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Antithrombotic effects of YM-60828, a newly synthesized factor Xa inhibitor, in rat thrombosis models and its effects on bleeding time

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1 The effects of YM-60828, a newly synthesized factor Xa inhibitor, were investigated to analyse the relationship between its antithrombotic effects and its prolongation of template bleeding time in rats. YM-60828 was compared with argatroban, heparin and dalteparin. All agents were intravenously administered as a bolus.

2 In *ex vivo* studies, YM-60828 and argatroban prolonged both prothrombin time and activated partial thromboplastin time in a dose-dependent manner, while heparin and dalteparin prolonged only activated partial thromboplastin time.

3 In a venous thrombosis model, all agents exerted antithrombotic effects in a dose-dependent manner. The ID_{50} values of YM-60828, argatroban, heparin and dalteparin were 0.0081 mg kg^{-1}, 0.011 mg kg^{-1}, 6.3 iu kg^{-1} and 4.7 iu kg^{-1}, respectively.

4 In an arterio-venous shunt model, all agents exerted antithrombotic effects in a dose-dependent manner. The ID_{50} values of YM-60828, argatroban, heparin and dalteparin were 0.010 mg kg^{-1}, 0.011 mg kg^{-1}, 10 iu kg^{-1} and 4.2 iu kg^{-1}, respectively.

5 In bleeding time studies, all agents prolonged template bleeding time in a dose-dependent manner. ED_2 values, the doses causing a 2 fold prolongation of bleeding time in the saline group, of YM-60828, argatroban, heparin and dalteparin were 0.76 mg kg^{-1}, 0.081 mg kg^{-1}, 18 iu kg^{-1} and 25 iu kg^{-1}, respectively.

6 The ratio (ED_2/ID_{50}) of YM-60828 was more than 30 fold greater than that of heparin and more than 10 fold greater than those of argatroban and dalteparin.

7 These data show that YM-60828 can exert its antithrombotic effects with little prolongation of bleeding time compared with the other currently used anticoagulant agents.

Keywords: YM-60828; argatroban; heparin; dalteparin; venous thrombosis; arterio-venous shunt; bleeding time

Introduction

Heparin and warfarin have been widely used in anticoagulant therapy. Despite their long history of use they possess problems such as difficulty controlling their anticoagulant activity and adverse effects on bleeding. Although agents that show potent anticoagulant activity via the specific inhibition of thrombin, the final product in the coagulation cascade (Rosenberg *et al*., 1975), have been recently developed (Lefkovits & Topol, 1994), their adverse effect on bleeding due to their effects on platelet function has not been mitigated. Therefore, agents which can be easily controlled and have little effect on bleeding are now being sought for clinical use.

The activated serine protease factor Xa (FXa) is the key enzyme at the convergent point of the intrinsic and extrinsic pathways of coagulation. It forms prothrombinase complex with factor Va, calcium and phospholipid to produce thrombin (Rosenberg *et al*., 1975). It is therefore thought that anticoagulant effects can be more efficiently exerted by inhibiting FXa rather than by inhibiting thrombin. Moreover, because FXa inhibitors affect coagulation specifically but not platelet function, this mechanism should notably decrease bleeding tendency. FXa-inhibiting peptides derived from natural products and DX-9065a, a synthetic and selective FXa inhibitor, have been shown to exert antithrombotic effects in various thrombosis models (Neeper *et al*., 1990; Schaffer *et al*., 1993; Yamazaki *et al*., 1994; Herbert *et al*., 1996). Moreover, DX-9065a inhibited thrombosis without affecting bleeding time (Hara *et al*., 1995).

YM-60828, an FXa inhibitor, has been newly synthesized in our laboratory. This compound inhibited human FXa with a K_i value of 1.3 nM (Taniuchi *et al*., unpublished data). In this study, the antithrombotic effects of YM-60828 in venous thrombosis and arterio-venous shunt models in rats and its prolongation of template bleeding time were compared with those of the currently used anticoagulant agents, argatroban (a specific thrombin inhibitor, Okamoto *et al*., 1981), heparin and dalteparin (a low molecular weight heparin, Hamano *et al*., 1992).

