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LB30057, an Orally Effective Direct Thrombin Inhibitor, Prevents Arterial and Venous Thrombosis in Rats and Dogs

Hee Dong Park, Hee Jin Kim, Yeong Soo Oh, In Chull Kim, Yong Zu Kim, Hyun Chul Koh[1], In Chul Shin[1], Yong Hee Lee, and Chang Ho Lee[1]

LG Biotech Research Institute, LG Chem. Daejeon 305-380, Korea and [1]Department of Pharmacology and Institute of Biomedical Sciences, Hanyang University, College of Medicine, Seoul 133-791, Korea

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The anti-thrombotic effects of LB30057, a direct thrombin inhibitor, were evaluated with in vivo rat and dog thrombosis models. In rats, 1 mg/kg of LB30057 inhibited half of the clot formations in the inferior vena cava at 5 minutes after intravenous application. When measured at 2 hours after oral application, 100 mg/kg prevented approximately half of the clot formations in the inferior vena cava and 50 mg/kg prolonged the mean occlusion time from 15.6±1.3 minutes to 47.2±8.3 minutes in the carotid artery. In dogs, the formation of thrombus in the jugular vein was reduced to half at a dose range of 20-30 mg/kg at 6 hours after oral application. In addition, the LB30057 dosage required to reduce venous clot formation by approximately 80-90% in dogs was only about 10% of that required for the same reduction in rats. This is probably due to the variation in its time-dependent blood concentration profiles in each species; for example, the plasma half-life of LB71350 in dogs was longer than that in rats (153.0±3.0 vs. 129.7±12.7 min at 30 mg/kg, i.v., respectively). AUC, T_{max}, C_{max}, and BA in dogs were 59, 8.9, 9.17, and 13.3 times higher than those in rats at oral 30 mg/kg, respectively. Taken together, these results suggest that LB30057 administered orally is effective in the prevention of arterial and venous thrombosis in rats and dogs. It therefore represents a good lead compound for investigations to discover a new, orally available, therapeutic agent for treating thrombotic diseases.

Key words: LB30057, Oral direct thrombin inhibitor, Anti-thrombotics, Arterial thrombosis, Venous thrombosis

INTRODUCTION

Currently available anti-thrombotic agents are heparin, low molecular heparin, and oral coumarins. Each has clinical limitations due to their mechanistic characteristics (Bussey *et al.*, 1993; Hull *et al.*, 1994; Buller *et al.*, 1998). In order to overcome these limitations and provide more effective ways of treatment, there is a serious need for new anti-thrombotics that can be available as an oral drug.

Thrombin plays a major role in thrombosis and coagulation procedures. Thus, extensive efforts have been made to develop potent, direct thrombin inhibitors as potential anti-thrombotic agents. Particularly, oral bioavailability has been an important issue for the successful discovery of a new, direct acting thrombin inhibitor.

Of the compounds with different modes of actions, intense research and development efforts have been focused on catalytic site-directed thrombin inhibitors (Cousins *et al.*, 1996; Matsuo *et al.*, 1997; Mehta *et al.*, 1998; Cook *et al.*, 1999). Among the many direct thrombin inhibitors, argatroban, which has reversible and fast-binding kinetics, has been shown to be clinically effective in treating chronic, peripheral arterial obstructive disease and acute ischemic stroke with its safe and effective anti-thrombotic action only when applied intravenously (Duval *et al.*, 1996; Matsuo *et al.*, 1997). Recently, several orally active, direct thrombin inhibitors have been discovered. The oral efficacy of L-374,087, pyrimidinone acetamide thrombin inhibitor, has been characterized as a prototype for the further development of orally active, direct thrombin inhibitors (Sanderson *et al.*, 1998; Cook *et al.*, 1999). Melagatran was reported to prevent or delay formation of electrically induced occlusive thrombus in the canine coronary artery when administered orally (Mehta *et al.*, 1998). Similarly, CVS 1123,

Correspondence to: Chang Ho Lee, Ph. D., Department of Pharmacology, Hanyang University, College of Medicine, 17 Hengdang-dong, Sungdong-Ku, Seoul 133-791, Korea
Tel: 82-2-2290-0655, Fax: 82-2-2292-6686
e-mail: jennysue@hanyang.ac.kr

a direct thrombin inhibitor, was reported to be effective in reducing the incidence of electrical stimulation-induced, arterial thrombosis in dogs when administered orally at 20 mg/kg every 8 hours (Cousins *et al.*, 1996; Rebello *et al.*, 1997).

Recently, systematic variation of the so-called P-pocket moiety of benzamidrazone-based, selective thrombin inhibitor has led to the discovery of LB30057 (Oh *et al.*, 1998). In our previous reports, we fully described the characteristics of LB30057, such as its in vitro biochemical activities, good selectivity and high potency, and its possession of slow-tight binding kinetic properties was established with *in vitro* chromogenic assay (Kim *et al.*, 1997; Oh *et al.*, 1998). It should be noted that maintaining an adequate concentration of LB30057 in the blood is therapeutically relevant in preventing thrombosis since its target exists in the blood. Therefore, we here described the anti-thrombotic efficacy of LB30057 with relation to blood disposition after intravenous and oral administration to rats and dogs. In order to evaluate its oral anti-thrombotic efficacy *in vivo*, thrombosis models in rats and dogs, which are widely used for evaluating the effects of anti-thrombotics, were used.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (250-300 g), supplied by LG Chem Biotech Institute Animal Facility (Daejeon, Korea) and male beagle dogs (8-12 kg), supplied by Hazleton Research Product Inc. (Calamazoo, MI, USA) were used for the experiments. All animals were housed and fed, *ad libitum*, with standard commercial food at the LG Chem. Animal Facility, in which the environment was well controlled (temperature; 20~22°C; humidity of 45-65%; 12 hours light and 12 hours dark). All the animals utilized for the oral application studies were fasted overnight prior to treatment.

