Meeting Report

Advances in Anticoagulant, Antithrombotic, and Thrombolytic Drugs: 10th Anniversary International Symposium, October 3–7, 1999

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International Business Communications’ (IBC) 10th Anniversary International Symposium on Advances in Anticoagulant, Antithrombotic, and Thrombolytic Drugs (AATD ’99) was held on October 3–7, 1999, at the Fairmont Copley Plaza Hotel in Boston, MA, USA. The meeting brought together prominent clinical and pharmaceutical industry investigators from around the world to discuss and review some of the most interesting developments and newest drugs in this rapidly evolving therapeutic area. Several selected presentations are briefly reviewed in this Meeting Report.

HEPARIN AND LOW MOLECULAR WEIGHT HEPARINS

Jawed Fareed (Loyola Univ. Medical Center, Maywood, IL, USA) commented on the expanding role of low molecular weight heparins (LMWHs) in the management of a variety of thrombotic, cardiovascular, and metastatic disorders. Several LMWHs are now available in Europe, but in the United States only three LMWHs have been approved and are marketed. They are ardeparin (Normiflo®) from American Home Products Corp., dalteparin (Fragmin®) from Pharmacia & Upjohn, Inc., and enoxaparin (Lovenox®) from Rhône-Poulenc Rorer, Inc. A fourth, tinzaparin (Innohep®), has been filed with the FDA by DuPont Pharmaceutical Co. and approval is pending. All LMWHs are produced by some type of chemical depolarization of unfractionated heparin derived from animals to yield a range of lower molecular weight species. Since the LMWHs produced by various manufacturers are prepared using different methods, each LMWH product has different chemical properties and potency. Because of these differences, Fareed argues that each LMWH should be considered unique and must therefore be evaluated individually for its efficacy and safety. Thus, different LMWHs are not interchangeable with one another in the clinic. This point is best illustrated by examining anti-factor Xa (anti-Xa) and anti-factor IIa (anti-IIa) or the antithrombin activities of each LMWH. Anti-Xa activities vary

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widely from almost 150 anti-Xa units/mg with dalteparin to about 60 anti-Xa units/mg with ardeparin. It has been suggested that LMWHs may be interchangeable if administered at equivalent anti-Xa dosages, but experiments have shown that anti-IIa activity varies considerably at equivalent anti-Xa dosages for the different LMWHs. At a dose of 200 anti-Xa units, anti-IIa activity varies from a low of about 60 units with enoxaparin and several other LMWHs to a high of just over 100 units with tinzaparin. Each LMWH can therefore be characterized by its unique anti-Xa/anti-IIa ratio. Clinical trials have shown that equivalent efficacy, or superior efficacy in certain indications, can be attained with different LMWHs in comparison with unfractionated heparin, but there have been few clinical trials that have directly compared different LMWHs. Thus, the question of which LMWH is best for a particular indication is still open. The FDA and WHO evaluated the available data, and both organizations consider every LMWH to be a distinct entity.

Sylvia Haas (Technical Univ. of Munich, Munich, Germany) discussed the use of LMWHs in the prevention of venous thromboembolism in nonsurgical patients. Medical patients often have an increased risk for venous thromboembolism, and appropriate therapy is therefore indicated. Unfractionated heparin has been the treatment of choice in the short term, although many patients are untreated until an event occurs. Several factors influence the risk for thromboembolism in unrelated medical conditions such as cancer, serious infectious disease, stroke, trauma, chronic renal failure, complications of pregnancy, and others. Based on the evidence, it is possible to stratify risk into high, moderate, and low categories, which can then be used to prepare a treatment plan. High risk factors include stroke, advanced age (>70 years), congestive heart failure, shock, and a previous history of deep vein thrombosis (DVT) or pulmonary embolism. Moderate risk factors include thrombophilia (such as factor V Leiden and other inherited genes that may predispose a patient to thrombosis), immobilization of patients with active disease, and cardiac failure. Patients with minor medical illnesses have a low risk for venous thromboembolism. Several clinical trials have evaluated heparin or LMWHs in medical patients, but most studies were flawed because of inadequate size or inadequate methods for detection of venous thromboembolism, or because the event rate was too low when the patients enrolled in the trials were not sick enough. A few well-designed studies were performed that suggest that prophylaxis under some conditions is indicated. One of these studies enrolled cancer patients who had an indwelling subclavian venous catheter. Patients were randomized to dalteparin or placebo for the prevention of catheter-related upper extremity DVT. This study was terminated early when it was observed that only 6% of the patients treated with dalteparin developed DVT compared with 62% of the patients in the placebo group. In two other studies, enoxaparin was shown to provide greater benefit than heparin in the clinical outcome of patients with congestive heart failure who are confined to their beds and patients who have had a stroke.

