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RECENT DEVELOPMENTS IN ANTITHROMBOTIC THERAPY

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ABSTRACT

Recently, many newer developments in the clinical management of thrombotic and cardiovascular disorders have occurred. In particular, several newer approaches for the prophylactic and therapeutic management of such disorders as venous thrombosis, stroke, acute myocardial infarction and prevention of post-interventional coronary and reocclusive syndromes have been introduced. These have only been possible due to the understanding of the molecular mechanisms involved in the thrombogenic process which play a pivotal role in the pathophysiology of thrombotic and cardiovascular disorders.

With the increased knowledge of the pathophysiology of thrombogenesis have come advances in drug treatment possibilities. Advances in biotechnology and separation techniques have contributed to the development of many newer antithrombotic, anticoagulant and thrombolytic drugs. Many new drugs and devices based on newer concepts are currently being tested in various clinical trials. Hirudin, hirulog, synthetic and recombinant GpIIb/IIIa targeting drugs and tissue factor pathway inhibitor (TFPI), are some examples.

From these current developments, it can be appreciated that antithrombotic drugs represent a wide spectrum of natural, synthetic, semisynthetic and biotechnology produced agents with marked differences in chemical composition, physicochemical properties, biochemical actions and pharmacologic effects. The use of physical means to treat thrombotic disorders and advanced means of drug delivery add to the expanding nature of treatment.

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Besides the development of new antithrombotic and anticoagulant drugs, a better understanding of the conventional anticoagulant and antithrombotic drugs such as heparin, warfarin and aspirin has led to the newer applications for these drugs. Furthermore, prophylactic and treatment protocols are now optimized using these conventional drugs. A renowned interest is clearly seen to develop an optimal approach for these agents.

The endogenous actions of the antithrombotic drugs are quite complex. It is no longer valid to assume that an antithrombotic drug must produce an anticoagulant action in blood as do the classical heparin and oral anticoagulants. Many of these new drugs do not produce any alteration of currently measurable blood clotting parameters, yet they are effective therapeutic agents because of their interactions with the elements of the blood and the vasculature. Another perspective is that several of these agents require endogenous transformation to become active products. Therefore, it becomes important to rely on the pharmacodynamic actions of these agents rather than on other in vitro characteristics to assess potency or efficacy. Hematologic and vascular modulation play a key role in the mediation of the antithrombotic actions of these drugs involving red cells, white cells, platelets, endothelial cells and blood proteins. Thus, an optimal antithrombotic drug/approach will include the targeting of all possible sites involved in thrombogenesis. Polytherapeutic approaches utilizing combinations of drugs may turn to be the most effective in the management of thrombotic disorders. A more optimal use of conventional antithrombotic drugs in monotherapeutic and polytherapeutic approaches will expand their use in the management of thrombotic and cardiovascular indications.

1. OVERVIEW

The newer developments in antithrombotic drugs are rather significant. Many advanced techniques to develop antithrombotic drugs are used at the present time. Advances in biotechnology and separation techniques have also contributed to the development of newer antithrombotic drugs [1-8]. These drugs may prove to have a better efficacy in the control of thrombogenesis and its treatment. Drugs and devices which have been or are being developed based on newer concepts can be classified into the following groups as listed below. The drugs marked with an asterisk may be useful in cardiovascular indications as their safety/efficacy index is claimed to be higher than conventional anticoagulants.

I. Heparin Related Drugs

1. *Low Molecular Weight Heparins.
2. *Medium Molecular Weight Heparins.
3. *High Molecular Weight Heparins.
4. Chemically Modified Heparins.
5. Dermatans.
6. *Heparans.
7. Semisynthetic Heparin Derivatives (Suleparoide).
8. Chemically Synthesized Antithrombotic Oligosaccharides.
9. Sulfated Dextrans.
10. Synthetic Hypersulfated Compounds.
11. Polyanionic Agents.
12. Marine Polysaccharides.
13. Heparinoids (Lomoparan).
14. Biotechnology derived heparin homologues.