Methods

Ex vivo *studies*

Non-fasted male Sprague-Dawley (SD) rats (200–420 g, Japan SLC, Hamamatsu, Japan) were anaesthetized by intraperitoneal injection of pentobarbitone (50 mg kg^{-1}). The jugular vein was cannulated and a 500 μl citrated (1 : 10 dilution, 3.8% sodium citrate) blood sample was collected from the jugular vein. The agents were administrated via the femoral vein as a bolus, with blood sampling performed before and 1 min after the administration of the agents. Platelet-poor plasma (PPP) was prepared by centrifugation (12 000 r.p.m. × 10 min, TCF-12, Iwaki, Japan) at room temperature. Anticoagulant activity was measured with a coagulometer

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(KC-10, Amelung, FRG). To measure prothrombin time (PT), 50 μl of PPP was incubated for 1 min at 37°C. Coagulation was induced by the introduction of 50 μl of PT reagent (Ortho-Clinical Diagnostic K.K., Tokyo, Japan). To measure activated partial thromboplastin time (APTT), 50 μl of PPP and APTT reagent (Ortho-Clinical Diagnostic K.K., Tokyo, Japan) were mixed and incubated for 3 min at 37°C. Coagulation was induced by the introduction of 50 μl of 20 mM $CaCl_2$ solution. Anticoagulant activity was expressed as the relative increase in coagulation time compared with that before the administration of the agent. Each experiment was performed three times.

Venous thrombosis model in rats

Thrombus formation was induced by the method of Reyers (Reyers *et al*., 1980). Non-fasted male SD rats (320–380 g, Japan SLC, Hamamatsu, Japan) were anaesthetized by intraperitoneal injection of pentobarbitone (50 mg kg^{-1}). The abdomen was surgically opened and the inferior vena cava was isolated. Venous thrombosis was induced by tight ligation with cotton thread of the inferior vena cava just below the left renal venous branch. All agents were administered via the femoral vein as a bolus 1 min before ligation of the vena cava. Two hours after ligation, the vena cava was clamped about 2 cm below the ligation and the vascular segment between the ligation and the clamp was longitudinally opened. The thrombus was gently removed and dissolved in 2 ml of 0.5 N NaOH. The protein content of the thrombus was measured by a photometric method by use of a dye-binding assay kit (Bio-Rad, Hercules, CA) and bovine serum albumin (BSA) as a protein standard.

Arterio-venous shunt model in rats

Non-fasted male SD rats (330–380 g, Japan SLC, Hamamatsu, Japan) were anaesthetized by intraperitoneal injection of urethane (0.96 g kg^{-1}). The left jugular vein and the right carotid artery were cannulated with a 12 cm long polyethylene tube (o.d. 0.965 mm, PE-50, Clay Adams, NJ, U.S.A.). These catheters were connected to the ends of a 10 cm long polyethylene tube (o.d. 1.52 mm, PE-100, Clay Adams, NJ, U.S.A.) containing a 2 cm long copper wire (o.d. 0.3 mm). All agents were administered via the femoral vein as a bolus 1 min before blood circulation in the shunt. Ten minutes after blood circulation started, the copper wire was gently removed and the thrombus attached to the wire was dissolved in 2 ml of 0.5 N NaOH. The protein content of the thrombus was measured by a photometric method by use of a dye-binding assay kit (Bio-Rad, Hercules, CA) and BSA as a standard protein.