Hans Klaus Breddin (International Institute of Thrombosis and Vascular Diseases, Germany) considered endpoint parameters in clinical trials of LMWHs in DVT. He asked whether phlebographical endpoints are relevant in clinical studies. The goal of anticoagulant therapy with LMWHs is to prevent clinically relevant pulmonary embolism and extension of existing DVT in patients who are at risk, but as many as 35 to 45% of patients may develop an asymptomatic or “silent” pulmonary embolism that can be detected only by phlebography and perfusion/ventilation lung scans. Asymptomatic DVT can also be evaluated in this manner. A few studies have already used this endpoint to evaluate the
efficacy of LMWH therapy. The CORTES clinical trial randomized patients with DVT confirmed by phlebography to one of the following three groups: 1) unfractionated heparin, 2) Clivarine® (repiriparin) once daily for 4 w, or 3) Clivarine® b.i.d. for 1 w plus oral anticoagulation. The endpoint was recurrence of thromboembolism within 90 d. The incidence of any clinically relevant or symptomatic events, DVT, pulmonary embolism, or both, was 6.4% in group 1, 3.5% in group 2, and 1.8% in group 3. As expected, the incidence of DVT or pulmonary embolism detected by phlebography was greater than the clinical event rate. Only 41% of patients in group 1 responded to therapy, defined as no evidence of new or recurrent thromboembolism detectable by phlebography, compared with 53.9% and 53.5% in groups 2 and 3, respectively. The outcome, however, was predictable since the incidence of clinical events and DVT or pulmonary embolism detected by phlebography were both greater in group 1 than in groups 2 and 3.

Russell Hull (Univ. of Calgary, Calgary, Alberta, Canada) presented the results of a clinical trial that enrolled patients with pulmonary embolism and underlying proximal DVT who were treated with LMWH. Pulmonary embolism is known to occur in 50% or more of patients with proximal DVT. Since LMWHs are effective in patients with DVT, Hull was interested in determining whether LMWHs would also be effective in preventing and treating acute submassive pulmonary embolism. This multicenter, double-blind trial enrolled 200 patients who were very ill with a variety of unrelated diseases (30% of patients had cancer). Ninety-seven patients were randomized to weight-adjusted, fixed-dose tinzaparin administered once daily and 103 patients to intravenous adjusted-dose heparin. The mortality rate was 8.7% and 6.2% in the heparin and tinzaparin groups, respectively. This difference was not significant, but the trial was small and some deaths could probably be attributed to the underlying disease. However, the incidence of recurrent venous thromboembolism was 6.7% in the heparin group and 0% in the tinzaparin group. This was a significant difference (P = 0.014).

Georg-Friedrich von Tempelhoff (City Hospital of Ruesselheim, Ruesselheim, Germany) reviewed the use of LMWHs during pregnancy. The incidence of venous thrombosis in the female population as a whole is 0.1 to 0.3%, but the incidence increases about sixfold in pregnant women. Physiological changes in pregnant women predispose women to venous thrombosis due to reduced blood flow to the periphery, elevated levels of a variety of coagulation factors and fibrinogen, reduced levels of protein S, and elevated levels of plasminogen activator inhibitors. The risk increases dramatically in women who have an inherited thrombophilia, such as protein C or protein S deficiencies, or carry the factor V Leiden mutation. Based on these and other risk factors, a treatment plan can be prepared for pregnant women stratified according to their cumulative risk factors. Heparin has been the anticoagulant of choice in the treatment of pregnant women, but LMWHs are far more convenient since they are administered once daily by subcutaneous injection and require no monitoring. von Tempelhoff concluded that the use of LMWHs in pregnancy is safe and efficacious.