Chemicals

LB30057 (Lot No.: TP-07, purity: 98.8%) was synthesized at LG Chem. (Daejeon, Korea). The structure of LB30057 is shown in Fig. 1. Heparin was purchased from Green Cross Co. (Seoul, Korea). Gelatin capsules (#00, #000, and #13) were purchased from Torpac Inc. (Fairfield, NJ, USA). Other chemicals (Merck, Darmstadt, Germany) for HPLC analysis were analytical grade. For administration, LB30057 was dissolved in 0.9% isotonic sterile saline (vehicle) at a proper concentration.

Measurement of blood concentration of LB30057 in rats and dogs

Sprague-Dawley rats were restrained individually in a

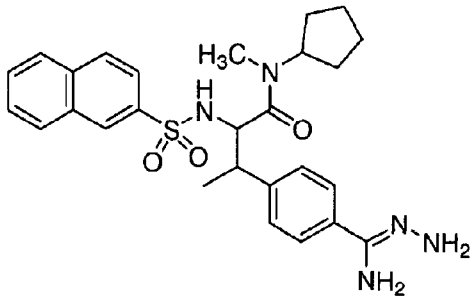

Fig. 1. Structure of LB30057.

Ballmann cage (DaeJong Instrument Company, Seoul, Korea). The femoral artery and the femoral vein (for intravenous administration only) of the rats were cannulated with polyethylene tubing (PE-50, Clay Adams, Parsippany, NJ, USA) under light ether anesthesia. After complete recovery from anesthesia, LB30057 at doses of 5, 10, and 30 mg/kg was injected intravenously via the femoral vein, and blood samples were collected from the femoral artery at 1, 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300, and 360 minutes after injection. For oral administration, doses of 30, 100, and 300 mg/5 mL/kg were administered via gavage, and blood samples were collected at 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300, and 360 minutes after administration.

Beagle dogs were housed individually in metabolic cages for blood disposition studies. LB30057 at doses of 10 and 30 mg/0.2 mL/kg was injected intravenously via the cephalic vein of the left leg or the same dosages (powder filled in gelatin capsule) were administered orally. Blood samples were collected via the cephalic vein at 1, 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, and 720 minutes after intravenous injection and at 10, 20, 30, 40, 45, 60, 90, 120, 180, 240, 360, 480, and 720 minutes after oral administration.

HPLC assays and calculation of pharmacokinetic parameters

Blood samples (approximately 250 μL aliquots) were withdrawn into heparinized tubes (25 U/mL), and immediately underwent HPLC analysis as previously described (Lee *et al.*, 1998). In brief, all blood samples were immediately deproteinized with 1.5 volume of methanol and 0.5 volume of 10% zinc sulfate, and analyzed by validated HPLC methods. The calibration curves were linear in the range of 0.05 to 100 μg/mL of LB30057 in the blood samples of experimental animals and the sample detection limit was 50 ng/mL. Data were plotted as blood concentration versus time. In case of intravenous injection, data were fitted to a two-exponential decay model with the non-linear regression program SIPHAR (Version, 4.0, Simed, Creteil Cedex, France). The half-life at terminal phase ($t_{1/2\beta}$, min), area

under the blood concentration-time curve (AUC, μg.min/ml), time-averaged total body clearance (CL, mL/min/kg), and apparent volume of distribution at steady-state (Vd_{ss}, mL/kg) were estimated. For oral administration, the model-independent parameters, such as peak concentration (C_{max} μg/mL), peak time (T_{max}, min), and bioavailability (BA, %) were estimated. AUC from time zero to time infinity was calculated by the trapezoidal rule-extrapolation method (Chiou *et al.*, 1978). CL and Vd_{ss} were estimated by the standard method described by Gibaldi and Perrier (Gibaldi *et al.*, 1982). BA was calculated by $(AUC_{p.o.} \times Dose_{i.v.})/(AUC_{i.v.} \times Dose_{p.o.})$.