Template bleeding time in rats

Template bleeding time in the auricle was measured by the method of MacDonald (MacDonald *et al*., 1994). Non-fasted male SD rats (300–400 g, Japan SLC, Hamamatsu, Japan) were anaesthetized by intraperitoneal injection of urethane (0.96 g kg^{-1}). All agents were administered via the femoral vein as a bolus 1 min before an incision of the ear. A template bleeding device (Simplate, Organon Teknika Co., Tokyo, Japan) was placed on the dorsal surface of the auricle and triggered. Blood flowing from the incision was gently wiped away with filter paper every 30 s. Bleeding time was measured as time elapsed until bleeding stopped. When template bleeding time was prolonged beyond 30 min, measurement was stopped and the bleeding time was recorded as 30 min.

Drugs

YM-60828 (Figure 1, [N-[4-[(1-acetimidoyl-4-piperidyl)oxy]-phenyl] -N- [7-amidino -2- naphthyl) methyl] sulphamoyl] acetic acid dihydrochloride) was synthesized at Yamanouchi Pharmaceutical Co. Heparin sodium, dalteparin sodium and argatroban were purchased from Takeda Chemical Industries (Shimizu, Osaka, Japan), Kissei Pharmaceutical Co. (Fragmin, Nagano, Japan) and Tokyo-Tanabe Pharmaceutical Co. (Novastan, Tokyo, Japan), respectively. YM-60828 was dissolved in saline before use. Heparin, dalteparin and argatroban were diluted with saline.

Statistical analysis

All data are presented as the mean ± s.e.mean. Statistical analysis was performed by Dunnett multiple comparison test for the venous thrombosis model or Steel test for the arterio-venous shunt model and template bleeding time, compared with the saline group. A P value of less than 0.05 was considered significant.

Ethical considerations

All experiments were performed in accordance with the regulations of the Animal Ethical Committee of Yamanouchi Pharmaceutical Co., Ltd.

Results

Ex vivo *studies*

YM-60828 and argatroban prolonged both PT and APTT in a dose-dependent manner. In contrast, heparin and dalteparin prolonged only APTT in a dose-dependent manner (Table 1). Argatroban prolonged coagulation time more than YM-60828 at a dose of 1 mg kg^{-1}. Heparin prolonged APTT by more than 10 fold that before administration of the agents at a dose of 100 iu kg^{-1}.

Venous thrombosis model in rats

Figure 2 represents the effects of the anticoagulant agents on thrombus formation in the venous thrombosis model in rats. The protein content of the thrombus formed in the saline group was 1.91 ± 0.20 mg (n = 10). All agents exerted antithrombotic effects in a dose-dependent manner (n = 6). Both YM-60828 and argatroban significantly inhibited thrombus formation at doses of 10 μg kg^{-1}. Both heparin and dalteparin exerted significant anti-thrombotic effects at doses of 10 iu kg^{-1}. Table 2 shows

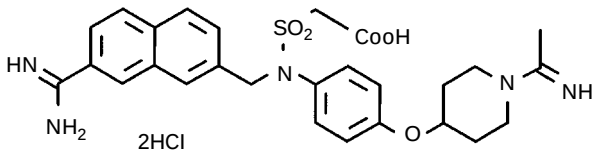

Figure 1 Structure of YM-60828, [N-[4-[(1-acetimidoyl-4-piperidyl)oxy]phenyl]-N-[(7-amidino-2-naphthyl)methyl]sulfamoyl]acetic acid dihydrochloride

the ID_{50} values estimated from the dose-inhibition curves. YM-60828 was as potent as argatroban in the venous thrombosis model.

Arterio-venous shunt model in rats

Figure 3 represents the effects of the anticoagulant agents on thrombus formation in the arterio-venous shunt model in rats. The protein content of the thrombus formed in the saline group was 1.08 ± 0.13 mg ($n=14$). All agents exerted antithrombotic effects in a dose-dependent manner ($n=6$). Both YM-60828 and argatroban inhibited thrombus formation significantly at doses of 30 μg kg^{-1}. Heparin and dalteparin exerted significant antithrombotic effects at doses of 10 iu kg^{-1} and 3 iu kg^{-1}, respectively. Table 2 shows ID_{50} values estimated from the dose-inhibition curves. YM-60828 was as potent as argatroban in the arterio-venous shunt model.