Meyer Michele Samama (Hotel Dieu Hospital, Paris, France), a keynote speaker, reiterated the theme that LMWHs are “not created equal.” Samama reviewed differences in molecular structure and manufacturing processes and then focused on variation in anti-Xa/anti-IIa ratios and pharmacokinetics of different LMWHs. The anti-Xa/anti-IIa ratio is close to 3.0 for most commercially available LMWHs, but it can range from about 1.5 for tinzaparin to 8.0 for bemiparin. Interestingly, anti-IIa activity is cleared faster by
the body so that the anti-Xa/anti-IIa ratio actually increases with time. With enoxaparin and tinzaparin, for example, the ratio is 3.3 and 1.7 after injection, respectively, increasing to about 7.0 and 4.0 after about 12 h. Another important difference among LMWHs is their ability to release tissue factor pathway inhibitor (TFPI) into the blood despite similar administered doses with respect to anti-Xa activity. TFPI levels after 1 h were almost three times higher in patients treated with dalteparin than those treated with enoxaparin.

**Elliott M. Antman** (Brigham & Women’s Hospital, Boston, MA, USA) reviewed several clinical trials that evaluated the use of LMWHs in the treatment of acute coronary syndromes. Perhaps the first significant clinical trial with LMWHs was FRISC (Fragmin during Instability in Coronary Artery Disease), which showed that twice daily administration of Fragmin® (dalteparin) to patients with unstable coronary artery disease for up to 45 d produced a significant reduction in death, new myocardial infarction (MI), and the need for intravenous heparin or revascularization when compared with placebo. FRIC (Fragmin in Unstable Coronary Artery Disease) extended these findings by showing that Fragmin® was equivalent in efficacy to an active control, unfractionated heparin, in the treatment of the acute phase of unstable angina and non-Q-wave myocardial infarction. ESSENCE (Efficacy and Safety of Subcutaneous Enoxaparin in Non-Q-wave Coronary Events) directly compared unfractionated heparin with Lovenox® (enoxaparin) in patients with unstable coronary artery disease. The results showed that enoxaparin produced a greater than 16% reduction in the relative risk of death, MI, or recurrent ischemia when compared with adjusted-dose heparin. Antman remarked that the therapeutic role of LMWHs in acute coronary syndromes will be better defined after the results of two ongoing clinical trials are known. HART-II will compare a bolus injection of enoxaparin plus infusion with heparin in patients with an acute MI following thrombolysis, and ENTIRE (TIMI 23) will compare enoxaparin with heparin in patients with an acute MI treated by thrombolysis with tenecteplase, a new single-bolus thrombolytic agent, with or without adjunctive therapy with a platelet GPIIb/IIIa antagonist, ReoPro® (abciximab).

**Andrew Nicolaides** (St. Mary’s Hospital, London, U.K.) reviewed the use of LMWHs in the treatment of stroke. There is an increased risk for DVT and pulmonary embolism in patients who have had a stroke, and 3 to 5% of early deaths in these patients are due to pulmonary embolism. However, clinical trials in the 1980s that compared anticoagulant therapy with placebo in stroke patients produced contradictory results with respect to efficacy and mortality rates. Because of the perceived risk of intracranial hemorrhage, heparin and other anticoagulants did not become widely used in stroke patients. The International Stroke Trial (IST) finally showed that low dose heparin significantly reduced the incidence of pulmonary embolism in stroke patients from 0.8% to 0.5%, but the design of IST was flawed. Then the FISS (Fraxiparine® (nadroparin) for the Treatment of Acute Ischemic Stroke) trial compared Fraxiparine® administered for 10 d with placebo in patients with ischemic stroke confirmed by CT scan. After 6 mo, the incidence of death and dependency was 45% in the Fraxiparine® group and 65% in the placebo group, a significant difference. At least two other clinical trials of LMWHs in stroke patients, FISS bis with Fraxiparine® and TAIST with tinzaparin, are underway. One other clinical trial, TOAST (Trial of Orgaran® (danaparoid) in Acute Stroke Treatment), compared a 7-d course of intravenous Orgaran® with placebo in patients with ischemic stroke. Patients were categorized according to the type of stroke, and this trial showed that in patients with stroke due to atherosclerosis Orgaran® significantly increased the incidence of a “favor-
able” clinical outcome to 43.4% of patients from 29.1% of patients in the placebo group. There was no significant difference in clinical outcomes of patients with cardioembolism, small vessel stroke, or stroke from undetermined causes. The results suggest that LMWH and anticoagulants may provide clinical benefit to stroke patients, but because of the possibility of different responses depending on the type of ischemic stroke, future clinical trials should stratify patients according to this criterion in order to determine the optimal therapy for each kind of stroke.

