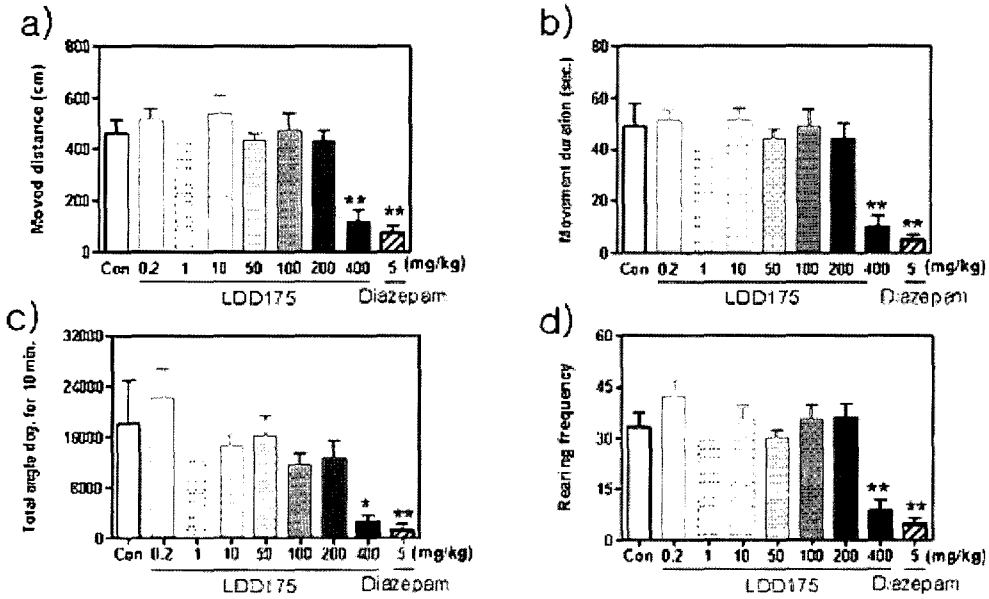

Fig. 2. Effects of LDD175(i.p.) on the locomotor activity in center area in mice (n=9~10). a) the distance moved for 10 min. b) the movement duration for 10 min. c) the number of turn angles for 10 min. d) the rearing frequency for 10 min. Data are expressed as the mean±S.E.M. (* p <0.05, ** p<0.01 compared to control group).

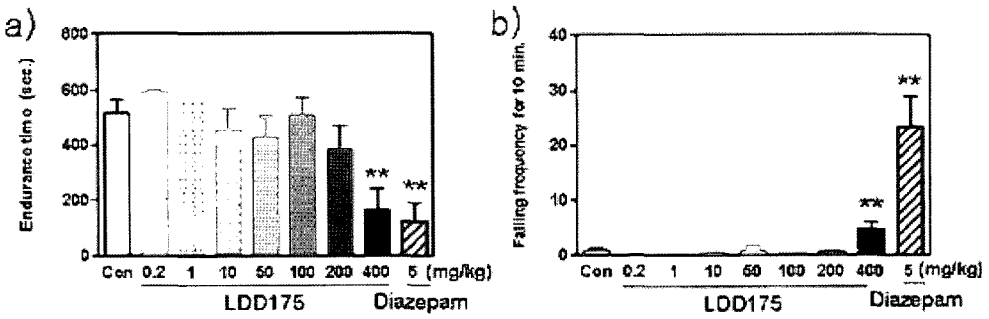

Fig. 3. Effects of LDD175(i.p.) on activities on the rotarod in mice (n=9~10). a) the running time until the first fall. b) the falling frequency for 10 min. Data are expressed as the mean±S.E.M. (* p <0.05, ** p<0.01 compared to control group).

DISCUSSION

The LD_{50} by i.p. injection of LDD175 was determined to be 2493 mg/kg and 4908 mg/kg in male and female mice, respectively (Table I). The LD_{50} determination is used to categorize the potential toxicity of chemical compounds or the relative degree of toxicity to an organism. In general, if the LD_{50} is > 15g/kg in the case of oral administration, it can be described as being practically nontoxic. If the LD_{50} is 5-15 g/kg, 0.5-5 g/kg, 50-500 mg/kg, 5-50 mg/kg and <5 mg/kg, it can be described as being slightly toxic, moderately toxic, very toxic, extremely toxic and super toxic (Loomis and Hayes, 1996; Gossel and Bricker, 1990). Most medicines belong to the range of moderately toxic or very toxic compounds. LDD175 belongs to the range of slightly toxic compounds. The LD_{50} of LDD175 was higher than the other BK_{Ca} channel openers, Tamoxifen (LD_{50} oral in mice = 1.2-2.5 g/kg) or a typical sedatives, phenobarbital (LD_{50} oral = 0.16 g/kg) (Budavari *et al.*, 1989). Therefore, LDD175 is relatively a safe chemical agent.

Reduction of weight gaining was observed at high dose group of LDD175 (Table II), and this effect was more pronounced in female. For the organ weights, in the case of the i.p. injection only, the spleen weight of LDD175 high dose treated groups was significantly higher and the kidney weight was lower than those of the control group (Table III). However, the animals did not show any specific changes in their general behavior or other physiological activities from 1 day after administration. No pathological alterations were grossly detected. There were neither histopathological changes noted with the change in the organ weights, nor were any gross morphological abnormalities detected in any organ.

The administration of LDD175 produced no clinical signs or adverse effects except for a decrease in movement in the high dose group. The intraperitoneal administration of LDD175 400 mg/kg decreased the locomotor activity, and the effect was predominant in the center area. Whereas the reduction of total locomotor activities means a simple sedation or dullness of behavior, reduction of locomotor activities in the center area means anxiety, avoidance or depression (Meyer and Quenzer, 2005; Fox *et al.*, 2001). Endurance time on the rotarod significantly and increased the falling frequency from rotarod over a 10 minute period. This effect disappeared 12 hours after administration and the efficacy was less than that of diazepam. LDD175 is a potent BK_{Ca} channel opener (Ha *et al.*, 2006). The activation of the BK_{Ca} channel in neurons is very important in the repolarization and after-hyperpolarization of the cell membrane during the action-potential in the early period after the release of potassium as a result of calcium entry (Sah, 1996; Adams *et al.*, 1982; Storm, 1987). Therefore, LDD175 can induce hyperpolarization in the membrane potential and produce an inhibitory postsynaptic potential of a neuron. The normal excitation of a neuron might be inhibited by LDD175 because the inhibitory post synaptic potential has moved the membrane potential farther away from the threshold for generation of the action potential generation (Meyer and Quenzer, 2005). Therefore, it is possible that LDD175 at high doses decreases the locomotor activity or activity on the rotarod because a high dose can non-selectively act on all tissues.

In conclusion, the acute toxicity of LDD175 is not serious even though it evoked a light decrease in weight gain, organ development and behavioral activity at high doses.

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