Stasis-induced venous thrombosis in anaesthetized rats and dogs

To evaluate the anti-thrombotic activity of LB30057 on venous thrombosis in rats and dogs, the method described by Millet *et al.* (1992) was used with modifications. In rats, after a mid line incision of the abdomen in the urethane-anesthetized (1.25 g/kg) rats, the inferior vena cava was carefully exposed with the removal of surrounding tissues and then followed by ligations of both vena iliolumbar and spermatic with silk threads. In order to induce clotting the vena cava was ligated just below the left renal vein. Thromboplastin (Simplastin®, Organon Teknika, Durham, NC, USA; one vial dissolved with 10 mL of distilled water; 0.5 mL/kg/min) was infused for 30 seconds via the left femoral vein through an infusion pump (Model 100, IITC Life Science, Woodland Hills, CA, USA). Then, another ligature, 16 mm apart from the first, was made just above the iliac vein. After 15 minutes, the clots were removed, blotted on wet filter paper and weighed. Various doses of LB30057 were administered at 5 minutes prior to (0.2, 0.5, 1, 3, and 5 mg/kg, intravenous bolus), and at 2 hours prior to (25, 50, 100, 200, 300, and 400 mg/kg, oral application), thromboplastin infusion. For generating clots in the pentobarbital sodium-anesthetized (35 mg/kg) beagle dogs, all of the small branches of the vein were ligated. Thromboplastin (0.4 mL in a vial reconstituted with 2.5 mL of distilled water) was infused to the jugular vein through one of its branch vessels with an infusion pump. At the start of infusion, the jugular vein was ligated at the proximal side and 30 seconds later, another ligature was made at the distal side 2 cm apart from the first. Fifteen minutes later, the clots were removed, blotted on wet filter paper and weighed. Oral applications of LB30057 with 10, 20 and 30 mg/kg in capsule formula were done at 3 and 6 hours before the induction of venous thrombosis. Blood samples were collected at the same time points and analyzed by HPLC.

Chemically-induced arterial thrombosis in anaesthetized rats

The method described by Broersma *et al.* (1991) was used. Briefly, after installing an ultrasonic blood flow probe (setting at 0.5 V with instrument T208, Transonic, Ithaca, USA) on the left carotid artery, electrical signals were recorded with Cyberamp 380 and AXO TAPE (Axon Instrument, Foster City, CA, USA). After stabilization of carotid blood flow (20 minutes), a strip of filter paper (Whatman #1) presoaked in 5% $FeCl_3$ in 1 N HCl was placed on the blood vessel down to the probe and the blood flow was monitored continuously until the occlusion took place. Occlusion time was defined as zero blood flow maintained for at least 2 minutes. Saline or LB30057 at 10, 25, 50, 200 and 400 mg/kg was administered orally 2 hours prior to the $FeCl_3$ application.

Statistical analysis

Statistical analysis was carried out by Dunnett test. Data were expressed as mean±S.E. (standard error of the mean) or S.D. (standard deviation) as indicated in the figure legends. P-values less than 0.05 were considered significant.

RESULTS

Blood concentration profiles of LB30057 in rats and dogs

Fig. 2-A shows the time-dependent blood concentration profiles of LB30057 after intravenous injection of 5, 10, and 30 mg/kg in rats. At all test doses, the blood concentration of LB30057 decreased exponentially within the first 5 minutes and decreased rather gradually after that. For example, at 5 mg/kg its blood concentration at 5 minutes was reduced to approximately one ninth of the initial blood level (from 9436±441 ng/mL at 1 minute to 1037±23 ng/mL at 5 minutes) and then reduced gradually to 57 ng/mL at 90 minutes after application.

Fig. 2-B shows the time-dependent concentration profile of LB30057 in the blood after oral administration to rats, which depicts a basically similar pattern in all test doses (30, 100, and 300 mg/kg). For example, at 100 mg/kg, its blood concentration gradually increased, reached a maximum concentration (2.59±0.49 μg/mL, C_{max}) at 13.3±3.3 minutes (T_{max}), and then decreased gradually and continuously (424±260 ng/mL at 120 minutes, 219±141 ng/mL at 360 minutes). In case of 30 mg/kg, LB30057 was not detected in the blood after 90 minutes.

Fig. 3-A shows the time-dependent blood concentration profile of LB30057 after intravenous injection in dogs. At test doses, its blood concentration exponentially decreased over the first 15 minutes and then reduced gradually. For example, at 30 mg/kg its blood concentration exponentially decreased in the first 15 minutes (from 230064±2507 ng/mL at 1 minute to 10377±235 ng/mL at 15 minutes) and

