MK-383 (L-700,462), a Selective Nonpeptide Platelet Glycoprotein IIb/IIIa Antagonist, Is Active in Man

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Background. Fibrinogen-dependent cross-linking of glycoprotein (GP) IIb/IIIa on activated platelets is the final mechanism leading to platelet aggregation. Inhibition of this mechanism may result in a novel antithrombotic agent. We studied the activity of MK-383 (L-700,462), a new, nonpeptide GPIIb/IIIa antagonist, in vitro and in vivo, in man.

Methods and Results. MK-383, a nonpeptide tyrosine derivative, dose-dependently inhibited fibrinogen-dependent platelet aggregation, in vitro. Binding of 125I-labeled fibrinogen to activated platelets was prevented in a competitive manner with an IC50 of 10±4.2 nmol/L. The activity and tolerability of MK-383 were evaluated in a two-part double-blind, placebo-controlled, dose-escalation study in healthy male subjects using 1- and 4-hour intravenous infusions. Effects on ADP- and collagen-induced ex vivo platelet aggregation (APA or CPA) and template bleeding time (TBT) were evaluated. Twenty-four subjects participated in the 1-hour part. Six received placebo and 18 MK-383 in doses ranging from 0.05 to 0.40 μg·kg⁻¹·min⁻¹. MK-383 inhibited platelet aggregation and prolonged bleeding time in a dose-dependent manner. APA and CPA were totally inhibited at the end of infusion of 0.4 μg·kg⁻¹·min⁻¹ and returned to 55% and 89% of baseline, respectively, at 3 hours after infusion. TBT was prolonged at this dose from 5.0±1.3 minutes predose to 22.7±6 minutes at the end of the infusion (P<.01) and was normalized by 3 hours after infusion. In the 4-hour infusion part, 15 subjects received MK-383 (0.1 to 0.2 μg·kg⁻¹·min⁻¹), and five received placebo. Complete inhibition of ex vivo platelet aggregation was seen at 0.15 and 0.2 μg·kg⁻¹·min⁻¹. At 0.2 μg·kg⁻¹·min⁻¹, TBT was prolonged from 4.4±1.2 to 23.9±4.3 minutes at the end of infusion (P<.01) and remained slightly prolonged 3 hours after infusion (7.2±1.8 minutes). No adverse effects were observed in any of the 33 subjects receiving MK-383.

Conclusions. The results from this study indicate that MK-383 appears to be well tolerated and active in man. It is the first nonpeptide GPIIb/IIIa antagonist that can be used to investigate the antithrombotic potential of this new class of antiplatelet agents.

Key Words • integrin • fibrinogen receptor • antithrombotic drug • antiplatelet agents • bleeding time

Platelets play an important role in the initiation of occlusion or reocclusion of arteries at the site of high-grade stenosis or ruptured atheromatous plaques, thereby contributing significantly to cerebrovascular, cardiovascular, and peripheral vascular diseases. Platelets are also important in acute restenosis after coronary angioplasty, thrombolysis, and carotid endarterectomy and to the formation of stenoses at anastomotic sites of vascular grafts and thrombus formation at sites of indwelling catheters.¹³ The final common mechanism in the formation of a platelet aggregate is the cross-linking of adjacent activated platelets by binding of fibrinogen to the glycoprotein complex GPIIb/IIIa.¹² This glycoprotein receptor is a potential target for antiplatelet agents. In this regard, monoclonal anti-GPIIb/IIIa antibodies are potent inhibitors of platelet aggregation and of platelet-dependent thrombus formation in experimental models,⁶-⁸ and the monoclonal antibody 7E3, which is active in man, is now undergoing extensive clinical investigation.⁹,¹⁰ Following the discovery that an Arg-Gly-Asp-(RGD) sequence in the α-chain of fibrinogen is the prime recognition site for its interaction with GPIIb/IIIa,¹¹ a series of peptides containing this or an analogous sequence has been designed that competes with fibrinogen and thus prevents platelet aggregation.¹²,¹³ Furthermore, a number of snake venom peptides (disintegrins) were found to be potent GPIIb/IIIa-blocking agents with antithrombotic potential¹⁴-¹⁸ by virtue of an RGD sequence. To increase the potency and selectivity, peptidomimetic¹⁹,²⁰ and nonpeptide RGD mimetics²¹ have been synthesized and are now available for clinical study.

Received February 18, 1993; revision accepted May 28, 1993.
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We report on the effects in man of one such nonpeptide tyrosine derivative GPIIb/IIIa antagonist, MK-383,\textsuperscript{21} testing the hypothesis that one or more dose levels of the drug would be well tolerated and would produce targeted levels of inhibition of platelet function as measured in vivo by bleeding time prolongation and ex vivo by inhibition of ADP- and collagen-induced platelet aggregation.