Table 1 Anticoagulant effects of anticoagulant agents (*ex vivo*)

			PT (relative-increase)	*APTT* (relative-increase)
YM-60828	0.1	mg kg^{-1}	1.3±0.0	1.1±0.0
	0.3		2.1±0.2	1.3±0.3
	1		2.9±0.1	1.9±0.1
Argatroban	0.1	mg kg^{-1}	1.2±0.1	1.3±0.1
	0.3		1.9±0.1	2.1±0.1
	1		4.1±0.4	4.2±0.4
Heparin	10	iu kg^{-1}	1.0±0.0	1.5±0.1
	30		1.1±0.0	2.5±0.4
	100		1.5±0.1	>10
Dalteparin	10	iu kg^{-1}	1.1±0.1	1.4±0.2
	30		1.1±0.0	1.6±0.1
	100		1.2±0.0	4.7±0.6

Data represent the relative increase in coagulation time compared with that before administration of the agent and are expressed as mean±s.e.mean ($n=3$). Agents were intravenously administered as a bolus 1 min before blood sampling.

Table 2 The ID_{50} and ED_2 values of the anticoagulant agents

	YM-60828 (mg kg^{-1})	*Argatroban* (mg kg^{-1})	*Heparin* (iu kg^{-1})	*Dalteparin* (iu kg^{-1})
ID_{50VT}	0.0081	0.011	6.3	4.7
ID_{50AV}	0.010	0.011	10	4.2
ED_2	0.76	0.081	18	25

ID_{50VT} dose causing 50% inhibition in the venous thrombosis model. ID_{50AV} dose causing 50% inhibition in the arterio-venous shunt model. ED_2 dose causing 2 fold prolongation of bleeding time in the saline group.

Template bleeding time in rats

Figure 4 represents the effect of the anticoagulant agents on template bleeding time. Template bleeding time in the saline group was 3.0 ± 0.3 min ($n=13$). All agents prolonged template bleeding time in a dose-dependent manner ($n=6$). YM-60828 and argatroban significantly prolonged bleeding

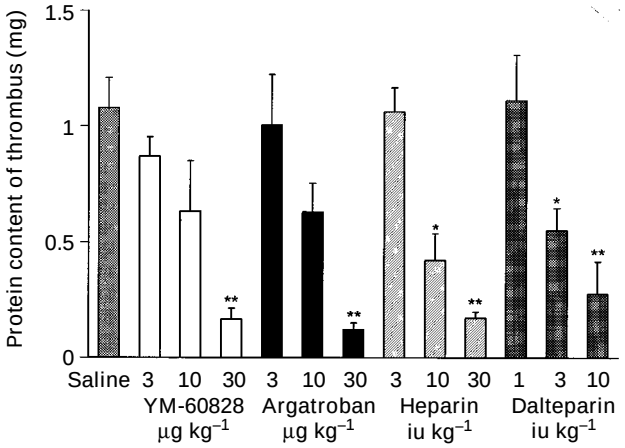

Figure 3 Antithrombotic effects of YM-60828 (open columns), argatroban (solid columns), heparin (hatched columns) and dalteparin (stippled columns) in an arterio-venous shunt model in rats. The agents were intravenously administered as a bolus 1 min before blood circulation in the shunt. Data are expressed as mean±s.e.mean ($n=6-14$). Statistical analysis was performed by Steel test. $^{*}P<0.05$, $^{**}P<0.01$ compared with the saline group.