II. Antiplatelet Drugs

1. *(Ticlopidine) and Related Antiplatelet Drugs.
2. *Pletaal and Related Phosphodiesterase Inhibitors.
3. Iloprost and other Prostanoid Modulators.
4. Eicosanoids and Related Drugs.
5. ω-3 Fatty Acids and Fish Oil Related Products.
6. Synthetic Peptides and Peptidomimetic Targeting Membrane Glycoproteins.
7. Peptides and Proteins Modulating Platelet Function.

III. Endothelial Lining Modulators

1. *Nucleic Acid Derivatives (Defibrotide and Aptamers).
2. Sulfomucopolysaccharide Mixtures.
3. DDAVP and Related Peptides.
4. Growth Factor Related Peptides.
5. Protein Digests.
6. Vitamins.

IV. Viscosity Modulators

1. Synthetic and Natural Polymers.
2. *Pentoxifylline.
3. Polyelectrolytes.
4. Venoms Defibrinating agents (Ancrod).

V. Biotechnology Based Proteins

1. T-PA and Mutants.
2. *Hirudin, Mutants and Fragments.

3. Protein C and Activated Protein C
4. Thrombomodulin.
5. Blood Vessel Derived Antithrombotic Drugs
6. Antithrombin III (AT).
7. Antithrombin III-Heparin Complex.
8. Recombinant Heparin Cofactor II.
9. Glycoprotein Targeting Proteins.
10. Protease Specific Inhibitors.
11. Recombinant Tissue Factor Pathway Inhibitor and Variants

VI. Peptides and Related Antithrombotic Peptides

1. *Hirulogs.
2. Efegatran
3. Argatroban.
4. Inogatran
5. Napsagatran
6. Borohydride Derivatives.

VII. Optimized Polytherapeutic Approaches

1. Heparin/Antiplatelet Drugs.
2. Coumadin/Antiplatelet Drugs.
3. Aspirin/Ticlopidine
4. Thrombolytic Agents/Heparin.
5. Thrombolytic Agents/Antiplatelet Drugs.
6. Thrombolytic Agents/Hirudin and Other Thrombin Inhibitors.
7. Recombinant Drug Conjugates.

8. Hirudin/Antithrombotic Agents.

9. Hirudin/Glycoprotein Targeting Antibodies.

VIII. Newer Drug Delivery Systems and Formulations

1. Oral.

2. Ointments.

3. Transdermal.

4. Sustained Release.

5. Transdermal.

6. Target Specific Antithrombotic Drugs (Antibody Directed).

7. Catheters and Devices Capable of Targeted Drug Delivery.

IX. Non-Thrombotic/Antithrombotic Biomaterials

1. Stents and Related Implantable Devices

2. Artificial Valves

3. Dialyzers

It can be appreciated from the above survey that antithrombotic drugs represent a wide spectrum of natural, synthetic, semi-synthetic and biotechnology produced agents with marked differences in chemical composition, physicochemical properties, biochemical actions and pharmacologic effects. Figure 1 depicts the wide heterogenicity in the structure of various natural, synthetic and recombinant antithrombotic agents. In addition, each of these drugs also exhibits wide degrees of functional heterogenicity. The use of physical means to treat thrombotic disorders, and advanced means of drug delivery, add to the expanding nature of this area.

The endogenous actions of the antithrombotic drugs are remarkably complex. It is no longer valid to assume that an antithrombotic drug must produce an anticoagulant action in blood as the conventional heparin and oral anticoagulants. Many of the drugs listed in various categories do not produce any alteration of blood clotting

parameters, yet they are effective therapeutic agents because of their interactions with the various elements of the vasculature and other blood components. Another perspective is that several of these agents require endogenous transformation to become active products. Therefore, it becomes important to rely on the pharmacodynamic actions of these agents rather than on their in vitro characteristics to assess potency or efficacy of the product. Hematologic modulation plays a key role in the mediation of the antithrombotic actions of these drugs involving red cells, white cells, platelets and blood proteins. This is particularly true for the case of trauma induced thrombotic disorders where multiple processes are involved in thrombogenesis.