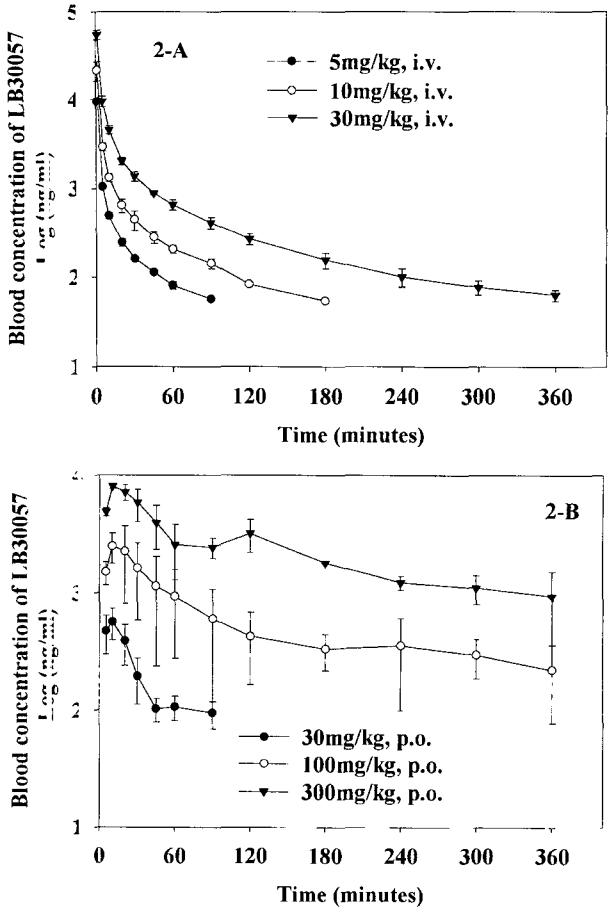

Fig. 2. Blood concentration of LB30057 after intravenous and oral administration in rats. LB30057 at 5, 10, and 30 mg/kg was injected intravenously and blood samples were collected at 1, 5, 10, 20, 30, 45 60, 90, 120, 180, 240, 300, and 360 minutes after injection (2-A). For oral administration, doses of 30, 100, and 300 mg/5 mL/kg were administered via gavage, and blood samples were collected at 5, 10, 20, 30, 45, 60 90, 120, 180, 240, 300, and 360 minutes after administration (2-B). Blood concentration (ng/mL) was measured as described in "Materials and Methods". Each value represents the mean±S.D. of 3 animals. The calibration curves were linear in the range of 0.05-100 μg/mL of LB30057 in the blood samples of experimental animals and the sample detection limit was 50 ng/mL.

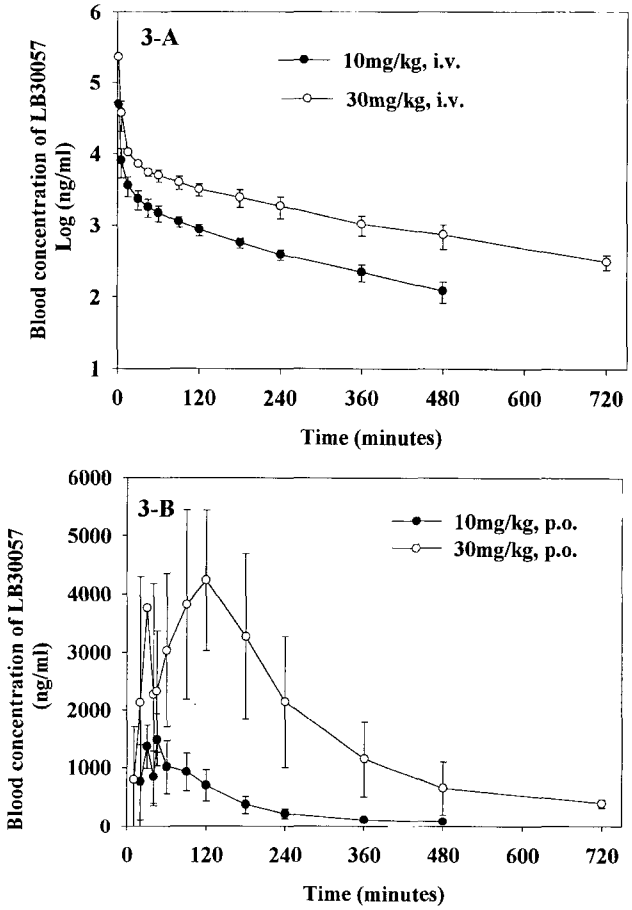

Fig. 3. Blood concentration of LB30057 after intravenous and oral administration in dogs. LB30057 at 10 and 30 mg/kg was injected intravenously and blood samples were collected at 1, 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, and 720 minutes after intravenous injection (3-A). LB30057 at 10 and 30 mg/kg (powder filled in gelatin capsule) was administered orally and blood samples were collected at 10, 20, 30, 40, 45, 60, 90, 120, 180, 240, 360, 480, and 720 minutes after administration (3-B). Blood concentration (ng/mL) was measured as described in "Materials and Methods". Each value represents the mean±S.D. of 2-4 animals. The calibration curves were linear in the range of 0.05-100 μg/mL of LB30057 in the blood samples of experimental animals and the sample detection limit was 50 ng/mL.

then reduced gradually up to 720 minutes (312±73 ng/mL).

Fig. 3-B shows the time-dependent blood concentration profile of LB30057 after oral administration of 10 and 30 mg/kg in dogs. At 10 mg/kg, its blood concentration increased gradually, showing T_{max} (min) and C_{max} (μg/mL) as 53.3±9.1 and 1.27±0.16, respectively, and then decreased gradually up to 480 minutes. At 30 mg/kg, a similar profile showed a peak at 89.0±10.7 and 5.14±0.26, respectively, with a gradual decline to 720 minutes.

Based on these observations, Table I summarizes several pharmacokinetic parameters of LB30057 after intravenous or oral administration in rats and dogs. For example, the plasma half-life of LB30057 in dogs was longer than that in rats with dose-dependent variation (rats vs. dogs: 47.0 ±2.4 vs. 114±9.5, and 129.7±12.7 vs. 153.0±3.0 minutes, at intravenous doses of 10 and 30 mg/kg, respectively). Especially, more dramatic pharmacokinetic differences between rats and dogs were observed when administered orally: i.e., at 30 mg/kg oral application, AUC, T_{max}, C_{max}, and BA in dogs were 59, 8.9, 9.17, and 13.3 times higher than those in rats, respectively.

Effects of LB30057 on stasis-induced venous thrombosis in rats and dogs

According to our preliminary results, for the induction of its dose-dependent anti-thrombotic effects in rats the blood

Table I. Pharmacokinetic parameters of LB30057 after intravenous or oral administration in rats and dogs.