Methods

In Vitro Studies

In vitro studies were carried out to characterize the pharmacologic profile of MK-383 with respect to inhibition of agonist-induced aggregation and fibrinogen binding.

Platelet aggregation. Blood from normal volunteers who denied having taken any drugs during the past 14 days was collected on 109 mmol/L trisodium citrate (1:10 vol). Platelet-rich plasma (PRP) and platelet-poor (PPP) were prepared by centrifugation for 10 minutes at 150g or for 15 minutes at 2000g, respectively. Aggregations were performed by a turbimetric method in an Elvi-840 dual-channel aggregometer (Elvi, Milan, Italy). Aggregation was induced with 2 μg/mL collagen (Hormon Chemie, München, Germany), 3.4 μmol/L ADP, 1 mmol/L arachidonic acid (Sigma Chemical Co, St Louis, Mo), 0.5 μmol/L U46619 (9,11-dideoxy 11α, 9α-epoxy methano-PGF\textsubscript{2α}; Upjohn Co, Kalamazoo, Mich), or 1.2 mg/mL ristocetin (Lundbeck, Copenhagen, Denmark). Aggregation induced by 0.2 U/mL thrombin (Roche, Brussels, Belgium) was studied in gel-filtered platelets prepared as described below. PRP samples were preincubated with different concentrations of MK-383 (1-tyrosine-N-(butylsulfonyl)-O-[4-(4-piperidinyl)butyl]monohydrochloride; Merck Research Laboratories, West Point, Pa) as indicated for 1 minute at 37°C.

Binding assay.\textsuperscript{125I}-labeling of human fibrinogen (a gift of Dr G. Marguerie, Grenoble, France), was done using iodogen reagent (Pierce Chemical Company, Rockford, Ill) as described.\textsuperscript{22} Platelets were isolated from fresh human blood drawn into acid/citrate/dextrose by differential centrifugation followed by gel filtration in a modified Tyrode buffer (pH 7.2) containing 2% bovine serum albumin, as previously described.\textsuperscript{23} Binding of \textsuperscript{125I}-fibrinogen (25 000 cpm/μg) to washed platelets was performed with 10⁶ cells/mL in the presence of 0.5 mmol/L calcium, 25 μmol/L ADP, and MK-383 at the indicated concentration. After an incubation period of 30 minutes, the bound ligand was separated from the free ligand by centrifugation of 80-μL aliquots of the reaction mixture through a 15% sucrose solution and was quantitated by measuring the radioactivity associated with platelets.

Volunteer Studies

A double-blind, placebo-controlled, two-part, dose-escalation study of 1- and 4-hour intravenous infusions of MK-383 was performed in healthy male subjects. The study was conducted according to the Declaration of Helsinki and after approval of the Ethical Committee of the University of Leuven. Volunteers were studied in panels of four: in each panel, three subjects received MK-383 and one received placebo. In the 1-hour study, a starting dose of 0.05 μg·kg\textsuperscript{-1}·min\textsuperscript{-1} was used; on the basis of animal experiments, this dose was expected to have no effect. Dose escalations were performed until targeted activity, defined as a bleeding time extension from baseline of 2.5-fold or more in at least two of three subjects receiving the study drug, was reached. A 1-hour infusion at the rate that achieved targeted activity was repeated in a second panel of four volunteers. Since infusion studies in dogs (results not shown) had shown significant changes in bleeding time between 1.0 and 3.0 μg·kg\textsuperscript{-1}·min\textsuperscript{-1}, suggesting the possibility of a steep dose-effect relationship, a conservative dose-escalation schedule was designed (0.05, 0.10, 0.15, 0.25, and 0.40 μg·kg\textsuperscript{-1}·min\textsuperscript{-1} [higher doses were planned but did not prove to be necessary]). In the 4-hour study, an initial infusion rate of one third of the 1-hour infusion rate that achieved targeted activity was used.

Bleeding time according to Ivy\textsuperscript{24} was performed using an automated template device (Simplate II; General Diagnostics, Morris Plains, NJ). The incisions were made on the volar side of the forearm, perpendicular to the elbow crease. All bleeding times were performed by the same operator. Bleeding times were performed the day before infusion of MK-383 and starting 10 minutes before the end of the infusion. When a prolongation of bleeding time more than twice individual baseline was found, bleeding time was repeated 3 hours after the end of the infusion.