2. HEPARIN AND LOW MOLECULAR WEIGHT HEPARIN.

2.1. Newer Applications of Unfractionated Heparin.

The unfractionated heparin is primarily used as an anticoagulant for both the therapeutic and surgical indications. Usually beef lung and porcine mucosal derived products are available for these indications. The unfractionated heparins have been recently used for the following additional therapeutic uses:

1. management of unstable angina
2. adjunct to chemotherapeutic agents
3. adjunct to anti-inflammatory drugs
4. modulatory agent for growth factors
5. treatment of hemodynamic disorders

The unfractionated heparins are heterogeneous in both of their chemical and functional properties. Based on the origin, the molecular weight of these anticoagulant drugs varies. The beef lung heparin preparations are usually of higher molecular weight, in contrast to the porcine mucosal heparin. However, the charge density and other characteristics, such as the serpin affinity is similar for both preparations. Several newer pharmacological effects, independent of the anticoagulant effects, have been described. These include glycosylation mediated functional modulation of macromolecules, growth factor modulation, release of endothelial products and interactions with various endogenous sites. More recently some of the components

of heparin are also known to modulate platelet functions by altering the P selection behavior on platelets.

Chemical modification of the unfractionated heparin, such as desulfation, deamination and coupling with various agents have resulted in products of non-anticoagulant nature with selective actions on enzymes and cellular receptors. More recently, heparin coated stents and other biomedical devices have also become available. These heparin derivatives are currently tested for such indications as sepsis, viral infections and the treatment of proliferative disorders.

2.2. The Development of Low Molecular Weight Heparins:

The development of low molecular weight heparin (LMWH) has added a new dimension to the clinical management of thrombotic disorders. These agents have revolutionized the prophylaxis of post-surgical thrombosis [9-11]. More recently these drugs have been used for the management of various thrombotic disorders associated with cardiovascular drugs. In particular, their relative effects on platelets are minimal in comparison to heparin. Thus, these agents are of value in platelet compromised patients. In addition to the low molecular weight heparins, several other agents such as the chemically synthesized analogues of heparin, non-heparin glycosaminoglycans such as dermatan sulfate, heparan sulfate and various other antithrombotic agents have also become available for the management of thrombotic disorders. In the US, however, most of these agents are in clinical trials and not yet available for general use in patients.

For the past few decades, heparin has been widely used for the prevention of post-operative thromboembolism [12-14]. However, there are several adverse side-effects associated with the use of heparin such as bleeding, heparin induced thrombocytopenia, heparin induced thrombosis [15,16] and osteoporosis [17]. In addition, the regimen of prophylactic heparin used in the prevention of deep venous thrombosis (DVT) is tedious, requiring 2 to 3 daily injections because of the limited bioavailability and short half-life of heparin when administered subcutaneously.

The many clinical problems associated with heparin led several investigators to study the structure of heparin and to identify the active component(s) of this agent. The observation that only low molecular weight heparins are absorbed after subcutaneous administration has led to the development of LMWHs. The bioavailability of LMWH component is much greater than the unfractionated heparin. Experimental studies revealed the first LMWH had a subcutaneous bioavailability of about 90% compared to 15 to 25% for unfractionated heparin (UFH) [18]. Furthermore, these agents exhibit a much longer biologic half-life in contrast to heparin [5]. Thus, LMWH preparations could be administered with a single daily injection making them easy to administer as a prophylactic agent. Eight LMWHs have already been approved for the prophylaxis of thrombosis in the European community. Several of these LMWHs are currently under clinical trials in the US. Enoxaparin® (Rhone Poulenc Rorer, Collegeville, PA USA) was approved in 1993 for use in post-orthopedic surgery prophylaxis of DVT. Another product, Fragmin (Pharmacia Upjohn, Daton, OH USA), was recently approved for prophylaxis of DVT in general surgery. Off label use of both products was tried by physicians in various areas including cardiovascular, hematologic and oncologic areas.