Treatment	$t_{1/2\beta}$ (min)	CL (mL/min/kg)	Vdss (mL/kg)	AUC (μg.min/mL)	T_{max} (min)	C_{max} (μg/mL)	BA (%)
5 mg.kg, i.v., rats	25.9±4.6	100.2±0.8	1760±318	49.9±0.4	–	–	–
10 mg.kg, i.v., rats	47.0±2.4	81.7±7.8	2058±379	124.8±13.0	–	–	–
30 mg.kg, i.v., rats	129.7±12.7	80.4±4.0	4470±508	374.5±18.0	–	–	–
30 mg.kg, p.o., rats	–	–	–	19.6±4.4	10.0±0.0	0.56±0.0	5.23±1.14*
100 mg.kg, p.o., rats	–	–	–	315.0±25.3	13.3±3.3	2.59±0.49	–
300 mg.kg, p.o., rats	–	–	–	1227.6±246.1	10.0±0.0	8.11±0.26	–
10 mg.kg, i.v., rats	114.0±9.5	18.5±1.8	2075±248	555.0±48.5	–	–	–
30 mg.kg, i.v., rats	153.0±3.0	12.7±0.9	1880±177	2374±158	–	–	–
10 mg.kg, p.o., rats	–	–	–	186.5±22.7	53.3±9.1	1.27±0.16	30.1±3.6**
30 mg.kg, p.o., rats	–	–	–	1156±129	89.0±10.7	5.14±0.26	69.4±7.1*

Values represent the mean±S.E.
*relative to 30 mg/kg, i.v.; **relative to 10 mg/kg, i.v.; –, not calculated
Blood concentrations below the detection limit (50 ng/ml) were not used in the pharmacokinetic calculations.

concentration of LB30057 should be approximately in the range of 200-1200 ng/mL. In fact, we observed that the blood concentration of LB30057 was 1037±23 ng/mL at 5 minutes after intravenous injection with 5 mg/kg (Fig. 2-A), and that its blood concentration was 424±260 ng/mL at 2 hours after oral application with 100 mg/kg (Fig. 2-B). Thus in the rat venous thrombosis model, experiments were performed at 5 minutes after intravenous injection with dosages ranging from 0.1 mg/kg to 5 mg/kg, and at 2 hours after oral application with 50, 100, 200, 300, and 400 mg/kg. In case of intravenous injection, clot formation was inhibited in a dose-dependent manner; 1 mg/kg of LB30057 reduced clot formation by half and 5 mg/kg by about 75% as compared with the control (Fig. 4-A). In case of oral application, a dose-dependent reduction in clot formation was also observed; 100 mg/kg of LB30057 effectively inhibited approximately 50% of the clot formations in rats as compared with control. In addition, inhibitory effects of about 80% were observed at 300 and 400 mg/kg, although its blood levels were much higher than that of 100 mg/kg (Figs. 2-B and 4-B).

In dogs, time-dependent inhibitory effects of LB30057 on clot formation in the jugular vein were evaluated after oral application with 10 and 30 mg/kg. We observed that its blood concentration reached a sub-maximal level (3270±1424 ng/mL) at 3 hours and a high residual level (1156±646 ng/mL) at 6 hours after oral application with 30 mg/kg (Fig. 3-B). Therefore, in order to prove the long duration of its anti-thrombotic effects on clot formation in the dog jugular vein, experiments were performed at 3 and 6 hours after oral application. In contrast to the inhibitory effects of LB30057 on venous thrombosis in rats, it effectively prevented clot formation in the dog jugular vein at much lower dosages and at much later time points after oral application. As shown in Fig. 5-A, about 23% of its

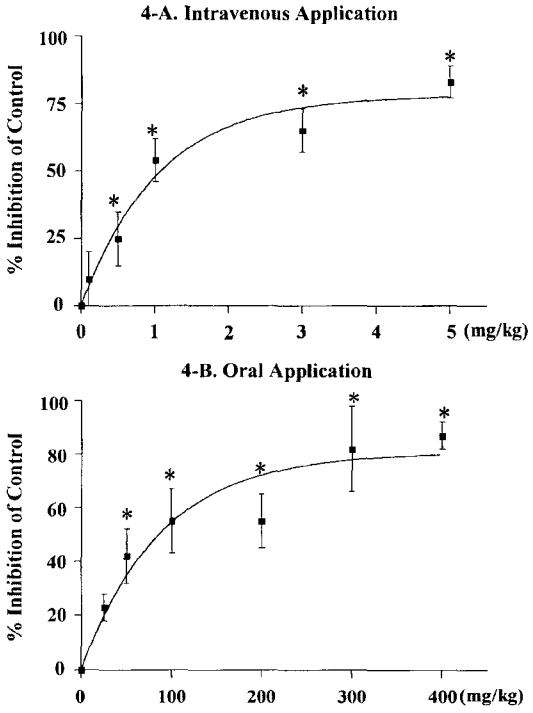

Fig. 4. Effects of intravenous and oral application of LB30057 on the thromboplastin and stasis-induced venous thrombosis in rats. Sprague-Dawley rats were treated with LB30057: 0.1, 0.5, 1, 3, and 5 mg/kg, i.v., at 5 minutes prior to thromboplastin infusion (4-A), or 25, 50, 100, 200, 300, and 400 mg/kg, orally, at 2 hours prior to thromboplastin infusion (4-B). Then thrombus formation was measured as described in "Materials and Methods". Each value represents the mean±S.E. of 4-6 animals and each data point shows percent inhibition of thrombus formation as compared with the saline-treated control group. Statistical analysis was carried out by Dunnett test and P-values less than 0.05 were considered significant, *.