Ajay Kakkar (Thrombosis Research Institute, Imperial College School of Medicine, London, U.K.) described the promising results of the use of heparin and LMWHs in prevention of DVT and pulmonary embolism in cancer patients. It is well known that cancer produces a hypercoagulable state that increases the risk for DVT and pulmonary embolism. Unfractionated heparin was shown to significantly reduce the incidence of pulmonary embolism in cancer patients as long ago as 1975. Since then, LMWHs and warfarin have been used to reduce the incidence of thromboembolism in cancer patients undergoing surgery or chemotherapy and in prophylaxis of venous catheter-associated thrombosis. More recently, it has been postulated that heparin may have intrinsic antitumor activity since heparin was shown to inhibit angiogenesis and thrombin and modulate apoptosis and oncogene expression. One study demonstrated that patients with small cell lung carcinoma respond better to chemotherapy if they are also treated with heparin. The mortality rate in these patients was also reduced if they were treated with a LMWH instead of heparin. To help determine definitively whether LMWH can reduce cancer mortality, a clinical trial enrolling 600 cancer patients with no underlying thrombosis has begun. This trial, called FAMOUS, will compare survival of cancer patients randomized to a LMWH or placebo. LMWH instead of heparin was chosen as the test agent in part because in this trial patients will be treated for 1 y.

**SYNTHETIC HEPARINS**

Jeanine Walenga (Loyola Univ. Medical Center, Maywood, IL, USA) described the development of synthetic pentasaccharides that are structurally based on the minimally active polysaccharide sequence of heparin. Synthetic pentasaccharides are selective factor Xa inhibitors and thereby inhibit thrombin generation without negatively modulating thrombin’s anticoagulant effects mediated by thrombomodulin and protein C. Synthetic pentasaccharides are also chemically homogeneous and have a very high bioavailability following intravenous or subcutaneous administration. A number of synthetic pentasaccharides have been developed and tested in preclinical studies. Several synthetic pentasaccharides, including SANORG 34006 (developed by Sanofi-Synthélabo in collaboration with Organon, the pharmaceutical subsidiary of Akzo Nobel N.V.), are potent factor Xa inhibitors at concentrations of 500 ng/mL. Unlike heparin, there is no danger of heparin-induced thrombocytopenia (HIT) with synthetic pentasaccharides and, in a variety of *in vitro* animal models of arterial, venous, and microvascular thrombosis, synthetic pentasaccharides have proven to be efficacious and safe.

Jean-Marc Herbert (Sanofi-Synthélabo, Toulouse, France) described the biochemical and pharmacological properties of SR123781, an oligosaccharide and heparin mimetic. A pentasaccharide domain with anti-Xa activity is linked to a pentasaccharide thrombin-binding domain with anti-IIa activity by a hexasaccharide linker in SR123781. The end
product is a linear oligosaccharide with 16 monosaccharide units (molecular weight = 4853) with both anti-Xa and anti-IIa activity. SR123781 requires antithrombin III for inhibition of thrombin and factor Xa, but it does not interact with heparin cofactor II. In addition, it does not bind to platelet factor 4, eliminating the risk for HIT. SR123781 exhibits 100% bioavailability after subcutaneous injection and has a half-life of about 10 h in humans. In a variety of animal models of arterial and venous thrombosis, SR123781 was more potent than heparin, selected LMWHs, and even synthetic pentasaccharides. No bleeding was observed at the drug’s active dose, suggesting that SR123781’s therapeutic index is excellent. Perhaps the major disadvantage of this drug is its complicated synthesis.