Low molecular weight components in unfractionated heparins have been identified long before the development of clinically effective agents. However, because of technologic problems, they were not made available for clinical use. The very first LMWH was obtained by a fractionation method [19]. However, this method only yielded a limited amount of the active product and was cost prohibitive. During the past decade, different chemical and enzymatic processes have been developed to obtain LMWH from the parent unfractionated heparin (UFH). The depolymerization process resulting in the formation of lower molecular weight heparins and heparin oligosaccharide is shown in Figure 2. Chemical, enzymatic, rediochemical and fractionation methods have been used for the production of these agents.

Table I lists some of the commercially available LMWHs. Fraxiparin was originally obtained by a fractionation method. However, it is now obtained by an optimized nitrous acid depolymerization [20]. Both products are currently available in the European market. Lovenox is obtained by benzylation followed by alkaline

depolymerization (ß-elimination). Large amounts of sodium bisulfite are added to prevent oxidation of the terminal groups, since Enoxaparine contains a double-bond at the reducing end [1]. A recent formulation does not contain any sodium bisulfate and the new formulation is claimed to exhibit pharmacological effects similar to the original product [7,8]. Fragmin is obtained by nitrous acid depolymerization followed by ion exchange chromatography. It markedly differs from the other LMWHs in physicochemical characteristics and pharmacologic profile [1]. Fluxum is obtained from beef mucosa by a peroxidative depolymerization process. This agent has been found to mediate some of its actions through non-AT III-mediated pathways [5]. Ardeparin (Wyeth), a product prepared by peroxidative digestion procedures by Hepar (KabiVitrum), has been developed by Wyeth laboratories for clinical use. The Novo LMWH (Logiparin) is prepared by enzymatic digestion using Flavobacterium heparinicum heparinase. This drug also contains large amounts of sodium bisulfite as an antioxidant [5]. Innohep® is also prepared by a heparinase digestion method and is an identical product to Logiparin. More recently, LMWHs are also being prepared by γ-radiation mediated cleavage. These products are claimed to retain their internal structure and exhibit similar AT III binding behavior as native heparin.

Embolex NM is a LMWH that is prepared by isoamyl nitrite digestion and is supplemented with dihydroergotamine. A monosubstance which does not contain dihydroergotamine has also been introduced. Reviparin (Clivarin) is a product of Knoll Laboratories (Ludwigshafen, Germany) which is also prepared by nitrous acid digestion. Bioparin is a LMWH from Bioiberica (Barcelona, Spain) and is prepared by a ß-elimination method. Boxol is a product of Rovi Pharmaceutical (Madrid, Spain) and is prepared by either ß-elimination or nitrous acid digestion method. Marked differences in the two different batches of this product have been observed. Miniparin is a product of Syntex Argentina that is prepared by a nitrous acid digestion method. Recent studies have shown that the individual LMWHs obtained from each process exhibit chemical and pharmacological differences [21-25]. Regulatory bodies such as the US FDA consider each LMWH preparation as a distinct drug. While the differences among these agents may not be obvious in the prophylactic studies, marked differences can be expected in clinical trials where these agents are used at higher dosesages.

2.3. Low Molecular Weight Heparins in the Management of Thrombosis

In the past, LMWHs have been used for the post-surgical prophylaxis of DVT. However, these agents are now also used in the treatment of pre-existing events utilizing both the subcutaneous [14] and intravenous (Breddin, personal communication) routes of administration.

Many randomized studies have compared LMWHs with UFH in patients undergoing abdominal surgery. In two studies a significant decrease in DVT was observed in patients receiving LMWHs compared with UFH [26-29]. There was no statistical difference in efficacy observed between the two treatments in the other studies [30-36,12]. In most studies there was no significant difference between the observed bleeding effects of the LMWHs and UFH. In all of these studies, LMWH was given as a single daily dose while UFH was administered 2 or 3 times daily. The results of these studies suggest that LMWHs are effective and well tolerated in patients undergoing general surgery. The advantage of LMWHs over heparin was primarily in the reduced number of injections per day.