inhibitory effect on clot formation was observed at 3 hours after oral application with 10 mg/kg. With the same dose,

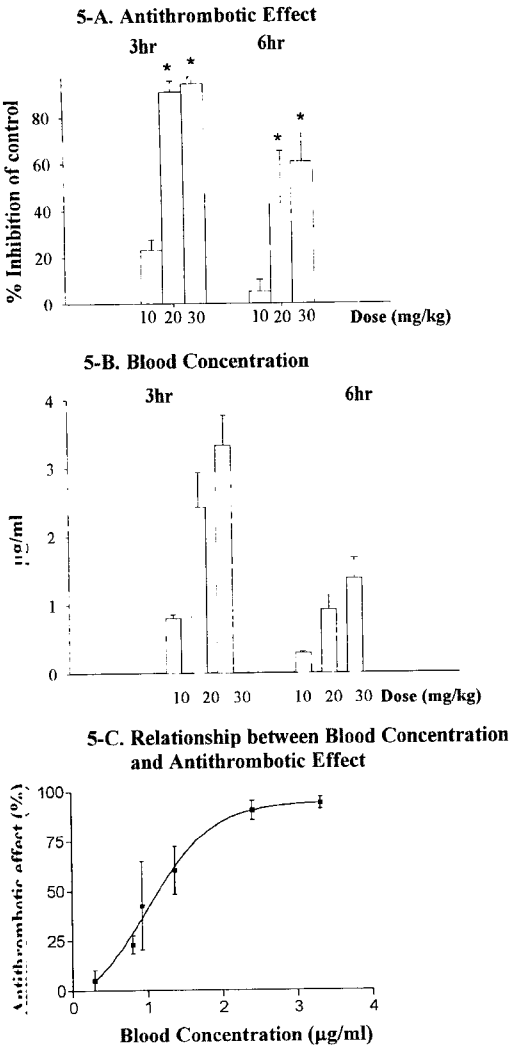

Fig. 5. Effects of oral administration of LB30057 on the thromboplastin and stasis-induced venous thrombosis in dogs. Beagle dogs were treated orally with LB30057 (10, 20, and 30 mg/kg) at 3 or 6 hours before thromboplastin injection and the formation of thrombus was measured (5-A) as described in "Materials and Methods". Blood samples were collected at each time point of clot formation assay and then the blood concentration (μg/mL) of LB30057 was measured (5-B). The relationship between blood concentration and anti-thrombotic activity of LB30057 is shown (5-C). Each value represents the mean±S.E. of 4-6 animals and each bar graph shows percent inhibition of thrombus formation as compared with the saline-treated control group. Statistical analysis was carried out by Dunnett test and P-values less than 0.05 were considered significant, *.

almost no inhibitory effect on clot formation was observed at 6 hours after oral application. However, approximately 90% inhibition of clot formation was observed at 3 hours after oral application with both 20 and 30 mg/kg. In addition inhibitory effects of 40-60% were still seen with 20 and 30 mg/kg even when measured at 6 hours after oral application. These observations can be explained by Fig. 5-B indicating that blood levels of LB30057 were decreased in both a dose- and a time-dependent manner and that the blood concentrations at 3 hours were much higher than those at 6 hours after oral application of all the doses. Based on correlation analysis, we determined that approximately 1 μg/mL blood concentration is necessary to produce an expect inhibitory effect of about 50% on the clots formed in the dog jugular vein, which is rather higher than that in rat venous thrombosis (Fig. 5-C).

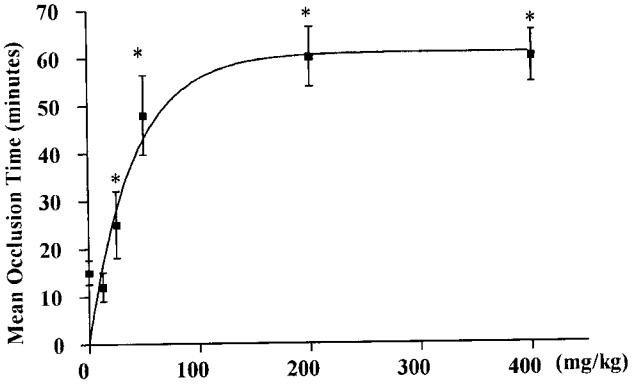

Fig. 6. Effects of oral administration of LB30057 on the $FeCl_3$-induced arterial thrombosis in rats. Sprague-Dawley rats were treated orally with LB30057 at 2 hours before $FeCl_3$ application and then mean occlusion times were measured as described in "Materials and Methods". Each value represents the mean±S.E. of 7-8 animals and each data point shows mean time to thrombus formation. Complete occlusion of the carotid artery occurred in all rats in the saline-treated group within 15.6±1.3 minutes. Statistical analysis was carried out by Dunnett test and P-values less than 0.05 were considered significant, *.