Scott D. Berkowitz (Duke Univ. Medical Center, Durham, NC, USA) described Phase II clinical trial results with orally available heparin. Emisphere Technologies, Inc. (Tarrytown, NY, USA), has discovered a delivery agent called sodium N-(8-[2-hydroxybenzoyl]amino)caprylate (SNAC) (molecular weight = 301) that enables gastrointestinal absorption of therapeutic amounts of unfractionated heparin. This combination, when administered orally in humans, increased APTT and induced the release of TFPI, just like intravenously administered heparin. The anti-Xa/anti-IIa ratios achieved were also similar to heparin. A Phase II dose-ranging trial was designed for patients undergoing total hip arthroplasty with no previous history of DVT. Two doses of SNAC with heparin were administered three times daily and compared with subcutaneous injections of heparin. There were no deaths, and the incidence of major bleeding events was the same in all groups (3.3% for the entire cohort). There was also a similar number of patients with confirmed venous thromboembolism in all three treatment groups. The results indicate that oral heparin is as efficacious and safe as subcutaneous heparin. A Phase III trial called PROTECT (Prophylaxis with Oral SNAC/Heparin against Thromboembolic Complications following Total Hip Replacement Surgery) is planned that will compare two doses of SNAC/heparin with a LMWH.

ANTITHROMBIN AND ANTI-Xa AGENTS

Gary C. Cupit (The Medicines Co., Cambridge, MA, USA) considered the advantages of bivalirudin, formerly called hirulog and now called Angiomax™, in primary coronary intervention. Angiomax™ is a peptidic direct thrombin inhibitor that is structurally based on hirudin, a naturally occurring thrombin inhibitor found in some species of leeches. Angiomax™ exhibits a very predictable dose-response following a bolus injection that is followed by infusion. It has a short half-life, which is an advantage since the time it takes to perform a coronary intervention procedure has been decreasing as devices are improved and cardiologists gain experience. In two heparin-controlled trials of Angiomax™ in patients undergoing primary coronary intervention, Angiomax™ proved to be superior to heparin in reducing the incidence of adverse outcomes (9.1% in the heparin group vs. 3.3% in the Angiomax™ group). The initial benefit was sustained at 6 mo. The risk for hemorrhage was also significantly decreased with Angiomax™ (9.3% vs. 3.7%). Two additional Phase III trials are underway with Angiomax™. CACHET (Comparison of Abciximab Complications with Hirulog Ischemic Events Trial) is enrolling patients undergoing elective percutaneous transluminal coronary angioplasty (PTCA) with or without stents to directly compare Angiomax™ with heparin in a more contemporary clinical setting.
setting with currently used heparin doses and abciximab (ReoPro®). HERO-2 (Hirulog Early Reperfusion/Occlusion-2) is a huge trial that will enroll about 17,000 patients with acute MI who will undergo thrombolysis with streptokinase (and aspirin). Angiomax™ will be directly compared with heparin as the anticoagulant regimen during thrombolysis. HERO-2 is sized to demonstrate a significant improvement in 30-d cumulative mortality in patients treated with Angiomax™.