The most extensively studied method of prophylaxis of DVT in orthopedic patients in recent years has been with LMWH. Evidence that this treatment is both effective and safe is quickly accumulating. Many randomized trials using venography or ^{125}I fibrinogen uptake as the endpoint have compared the efficacy of LMWHs in this indication with several standard protocols. Although the different results obtained in these studies is probably due to the different dosages selected for standard drugs as well as the difference in the dosages and composition of the different LMWHs, these agents have exhibited comparable or better efficacy.

Many surgeons and physicians around the world now appreciate that a large number of cases of venous thromboembolism, particularly those following surgery, can be avoided by correct use of prophylaxis. With additional education and with the introduction of newer and more efficacious agents, it should become routine

for all patients undergoing surgery to be assessed for their risk of venous thrombosis and protected accordingly.

Of the many drugs used as prophylactic antithrombotic agents, heparin has a long history as therapy for both DVT and pulmonary embolism (PE). Many studies have shown that in moderate and high risk patients, heparin can prevent postoperative DVT and PE [9,37-43]. Now, with the introduction of LMWHs, these benefits can be had together with easier dosing and potentially less risk of bleeding.

Many surgeons still, however, harbor fears and doubts about using thromboprophylaxis. One of the most common fears is that of bleeding. There are still some surgeons who are not convinced that the benefits of prophylaxis outweigh the risks.

During the 1980's there have been great advances in our understanding of how heparin-like compounds work. It is known that LMWHs are less anticoagulant (in vitro clot inhibiting) in their actions than heparin but retain their antithrombotic (in vivo thrombosis inhibiting) potential [44]. This makes the potential risk of bleeding less with LMWHs than with heparin when properly used particularly with patient adjusted dosages. However, minor wound bleeding after a successful operation is preferable to death caused by a PE. An updated account of the cost, safety and efficacy issues related to the prophylactic and therapeutic use of LMWHs has been published recently (45,46).

In the US, post-surgical and medical thromboembolic disorders affect over one-million Americans yearly requiring hospitalization. Approximately 10% of these individuals develop serious PE. Due to this problem, the US National Institutes of Health called a consensus meeting in March 1986 to discuss the magnitude of thromboembolic disorders and the need for prophylactic therapy.

The consensus conference was effective in identifying the magnitude of medical and post-surgical

thromboembolic disorders and made a strong recommendation to use prophylactic measures. Although the consensus meeting reviewed various pharmacologic and physical methods for the prophylaxis of thromboembolic disease, it specifically discussed the use of low dose heparin therapy for prophylaxis and made the following recommendations:

1. Low dose heparin can be used for the prophylaxis of DVT in general surgical patients (patients with medium risk).
2. Individualized dosages of low dose heparin should be given to high risk patients (trauma and orthopedic surgery).
3. None of the available (1986) prophylactic regimens were considered optimal.

At the time the initial recommendations were made, only one LMWH was commercially available in France and very limited information was available in the US on these new drugs. Subsequent consensus companies of the American College of Chest Physicians in 1989, 1992 and 1995 have endorsed the use of LMWHs in various prophylactic indications. In addition, other organizations such as the International Union of Angiology have also endorsed these agents for similar indications. Today the LMWHs are commonly used in many European countries for the prophylaxis of thromboembolic disorders in both surgical and medical patients. Several LMWHs are currently being evaluated in Phase II and Phase III trials in the US and two products are currently approved. The subsequent consensus statements have endorsed the use of LMWHs in various indications.

Available clinical data suggests that LMWHs can be safely substituted for low-dose heparin. To validate this, several comparative trials throughout the world on low dose heparin and LMWHs are in progress. In several European clinical trials, the efficacy of LMWHs in the prevention of post-surgical DVT has now been proven, and these agents are considered to be the drug of choice for this indication [26,33,48,49]. In a recent review, Hirsh has discussed the use of LMWHs for therapeutic indications [46].