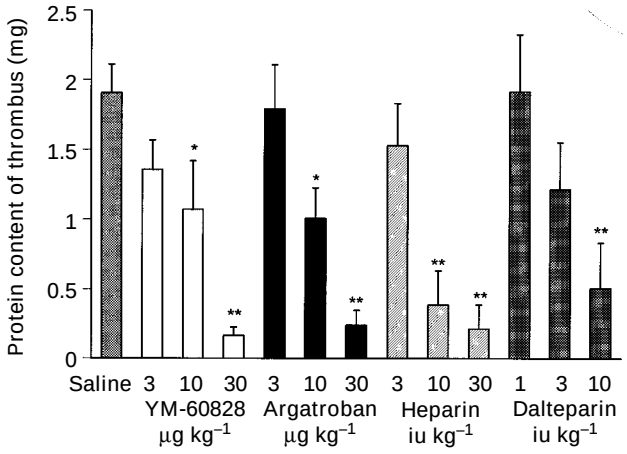

Figure 2 Antithrombotic effects of YM-60828 (open columns), argatroban (solid columns), heparin (hatched columns) and dalteparin (stippled columns) in a venous thrombosis model in rats. The agents were intravenously administered as a bolus 1 min before ligation of the inferior vena cava. Data are expressed as mean± s.e.mean ($n=6-10$). Statistical analysis was performed by Dunnett's multiple comparison test. $^{*}P<0.05$, $^{**}P<0.01$ compared with the saline group.

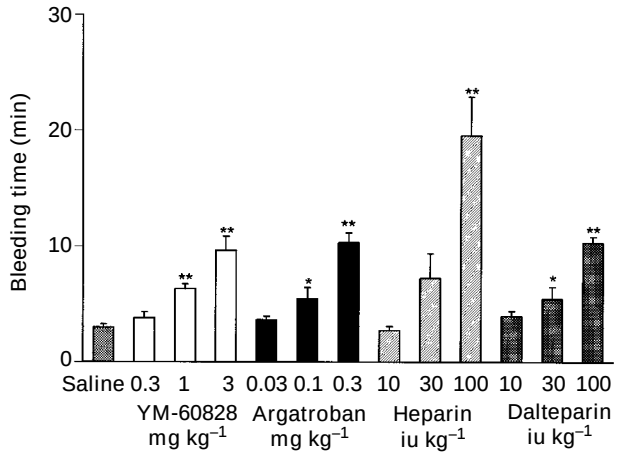

Figure 4 Effects of YM-60828 (open columns), argatroban (solid columns), heparin (hatched columns) and dalteparin (stippled columns) on template bleeding time in rats. The agents were intravenously administered as a bolus 1 min before incision of the ear. Data are expressed as mean±s.e.mean ($n=6-13$). Statistical analysis was performed by Steel test. $^{*}P<0.05$, $^{**}P<0.01$ compared with the saline group.

Table 3 Risk-benefit ratio of the anticoagulant agents

	YM-60828	*Argatroban*	*Heparin*	*Dalteparin*
ED_2/ID_{50VT}	94	7.4	2.9	5.3
ED_2/ID_{50AV}	76	7.4	1.8	6.0

ID_{50VT} dose causing 50% inhibition in the venous thrombosis model. ID_{50AV} dose causing 50% inhibition in the arterio-venous shunt model. ED_2 dose causing 2 fold prolongation of bleeding time in the saline group.

time at doses of 1 mg kg^{-1} and 0.1 mg kg^{-1}, respectively. Heparin and dalteparin significantly prolonged bleeding time at doses of 100 iu kg^{-1} and 30 iu kg^{-1}, respectively. Table 2 shows ED_2 values, the doses causing a 2 fold prolongation of bleeding time in the saline group, estimated from the dose-response curves. YM-60828 was about 10 fold less potent than argatroban in this test.

Risk-benefit ratio of the anticoagulant agents

Table 3 shows ED_2/ID_{50} of the anticoagulant agents as the risk-benefit ratio. The ratio of YM-60828 was more than 30 fold greater than that of heparin and more than 10 fold greater than those of argatroban and dalteparin.