Brigitte Kaiser (Friedrich Schiller Univ. Jena, Erfurt, Germany) discussed the rationale for the development of factor Xa inhibitors, including recent findings that have identified factor Xa to be a potent mitogen for vascular smooth muscle. Factor Xa occupies a central position in the coagulation cascade. Together with factor Va, calcium, and phospholipids, factor Xa is the protease that generates thrombin from prothrombin. Factor Xa also binds to intravascular thrombi and injured vascular walls to prolong thrombin generation. It also binds to a specific factor Xa receptor, the effector cell protease receptor-1 (EPR-1), which mediates factor Xa’s mitogenic activity. EPR-1 is expressed in cultured smooth muscle cells and smooth muscle cells harvested from the rabbit carotid artery after injury. Factor Xa inhibitors have been shown to reduce angiographic restenosis in a focal femoral atherosclerosis model in rabbits and to reduce neointimal proliferation in porcine coronary artery and rat carotid artery balloon injury models. A wide variety of factor Xa inhibitors are in development by many companies. Most factor Xa inhibitors are direct, small molecule, synthetic inhibitors, while others, such as pentasaccharide, are indirect inhibitors that require antithrombin III. Each one inhibits thrombin generation in a time- and dose-dependent manner in human whole blood activated by recombinant factor VIIa. In addition to animal models of restenosis, factor Xa inhibitors have demonstrated efficacy in animal models of arterial, coronary, and venous thrombosis; arteriovenous shunt thrombosis; disseminated intravascular coagulation; thrombolysis and reocclusion; and hemodialysis.

BIOTECHNOLOGY-DERIVED PRODUCTS AND ANTIBODIES

Bruce W. Grinnell (Eli Lilly & Co., Indianapolis, IN, USA) presented recent developments with recombinant human protein C. Native protein C is a vitamin K-dependent plasma serine protease activated by the thrombin-thrombomodulin complex on the surface of endothelial cells. Activated protein C is a potent inhibitor of thrombin formation since it inactivates factors Va and VIIIa. It is also profibrinolytic as it neutralizes plasminogen activator inhibitor-1 (PAI-1) and inhibits thrombin-activated fibrinolysis inhibitor (TAFI). Lilly has developed a human kidney 293 cell line that can produce recombinant human activated protein C (rhAPC; LY203638). Lilly has also modified the primary sequence of protein C in order to improve the recombinant protein’s pharmacokinetics and activity, advancing the recombinant version of the native protein into clinical trials. In a variety of animal models of arterial and venous thrombosis, rhAPC is effective, but it was particularly effective in preventing death in models of sepsis. Subsequently, Lilly has advanced rhAPC into Phase III clinical trials of sepsis, which are currently ongoing. In an earlier Phase II clinical trial, the 28-d mortality in patients with sepsis was 34% in placebo-treated patients and 35% and 21% in patients treated with a low and high dose of rhAPC, respectively. Though the trend suggested that high dose rhAPC could reduce mortality, the difference was not significant since this trial did not have sufficient power to detect a...
difference in mortality. The generation of antiinflammatory markers indicative of sepsis was suppressed in the rhAPC-treated patients.

**Mitsunobu Mohri** (Asahi Chemical Industry Co., Ltd., Tagata, Shizuoka, Japan) described his work with recombinant human soluble thrombomodulin (ART-123). ART-123 is a recombinant version of the entire extracellular domain of human thrombomodulin. It is a potent activator of protein C and therefore, indirectly, it is a potent inhibitor of thrombin \( K_i = 22 \text{ nmol} \) and factor Va \( \text{IC}_{50} = 0.052 \text{ } \mu\text{g/mL} \). ART-123 is also a safe drug that has little effect on APTT at therapeutic plasma levels. The ratio of the concentration required to double APTT to the concentration that inhibits thrombin generation by 50% is 530. In comparison, this ratio is 37 for APC, 14 for dalteparin, 11 for heparin, 2.9 for hirudin, and 2.2 for argatroban. ART-123 is an effective and safe anticoagulant in a variety of animal models. It also exhibits very high bioavailability (nearly 100%) and a long half-life (16 to 28 h) after subcutaneous injection.

**Giuseppe Cella** (Padova Univ. Medical School, Padova, Italy) and **Roberto Porta** (CRINOS Pharmaceuticals SpA, Villa Guardia, Italy) presented an update on the pharmacological activity of defibrotide, a polynucleotide derived from porcine DNA. Defibrotide is a heterogeneous mixture of polynucleotides of 15 to 30 kDa that has anticoagulant, antiinflammatory, antiischemic, and antiatherosclerotic activities due to its highly polyanionic structure. Specific nucleotide sequences within the heterogeneous mixture are believed responsible for defibrotide’s diverse activities. Two minimal nucleotide sequences (aptamers) with potent thrombin inhibitory activity have been discovered. They are the following:

\[
5'\text{--GGTTGG--ATT--GGTTGG--3'}
\]

and

\[
5'\text{--GGTTGG--ATC--GGTTGG--3'}
\]