Effects of LB30057 on arterial thrombosis in rats

The duration of carotid artery blood flow in rats treated with LB30057 is shown in Fig. 6. Complete occlusion of the carotid artery occurred in all rats in the saline-treated group within 15.6±1.3 minutes. After the formation of the occlusive thrombus, there was no incidence of spontaneous dissolution of thrombus in any of the rats during the observation period in this group. Mean time to occlusive thrombus formation was significantly prolonged in a dose-dependent manner by the treatment of LB30057 (partial and full occlusion, 24.8±7.2 minutes in rats given 25 mg/kg and 47.2±8.3 minutes in rats given 50 mg/kg LB30057, both $p<0.05$, as compared with that in the saline-treated group) and reached plateau levels at one hour (60±6.2 and 60±5 minutes in the rats treated with 200 and 400 mg/kg, respectively). In fact, once thrombus was formed, it was not dissolved at even high concentrations of LB30057 in the blood with the thrombus formation merely being retarded to a certain extent via the direct inhibition of thrombin. Therefore, no further prolongation of mean occlusion time was observed at 400 mg/kg of LB30057, as compared with that at 200 mg/kg. Overall, oral LB30057 significantly prevented arterial thrombus formation and

thus prolonged the mean occlusion time in the carotid artery.

DISCUSSION

Venous thrombosis is usually formed in regions of slow or disturbed flow and remains a major complication in patients undergoing highly risky surgical procedures such as orthopedic surgery and venous stasis (Wallis *et al.*, 1989; Agnelli *et al.*, 1997). In contrast to venous thrombosis, arterial thrombosis forms in high shearing settings such as vascular-wall injury. Disruption of the endothelium causes accumulation of platelets to the sub-endothelial matrix accompanied by local enhancement of the blood-clotting mechanism (Fuster *et al.*, 1992; Weitz *et al.*, 1995).

The purpose of our study was to assess the oral anti-thrombotic activity of LB30057 utilizing widely used animal models of thrombosis and to explore any relationship between its anti-thrombotic activity and blood concentration in experimental animals. In a rat model for venous thrombosis induced by injection of thromboplastin followed by vascular stasis, our results have shown that inhibition of thrombin by LB30057 significantly decreases *in vivo* thrombus formation. However, it should be noted that in this model complete occlusion of the vessel only partially reflects the pathological situation due to the lack of perfusion of the growing clots. It is known that the effects of anti-thrombotics strongly depend on the nature of the thrombus formation (Verstraete *et al.*, 1995). Accordingly, the effect of LB30057 on chemical-induced arterial thrombosis, which is associated with activation and subsequent aggregation of platelets, was evaluated. In the absence of effective anti-thrombotic therapy, occlusive, platelet-rich arterial thrombus developed at the site of vessel injury and blood flow decreased. Measurement of arterial blood flow allowed us to examine the potential therapeutic effects of LB30057 and we observed that LB30057 is effective in disrupting clot formation in damaged blood vessels at a similar dose range as that used for reducing clot formation in the vein.

In addition, the effects of LB30057 on the production of jugular vein thrombosis in an anesthetized dog were investigated to determine if there is any variation in its anti-thrombotic effects among different species. Interestingly, the dose of LB30057 in the dog preparation necessary to reduce venous clot formation by approximately 80% was only about 1/10 of that required for the same reduction in the rat preparation. This is probably due to the fact that its pharmacokinetic behavior varies in each species. For example, when comparing more directly its blood concentrations at 2 hours, after treating dogs with 30 mg/kg orally and rats with 300 mg/kg orally the concentration of LB30057 in dog blood was maintained at a much higher level than that in rat blood (4231±1204 ng/mL at 30 mg/kg in dogs versus 3203±992 ng/mL at 300 mg/kg in rats). To summarize, a higher blood level of LB30057 was maintained in dogs than that in rats when the same dosage was applied via the same route of administration.

Thus far, several important pharmacokinetic parameters including oral bioavailability and plasma half-life were assessed to explain the fact that there is a certain correlation between blood concentration profiles and anti-thrombotic effects of LB30057 in rats and dogs. However, extensive studies on the hepatic metabolism and renal excretion should be carried out in order to explain the mechanism of changes in pharmacokinetic parameters at various administration conditions; studies which are beyond the scope of this report. In addition, more detailed pharmacokinetic experiments should be performed in other species, particularly in monkeys, and also further studies are necessary to fully assess the pharmacology of LB30057 in order to predict its clinical value in humans.

In terms of the non-linear pharmacokinetics and the balance between risks and benefits of this anti-thrombotic agent, the adverse event of bleeding may be the greatest clinical concern. However, there were no changes in aPTT and PT, which reflect the incidence of major or severe bleeding episodes, hemorrhagic stroke and repeated myocardial infarction at the dose of LB30057 most effective in treating rat arterial and venous thrombosis (200 mg/kg, oral, separate manuscript in preparation).

In summary, LB30057 administered orally is effective in the prevention of arterial and venous thrombosis in rats and dogs. It therefore represents a good lead compound for investigations to discover a new, orally available, therapeutic agent for treating thrombotic diseases.

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REFERENCES

Agnelli, G. and Sonaglia, F., Prevention of venous thromboembolism in high risk patients. *Haematologica*, 82, 496-502 (1997).

Broersma, R. J., Kutcher, L. V. and Heminger, E. F., The effect of thrombin inhibitor in a rat arterial thrombosis model. *Thromb. Res.*, 64, 405-412 (1991).

[1]LG Biotech Research Institute, LG Chem. Daejeon 305-380, Korea.

[2]Department of Pharmacology and Institute of Biomedical Sciences, Hanyang University, College of Medicine, Seoul 133-791, Korea.