Blood for platelet aggregation was collected on trisodium citrate 129 mmol/L (1:10 vol; Vacutainer, Becton Dickinson, UK) by a separate venipuncture at each time point. Platelet aggregation was measured predose and prior to discharge in each subject. In the 1-hour part of the study, aggregations were performed 30, 60, 120, and 180 minutes after start of infusion. In the 4-hour part, aggregation was measured 60, 120, 180, 240, 280, and 360 minutes after start of infusion. PRP was prepared as above, and aggregation was induced by fixed concentrations of ADP (3.4 μmol/L) and collagen (2 μg/mL). These concentrations were determined as the minimal concentrations of these agonists that give full response in more than 90% of normal subjects. Aggregation was followed for 4 minutes after addition of agonist, and the maximum percent increase of light transmission obtained during this period (maximal amplitude) was calculated. Results were expressed as percent inhibition from baseline. Plasma levels of MK-383 were determined in lithium heparin anticoagulated plasma, using radioimmunoassay methodology.

For clinical safety assessment, the following parameters were studied: ECG, vital signs (blood pressure, heart rate, temperature, respirations), hematology (hemoglobin, hematocrit, red blood cell count, total and differential white blood cell count, platelet count, prothrombin time, partial thromboplastin time), blood chemistry (SGOT, SGPT, serum alkaline phosphatase, serum total bilirubin, serum creatinine, urea, fasting blood sugar, uric acid, sodium, potassium, fibrinogen), urinalysis (pH, protein, sugar, microscopy), and stool for occult blood.

Statistical analysis. A one-way ANOVA model was used to test for between-dose differences in percent change from baseline for ADP- and collagen-induced platelet aggregation. The least-squares estimates from this model were also used to test the null hypothesis of
no change from baseline for each dose level. The matched-pairs t test was used to test the null hypothesis of no difference in percent change from baseline between selected time points for each dose level.

Analysis of bleeding time extension was performed as above on log-transformed data.

**Results**

**In Vitro Studies**

MK-383 dose-dependently inhibited platelet aggregation induced by ADP, collagen, arachidonic acid, the thromboxane analogue U46619, and thrombin but not by ristocetin. IC\textsubscript{50} values for aggregation induced by 2 μg/mL collagen or 3.4 μmol/L ADP were 66±8 and 39±4 nmol/L (mean±SEM, n=4), respectively (Fig 1).

Binding of \textsuperscript{125}I-labeled human fibrinogen to ADP-stimulated platelets could be prevented by MK-383 with an IC\textsubscript{50} of 10.0±4.2 nmol/L. Double reciprocal plots of fibrinogen-binding curves in the absence or presence of MK-383 revealed that the inhibition was competitive with a K\textsubscript{i} of 2.1±1.0 nmol/L.

**Volunteer Studies**

In the 1-hour infusion part of the study, 6 subjects received placebo and 18 received MK-383 in doses ranging from 0.05 to 0.40 μg·kg\(^{-1}\)·min\(^{-1}\). The lowest dose, 0.05 μg·kg\(^{-1}\)·min\(^{-1}\), produced no apparent effects on bleeding time. Activity of MK-383 was apparent at the other doses. A dose-dependent prolongation of the bleeding time was seen when bleeding time was measured, 50 minutes after the start of the infusion (Table). This extension was statistically significant at the 0.10 μg·kg\(^{-1}\)·min\(^{-1}\) infusion rate (P<.01). A mean 4.6-fold (5.0±1.3 to 22.7±6 minutes, n=3) prolongation was obtained with 0.40 μg·kg\(^{-1}\)·min\(^{-1}\). Bleeding times had returned to normal 3 hours after the end of the 1-hour infusion (Fig 2).

Ex vivo platelet aggregation induced by either collagen or ADP was significantly inhibited (P<.01) 30 minutes following the start of the infusion of 0.10 μg·kg\(^{-1}\)·min\(^{-1}\) MK-383 (Fig 2). Complete inhibition of both collagen- and ADP-induced platelet aggregation was seen after 30 and 60 minutes at the infusion rate of 0.40 μg·kg\(^{-1}\)·min\(^{-1}\) with recovery to 89±4% and 55±12% of predose response at 4 hours after the start of infusion. No adverse effects were seen with any of the safety parameters; no changes in platelet counts were found during or after the infusion.

Based on the results of the 1-hour study, a 4-hour infusion study was completed in which 5 subjects received placebo and 15 subjects were treated with MK-383 at doses of 0.10 to 0.20 μg·kg\(^{-1}\)·min\(^{-1}\). At 0.20 μg·kg\(^{-1}\)·min\(^{-1}\), the bleeding time increased from 4.4±1.2 to 23.9±4.3 minutes (P<.01) at the end of the infusion (Table) and remained slightly prolonged 3 hours after infusion (7.2±1.8 minutes) (Fig 3). The plasma half-life of MK-383 was approximately 90 minutes; pharmacokinetics of the compound will be addressed in detail elsewhere. Dose-dependent rapid-onset inhibition of both collagen- and ADP-induced aggregation was seen with a near-complete inhibition of aggregation during the infusion of 0.15 and 0.20 μg·kg\(^{-1}\)·min\(^{-1}\).
Figure 2. Plots of bleeding time extension over baseline (A), MK-383 plasma levels (B), and ex vivo platelet aggregation induced by 3.4 μmol/L ADP (C) or 2 μg/mL collagen (D) before, during, and after a 1-hour infusion of placebo (○) or 0.05 (▲), 0.10 (□), 0.15 (▲), 0.25 (◇), or 0.40 (◇) μg·kg⁻¹·min⁻¹ MK-383 to six, three, three, three, six, and three volunteers, respectively.