Both aptamers were potent inhibitors of platelet aggregation and thromboxane formation as well as thrombin-induced cytosolic calcium mobilization. Defibrotide’s anticoagulant and antiplatelet activities could also be attributed, at least in part, to an aptamer that was found to be an inhibitor of cathepsin G, a protease that is also a platelet agonist. This aptamer has the sequence:

\[
5'\text{--GGTCGAGGAGCCTAGCAGCT--GGTTGGCGTGGTGAGG--3'}
\]

Inhibition of cathepsin G-mediated platelet aggregation is observed at concentrations less than 100 \( \mu\text{mol} \).

**Giora Z. Feuerstein** (DuPont Pharmaceuticals Co., Wilmington, DE, USA) described the anticoagulant properties of an anti-factor IX/IXa monoclonal antibody developed while Feuerstein was at SmithKline Beecham Pharmaceuticals (King of Prussia, PA, USA). This antibody, called BC2 and given the code number SB249415, has a high affinity \( K_d = 10 \text{ nmol} \) for the Gla domain of factor IX. BC2 was evaluated in a rat model of venous thrombosis. In this model, a venous thrombus is formed in a “vena cava sac” with a nonocclusive stenosis created by ligation over a needle that is subsequently withdrawn. A single bolus intravenous injection of BC2 reduced thrombus mass four- to ninefold with virtually no increase in activated partial thromboplastin time (APTT) (maxi-
mal APTT was 85 to 90 s). At comparable anticoagulant efficacy, heparin increased APTT two- to 17-fold (up to 730 s). BC2 was also effective in a rat carotid artery thrombosis model. In this model, BC2 exhibited marked synergy with aspirin. At concentrations that by themselves caused no reduction in thrombus weight, BC2 with aspirin completely inhibited thrombus formation without a significant increase in APTT. BC2 also prevented platelet deposition in endarterectomized aorta and Dacron vascular grafts. In a thrombolysis model, BC2 was more effective than heparin in accelerating reperfusion induced by t-PA, and it was more effective in preventing reclosure after 60 min. The data suggest that inhibition of factor IXa may provide clinically relevant anticoagulation with a much lower risk for bleeding than heparin. SmithKline Beecham has developed a humanized Fab fragment that retained anticoagulant activity and is proceeding with clinical development.

**THROMBOLYTIC DRUGS**

**Victor Gurewich** (Beth Israel Deaconess Medical Center, Boston, MA, USA) presented his work with a mutant version of recombinant prourokinase (pro-UK). Experiments in gene knockout mice indicate that the dominant physiological fibrinolytic agent is pro-UK, not t-PA. Pro-UK is a zymogen in blood and binds tightly to platelets by specific receptors. Fibrin specificity is derived from pro-UK’s selective activation of plasminogen bound only to C-terminal lysines on the E-domain of fibrin. Pro-UK is then locally activated to urokinase (UK), resulting in local amplification of fibrinolysis. Once converted to UK, all of the advantages of pro-UK are lost. UK is a fully active protease with no fibrin specificity that does not bind to platelets. Unfortunately, infusion of pro-UK leads to rapid conversion to UK, greatly limiting its utility as a thrombolytic agent because of compromised efficacy and safety. Gurewich developed a mutant version of pro-UK that has a single amino acid substitution, a lysine to histidine at position 300. This mutant pro-UK, called M5, is much more stable in plasma and retains all of the advantages of pro-UK. M5 produced more rapid fibrin-specific thrombolysis of clotted human blood *in vitro* than did pro-UK or t-PA. Similarly, M5 was more effective than pro-UK or t-PA in an *in vivo* thrombolysis model in dogs. Potential bleeding complications were evaluated by making standard incisions in these dogs and measuring blood loss through the cut. There was no difference in total blood loss with M5 and placebo, while pro-UK and t-PA resulted in significantly greater blood loss. The safety of M5 is probably derived from a difference in the fibrin composition of hemostatic or “good” thrombi and occlusive or “bad” thrombi. Hemostatic thrombi are believed to be composed of largely intact fibrin, while occlusive thrombi contain partially degraded fibrin. The former has internal lysines that bind plasminogen, while the latter also contains C-terminal lysines that also bind plasminogen. Plasminogen bound to internal lysines can be activated by t-PA and UK but not by pro-UK or M5. Plasminogen bound to C-terminal lysines is the only fibrin-bound plasminogen that can be activated by pro-UK or M5; thus, only occlusive thrombi are susceptible to lysis by M5.