Discussion

In this study, we investigated the antithrombotic effects of YM-60828, a newly synthesized FXa inhibitor, in the venous thrombosis and the arterio-venous shunt models in rats and its prolongation of template bleeding time in comparison with those of the currently used anticoagulant agents, argatroban, heparin and dalteparin.

This study employed the venous thrombosis and the arterio-venous shunt models. The thrombus formed in the venous thrombosis model is composed largely of fibrin and contains a few platelets (Reyers *et al*., 1980). In contrast, the arterio-venous shunt model is a mixed thrombus model containing both fibrin and platelets. However, the total size of the thrombus depends on the formation of a fibrin thrombus (Peters *et al*., 1991). Therefore, the models used in this study are likely to be suitable for the evaluation of the antithrombotic activity of the anticoagulant agents.

YM-60828 exerted potent antithrombotic effects in these thrombosis models, but had little effect on bleeding time. The risk-benefit ratio of YM-60828 was more than 30 fold greater than that of heparin and more than 10 fold greater than those of argatroban and dalteparin. Thrombin not only cleaves fibrinogen but also potently activates platelets (Lefkovits & Topol, 1994) and its affinity to platelets is 10,000 fold higher than that to fibrinogen (Higgins *et al*., 1983; Berndt *et al*., 1986). Therefore, even at the effective dose of YM-60828, sufficient thrombin may be still produced to activate and aggregate platelets for primary haemostasis, although it is short for the cleavage of fibrinogen. In fact, DX-9065a, another FXa inhibitor, also exerts its antithrombotic effect without prolonging bleeding time (Hara *et al*., 1995; Herbert *et al*., 1996).

Dalteparin exerted antithrombotic effects of equal potency to that of heparin, but did not prolong bleeding time as greatly as heparin. Heparin is a glycosaminoglycan with an average molecular weight of 10,000 – 15,000 (Andersson *et al*., 1979). Heparin converts antithrombin III, which exerts antithrombotic activity (Rosenberg & Damus, 1973). Since heparin shows not only anti-FXa activity but also antithrombin activity, the use of heparin involves some problems such as an increased bleeding tendency. Low molecular weight heparins with a molecular weight of 2,000 – 9,000 have been found to have antithrombotic properties linked to FXa inhibition rather than antithrombin activity (Holmer *et al*., 1982; Carter *et al*., 1982). Dalteparin is a low molecular weight heparin and is expected to decrease the adverse effect on bleeding because it exerts anti-FXa activity comparable to that of normal heparin but does not have the antithrombin activity of normal heparin (Hamano *et al*., 1992). The results of the present study support these previous findings. Dalteparin also prolonged APTT less than heparin. These results are consistent with the previous observation that dalteparin showed a weaker prolongation of APTT but had an antithrombotic effect as potent as that of normal heparin (Hamano *et al*., 1989). However, the risk-benefit ratio of dalteparin is only 2 to 3 fold greater than that of heparin, which means that the safety of dalteparin is similar to that of heparin.

Thrombin is the final product in the coagulation cascade (Rosenberg *et al*., 1975). Therefore, agents that specifically inhibit thrombin are thought to have potent anticoagulant activity. Recently such agents have been vigorously developed (Lefkovits & Topol, 1994). Among these, a specific thrombin inhibitor, argatroban, has been shown to have potent antithrombotic effects (Imura *et al*., 1992; Berry *et al*., 1994). Indeed, in this study, argatroban exerted antithrombotic effects as potent as those of YM-60828. However, argatroban prolonged bleeding time at a dose 10 fold lower than YM-60828. Moreover, argatroban prolonged bleeding time at a dose of 0.1 mg kg^{-1}, at which coagulation time was little prolonged, whereas the other anticoagulant agents prolonged coagulation time markedly at the dose at which they significantly prolonged bleeding time. The results for argatroban are in accord with those of a previous study (Berry *et al*., 1994). These data suggest that argatroban inhibits platelet aggregation at a much lower dose than coagulation and may therefore increase bleeding tendency by inhibiting platelet aggregation induced by thrombin, which is crucial for haemostasis. In effect, the risk-benefit ratio of argatroban, like that of dalteparin, is only 2 to 3 fold greater than that of heparin.