Discussion

We report on the first administration to man of a nonpeptide GPIIb/IIIa-blocking agent. In preliminary in vitro studies, it was shown that MK-383 selectively inhibits GPIIb/IIIa by the finding that fibrinogen-dependent platelet aggregation induced by a variety of agonists was inhibited. However, ristocetin-induced agglutination, which proceeds through binding of von Willebrand factor to GPIb, was unaffected. Furthermore, the compound dose-dependently inhibited fibrinogen binding to activated platelets in a competitive manner.

Administration of MK-383 to human volunteers during either a 1-hour (maximum dose, 0.40 μg·kg⁻¹·min⁻¹) or a 4-hour (maximum dose, 0.20 μg·kg⁻¹·min⁻¹) intravenous infusion resulted in marked effects on ex vivo platelet aggregation. At these doses, plasma drug concentrations of MK-383 were comparable to those needed for in vitro activity. As a presumed consequence of the inhibition of platelet function, the bleeding times were prolonged. However, the concentration-effect relationship for extension of bleeding time was to the right of the relationship for inhibition of ADP aggregation. This different sensitivity has been observed with a variety of GPIIb/IIIa inhibitors in both animal models and man, which indicates that inhibition of platelet aggregation can occur without major effects on bleeding time. Coller et al. have shown a correlation between the number of GPIIb/IIIa receptors blocked by the monoclonal antibody 7E3 and the bleeding time: when 8000 to 15 000 residual receptors were available, platelet aggregation was markedly decreased, whereas the bleeding time was only mildly affected. The bleeding time, however, rapidly prolonged when the number of residual GPIIb/IIIa receptors decreased below 10 000. The same mechanism may explain the rather large differences in bleeding times that were seen with small differences in plasma levels of MK-383 around 45 ng/mL. In this respect, it is noteworthy that in some thrombosis models, GPIIb/IIIa antibodies proved to be effective at levels that only mildly affected bleeding times. These differential effects could, however, depend on the nature of the thrombogenic challenge since in more severe thrombosis models, near-complete inhibition of GPIIb/IIIa receptors is required for efficacy.

Based on the findings of this study, MK-383 has the potential to be useful in treatment of patients with or at...
risk for platelet-dependent thrombus formation. This nonpeptide inhibitor also has obvious potential advantages compared with monoclonal antibodies. Following treatment with antibodies, the bleeding time can be prolonged for 12 to 24 hours, and inhibition of platelet aggregation may persist over at least 3 days. The reversal of the effects caused by MK-383 was essentially complete within 3 hours. Furthermore, compounds such as MK-383 are expected to be much less, if at all, immunogenic, whereas this may represent a major problem with antibody treatment. However, this aspect of the antibodies may, to some extent, be circumvented by producing a "humanized" chimeric antibody.

Finally, all GPIIb/IIIa inhibitors available at present have to be administered intravenously, which limits their use to acute thrombotic situations such as reocclusion following thrombolysis. To be useful for preventive therapy, an orally active compound is needed. The development of this still orally inactive but well tolerated and effective nonpeptide inhibitor, MK-383, is one step closer toward this goal.

Acknowledgments

Supported in part by a sponsored research agreement between the University of Leuven and Merck Research Laboratories.

We wish to thank C. Wittevrongel and E. Van Houtte for excellent technical assistance and J. Vangoetsenhoven and B. Verheyden for careful preparation of the manuscript.

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FIG 3. Plots of bleeding time extension over baseline (A), MK-383 plasma levels (B), and ex vivo platelet aggregation induced by 3.4 μmol/L ADP (C) or 2 μg/mL collagen (D) before, during, and after a 4-hour infusion of placebo (●) or 0.10 (○), 0.15 (△), or 0.20 μg·kg⁻¹·min⁻¹ (▼) MK-383 to five, three, nine, and three volunteers, respectively.

FIG 4. Scatterplot of fold prolongation of bleeding times (○) and inhibition of ex vivo ADP-induced platelet aggregation (PA) (●) as a function of plasma levels attained during infusion of MK-383 at all time points and doses given.


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Circulation. 1993;88:1512-1517
doi: 10.1161/01.CIR.88.4.1512

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