Buller, H. R., Kraaijenhagen, R. A. and Koopman, M. M., Early discharge strategies following venous thrombosis. *Vasc. Med.* 3, 47-50 (1998).

Bussey, H. I., How to monitor the dosage of warfarin. *Heart Dis. Stroke*, 2, 388-392 (1993).

Chiou, W. L., 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. Pharmacokin. Biopharm.*, 6, 539-546 (1978).

Cook, J. J., Gardell, S. J., Holahan, M. A., Sitko, G. R., Stump, G. L., Wallace, A. A., Gilberto, D. B., Hare, T. R., Krueger, J. A. Dyer, D. L., Sanderson, P. E., Vacca, J. P., Shafer, J. A. and Lynch, J. J., Antithrombotic efficacy of thrombin inhibitor L-374, 087: intravenous activity in a primate model of venous thrombosis extension and oral activity in a canine model of primary venous and coronary artery thrombosis. *J. Pharmacol. Exp. Ther.*, 289, 503-510 (1999).

Cousins G. R., Friederich, G. S., Sudo, Y., Rebello, S. S., Rote, W. E., Vlasuk, G. P., Nolan, T. G., Mendoza, C. and Lucchesi, B. R. Orally effective CVS-1123 prevents coronary artery thrombosis in the conscious dog. *Circulation*, 94, 1705-1712 (1996).

Duval, N., Lunven, C., OBrien, D. P., Grosset, A., OConnor, S. and Berry, C., Antithrombotic action of the thrombin inhibitor, argatroban, in a canine model of coronary cyclic flow: comparison with heparin. *Br. J. Pharmacol.*, 118, 727-733 (1996).

Fuster, V., Badimon, L. and Badimon, J. J., The pathogenesis of coronary artery disease and the acute coronary syndromes. *N. Engl. J. Med.*, 326, 242-250 (1992.).

Gibaldi, M. and Perrier, D., *Pharmacokinetics*. Marcel Dekker Co. New York, pp. 293-332 (1982).

Hull, R. D. and Pineo, G. F., Therapeutic use of low molecular weight heparins: knowledge to date and their application to therapy. *Semin. Thromb. Hemost.*, 20, 339-344 (1994).

Kim, S. S., Hwang, S. Y., Kim, Y. K., Yun, M. K. and Oh, Y.S., Rational design of selective thrombin inhibitors. *Bioorg. & Med. Chem. Lett.*, 7, 769-774 (1997).

Lee, S. H., Choi, Y. J., Jeong, Y. N., Seo, M. K. and Lee, Y. H., High-performance liquid chromatographic determination of a new oral thrombin inhibitor in the blood of rats and dogs. *J. Chromatogr. Biomed. Sci. Appl.*, 714, 379-383 (1998).

Matsuo, T., Koide, M. and Kario, K., Development of argatroban, a direct thrombin inhibitor and its clinical application. *Semin. Thromb. Hemost.*, 23, 517-522 (1997).

Mehta, J. L., Chen, L., Nichols, W. W., Mattsson, C., Gustafsson, D. and Saldeen, T. G., Melagatran, an oral active-site inhibitor of thrombin, prevents or delays formation of electrically induced occlusive thrombus in the canine coronary artery. *J. Cardiovasc Pharmacol.*, 3, 345-351 (1998).

Millet, J., Theveniaux, J. and Brown, N. L., The venous antithrombotic effect of LF 1351 in the rat following oral administration. *Thromb. Haemostat.*, 67, 176-179 (1992).

Oh, Y. S., Yun, M. K., Hwang, S. Y., Hong, S. W., Shin, Y., Lee, K., Yoon, K. H., Yoo, Y. J., Kim, D. S., Lee, S. H., Lee, Y. H., Park, H. D., Lee, C. H., Lee, S. K. and Kim, S. S., Discovery of LB30057, a benzamidrazone-based selective oral thrombin inhibitor. *Bioorg. & Med. Chem. Lett.*, 8, 631-634 (1998).

Rebello, S. S., Miller, B. V., Basker, G. C. and Lucchesi, B. R., CVS-1123, a direct thrombin inhibitor, prevents occlusive arterial and venous thrombosis in a canine model of vascular injury. *J. Cardiovasc. Pharmacol.*, 29, 240-249 (1997).

Sanderson, P., E., Cutrona, K. J., Dorsey, B. B., Dyer, D. L., McDonough, C. M., Nylor-Olson, A. M., Chen, I. W., Cook, J. J., Gardel, S. J., Krueger, J. A., Lewis, S. D., Lin, J. H., Lucas, B. J., Lyle, E. A., Lynch, J. J., Stranieri, M. T., Vastag, K., Shafer, J. A. and Vacca, J. P., L-374,087, an efficacious, orally bioavailable, pyrimidinone acetamide thrombin inhibitor. *Bioorg. & Med. Chem. Lett.*, 8, 817-822 (1998).

Verstraete, M. and Zoldhelyi, P., Novel antithrombotic drugs in development. *Drugs*, 49, 856-884 (1995).

Wallis, R. B., Inhibition of thrombin, a key step in thrombosis. *Drugs of Today*, 25, 597-605 (1989).

Weitz, J. I., Calif, R. M., Ginsberg, J. S., Hirsh, J. and Theroux, P., New antithrombotics. *Chest*, 108, Supplement 471S-485S (1995).