In comparison with the other anticoagulant agents, YM-60828 exerted significant antithrombotic effects even at a dose at which coagulation time was prolonged only slightly. As demonstrated in previous studies, other FXa inhibitors also exert antithrombotic effects without prolonging coagulation time (Sitko *et al*., 1992; Hara *et al*., 1995). Clinically, administration of agents such as warfarin and heparin is strictly controlled due to fear of bleeding. The monitoring of coagulation time of peripheral blood is troublesome and is a major factor preventing the wide use of conventional anticoagulant agents. Although the mechanism by which YM-60828 exerts its antithrombotic effect without prolonging coagulation time has yet to be clarified in detail, the lack of the need to monitor coagulation time would, if confirmed in man, seem to be of profound clinical merit.

In conclusion, YM-60828 exerted antithrombotic effects with little prolongation of bleeding time. In view of its reduced risk of bleeding, it may prove to be a safer agent than the other currently used anticoagulant agents.

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References

ANDERSSON, L.O., BARROWCLIFFE, T.W., HOLMER, E., JOHNSON, E.A. & SODERSTROM, G. (1979). Molecular weight dependency of the heparin potentiated inhibition of thrombin and activated factor X. Effect of heparin neutralization in plasma. *Thromb. Res.*, **15,** 531 – 541.

BERNDT, M.C., GREGORY, C., DOWDEN, G. & CASTALDI, P.A. (1986). Thrombin interaction with platelet membrane proteins. *Ann. New York Acad. Sci.*, **485,** 374 – 386.

BERRY, C.N., GIRARD, D., LOCHOT, S. & LECOFFRE, C. (1994). Antithrombotic actions of argatroban in rat models of venous, mixed' and arterial thrombosis, and its effects on the tail transection bleeding time. *Br. J. Pharmacol.*, **113,** 1209 – 1214.

CARTER, C.J., KELTON, J.G., HIRSH, J., CERSKUS, A., SANTOS, A.V. & GENT, M. (1982). The relationship between the hemorrhagic and antithrombotic properties of low molecular weight heparin in rabbits. *Blood*, **59,** 1239 – 1245.

HAMANO, S., KOMATSU, H., IKEDA, S., TAKAHASHI, K., OGUMA, Y. & SAKURAGAWA, N. (1989). Beneficial effect of low molecular weight heparin on the hemodialysis model in dogs. *Thromb. Res.*, **55,** 439 – 449.

HAMANO, S., NISHIYAMA, M., KOMATSU, H., MIYATA, H., IKEDA, S. & SAKURAGAWA, N. (1992). Study of anticoagulant mechanism of low molecular weight heparin. *Thromb. Res.*, **65,** 801 – 808.

HARA, T., YOKOYAMA, A., TANABE, K., ISHIHARA, H. & IWAMOTO, M. (1995). DX-9065a, an orally active, specific inhibitor of factor Xa, inhibits thrombosis without affecting bleeding time in rats. *Thromb. Haemost.*, **74,** 635 – 639.

HERBERT, J.M., BERNAT, A., DOL, F., HERAULT, J.P., CREPON, B. & LORMEAU, J.C. (1996). DX 9065A, a novel, synthetic, selective and orally active inhibitor of factor Xa: in vitro and in vivo studies. *J. Pharmacol. Exp. Ther.*, **276,** 1030 – 1038.

HIGGINS, D.L., LEWIS, S.D. & SHAFER, J.A. (1983). Steady state kinetic parameters for the thrombin catalyzed conversion of human fibrinogen to fibrin. *J. Biol. Chem.*, **258,** 9276 – 9282.

HOLMER, E., MATTSON, C. & NILSSON, S. (1982). Anticoagulant and antithrombotic effects of herapin and low molecular weight heparin fragments in rabbits. *Thromb. Res.*, **25,** 475 – 485.