**Charles W. Francis** (Univ. of Rochester, Rochester, NY, USA) described how 40 kHz ultrasound accelerates thrombolysis and improves tissue perfusion in a rabbit femoral artery thrombosis model. Previous studies have shown that ultrasound accelerates fibrinolysis with all available thrombolytic agents in an intensity-dependent fashion. The higher the intensity, the faster the reperfusion. The application of ultrasound has three
Principal effects: 1) it improves drug transport through the fibrin clot, enabling greater penetration of plasminogen activator into the clot; 2) it increases the binding of t-PA to fibrin so that more t-PA gets into a clot; and 3) it reversibly disaggregates fibrin into a larger number of thinner strands. Ultrasound, however, produces heat, and the higher the frequency, the more heat is created, increasing the chance of thermal injury to tissues. Ultrasound at lower frequencies also penetrates tissues better, and studies have shown that ultrasound applied at 40 kHz enhances thrombolysis more than does ultrasound at 1 MHz. Francis used a rabbit femoral artery occlusion model of reperfusion to examine the effects of 40 kHz ultrasound. He confirmed that 40 kHz ultrasound accelerates fibrinolysis more than does 1 MHz and that equivalent fibrinolysis can be attained with much less power and less generated heat. Interestingly, ultrasound by itself, with no added plasminogen activator, increased tissue capillary perfusion and reversed acidosis induced by ischemia. Thus, ultrasound may have beneficial effects in addition to accelerating fibrinolysis by plasminogen activators.

Randall W. Moreadith (ThromboGene, Ltd., Chapel Hill, NC, USA) described recent studies with recombinant staphylokinase, a new thrombolytic agent derived from bacteria. The fibrinolytic properties of a $M_r$ 15,500 protein secreted from *Staphylococcus aureus* were first described in 1948. The protein, called staphylokinase or Sak, was cloned and characterized in the 1980s. One reason for this prolonged delay was that in early studies in dogs the administration of Sak caused hemorrhagic diathesis and death. In the late 1980s, the biochemistry of this agent was reinvestigated and its remarkable fibrin specificity was discovered. It was learned that plasmin forms a 1:1 complex with native, inactive Sak and then cleaves the first 10 amino acids to yield an active enzyme. Thus, Sak is actually a prodrug. Importantly, the plasmin:Sak complex has a high affinity for plasmin and plasminogen at the fibrin surface and to antiplasmin in the fluid phase. As soon as the plasmin:Sak complex leaves the fibrin surface, it is inactivated by antiplasmin. Consequently, there is no systemic depletion of fibrinogen or plasminogen. In early, small clinical trials, recombinant Sak was shown to be equivalent to front-loaded or accelerated recombinant t-PA in inducing reperfusion. The number of patients that achieved TIMI grade 3 flow was actually greater with recombinant Sak. However, the age of single-bolus administration of a thrombolytic agent is fast approaching and, to keep up with the state-of-the-art, Sak was modified for a longer half-life to enable single-bolus dosing. Since Sak lacks a cysteine, ThromboGene engineered a cysteine into position 3 and attached polyethylene glycol (PEG) to the cysteine, producing PEGylated Sak. This changed the pattern and half-life of elimination of the protein. Native Sak exhibits monophasic elimination with a half-life of about 3 min. PEGylated Sak has biphasic elimination, with an alpha and beta half-life of 15 and 40 min, respectively. The thrombolytic activity was not dramatically altered by this modification. A pilot clinical trial of PEGylated Sak is currently underway.