IMURA, Y., STASSEN, J.M. & COLLEN, D. (1992). Comparative antithrombotic effects of heparin, recombinant hirudin and argatroban in a hamster femoral vein platelet-rich mural thrombosis model. *J. Pharmacol. Exp. Ther.*, **261,** 895 – 898.

LEFKOVITS, J. & TOPOL, E.J. (1994). Direct thrombin inhibitors in cardiovascular medicine. *Circulation*, **90,** 1522 – 1536.

MACDONALD, J.D., REMINGTON, B.J. & RODGERS, G.M. (1994). The skin bleeding time test as a predictor of brain bleeding time in a rat model. *Thromb. Res.*, **76,** 535 – 540.

NEEPER, M.P., WAXMAN, L., SMITH, D.E., SCHULMAN, C.A., SARDANA, M., ELLIS, R.W., SCHAFFER, L.W., SIEGL, P.K. & VLASUK, G.P. (1990). Characterization of recombinant tick anticoagulant peptide. A highly selective inhibitor of blood coagulation factor Xa. *J. Biol. Chem.*, **265,** 17746 – 17752.

OKAMOTO, S., HIJIKATA, A., KIKUMOTO, R., TONOMURA, S., HARA, H., NINOMIYA, K., MARUYAMA, A., SUGANO, M. & TAMAO, Y. (1981). Potent inhibition of thrombin by the newly synthesized arginine derivative No. 805. The importance of stereo-structure of its hydrophobic carboxamide portion. *Biochem. Biophys. Res. Commun.*, **101,** 440 – 446.

PETERS, R.F., LEES, C.M., MITCHELL, K.A., TWEED, M.F., TALBOT, M.D. & WALLIS, R.B. (1991). The characterization of thrombus development in an improved model of arterio-venous shunt thrombosis in the rat and the effects of recombinant desulphatohirudin (CGP 39393), heparin, and iloprost. *Thromb. Haemost.*, **65,** 268 – 274.

REYERS, I., MUSSONI, L., DONATI, M.B. & DE GAETANO, G. (1980). Failure of aspirin at different doses to modify experimental thrombosis in rats. *Thromb. Res.*, **18,** 669 – 674.

ROSENBERG, J.S., BEELER, D.L. & ROSENBERG, R.D. (1975). Activation of human prothrombin by highly purified human factors V and Xa in presence of human antithrombin. *J. Biol. Chem.*, **250,** 1607 – 1617.

ROSENBERG, R.D. & DAMUS, P.S. (1973). Purification and mechanism of action of human antithrombin-heparin cofactor. *J. Biol. Chem.*, **248,** 6498 – 6505.

SCHAFFER, L.W., DAVIDSON, J.T., SIEGL, P.K., GOULD, R.J., NUTT, R.F., BRADY, S.F. & CONNOLLY, T.M. (1993). Selective factor Xa inhibition by recombinant antistasin prevents vascular graft thrombosis in baboons. *Arterioscler. Thromb.*, **12,** 879 – 885.

SITKO, G.R., RAMJIT, D.R., STABILITO, I.I., LEHMAN, D., LYNCH, J.L. & VLASUK, G.P. (1992). Conjunctive enhancement of enzymatic thrombolysis and prevention of thrombotic reocclusion with the selective factor Xa inhibitor, tick anticoagulant peptide. *Circulation*, **85,** 805 – 815.

YAMAZAKI, M., ASAKURA, H., AOSHIMA, K., SAITO, M., JOKAJI, H., UOTANI, C., KUMABASHIRI, I., MORISHITA, E., IKEDA, T. & MATSUDA, T. (1994). Effects of DX-9065a, an orally active, newly synthesized and specific inhibitor of factor Xa, against experimental disseminated intravascular coagulation in rats. *Thromb. Haemost.*, **72,** 393 – 396.

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