When used for prophylactic treatment (subcutaneous), most LMWHs mediate their actions in a similar manner; however, their efficacy and tolerability profiles differ markedly as discussed above, and the recommended dosages for the various products differ. Because of the differences between products, such practices as standardization by a single in vitro assay and assignment of a single INN designation are deemed invalid for LMWHs as a group. The individualized agent approach to all LMWHs has recently been adopted by the World Health Organization (WHO), US Food and Drug Administration (FDA) and the Scientific and Standardization Subcommittee of the International Society of Thrombosis and Hemostasis (ISTH). The recognition of the individuality of each of the LMWHs is extremely important to avoid excessively high or low doses of a product. Dose finding studies are essential and will have a major impact on the prophylactic and therapeutic acceptance of LMWHs.

Having satisfactorily passed their first step in clinical use, LMWHs are now being applied to other clinical situations. LMWHs are moving into the area of established DVT and therapeutic treatment of thrombosis. LMWHs may be therapeutic alternatives to heparin in some, but not all patients who develop a sensitivity to heparin or who develop heparin-induced thrombocytopenia. At optimal dosages, these agents produce their antithrombotic effects, but whether they have fewer adverse effects than standard heparin may vary with the product and is unproven for many of them. LMWHs have proven to be equally effective as heparin in general surgery and orthopedic surgery [28,32,33,43,50].

Based on data from several studies, it is proposed that in addition to their antithrombotic effects, the LMWH mediated profibrinolytic effects may be responsible for the therapeutic actions of these agents [8,51,52]. However, this claim requires verification in well-designed experimental and clinical studies.

More recently, LMWHs are being used for specific indications such as percutaneous transluminal coronary angioplasty (PTCA), as adjuncts to thrombolytic agents in disseminated intravascular coagulation (DIC) and the hypercoagulable state. A recent study has reported on the inhibition of cellular proliferation after

experimental balloon angioplasty by LMWH in rabbits [53]. LMWHs are currently evaluated for their efficacy in such cardiovascular indications as unstable angina, adjunct drugs for the management of acute myocardial infarction, coronary stenting and atrial fibrilation. It is expected that these agents will find an expanded use in cardiovascular areas.

3. CURRENTLY USED ANTITHROMBOTIC AGENTS

For the prophylaxis of medical and surgical thromboembolic disorders, several pharmacological means other than heparin and certain physical methods are currently being used clinically. Oral anticoagulants are often used for the prophylaxis of thrombosis. Patient compliance is generally good, as one oral dosage is sufficient for the daily prophylaxis of thrombotic complications; however, the need for laboratory monitoring and dosage adjustment are drawbacks to this mode of therapy. With optimal monitoring using the INR, newer trials on the oral anticoagulants are in progress at this time. Dextrans are generally administered intravenously. However, prolonged usage of dextran often results in hypervolemia, bleeding and platelet dysfunction. While aspirin is useful for prophylaxis of arterial thrombosis, it is of questionable value in the prophylaxis of venous thrombosis. Furthermore, it may cause bleeding or gastric ulcers. More recently, sequential compression devices have been used for the prophylaxis of postsurgical thrombosis. Several advantages of these mechanical devices include minor or no adverse effects, no pharmacological manipulation and activation of the patient's own physiological systems. However, these devices are bulky, patient compliance is not as high as desired and there is questionable efficacy in high risk patients. While aspirin is not commonly used for the treatment of venous thromboembolism, it is widely used in the management of arterial thrombosis and cardiovascular disorders associated therapeutic indications. Aspirin in combination with oral anticoagulants and other antiplatelet agents such as ticlopidine is proven to be very useful.

4. NEWER HEPARINOMIMETIC DRUGS

4.1. Heparin Related and Other Antithrombotic Agents

Beside the above clinically used approaches, several newer drugs to prevent thromboembolic disorders have

been or are being developed. A list of some of these newer drugs is given in Table V. Most of these drugs are in their early phases of development and it will take some time before their clinical efficacy is proven.

A very-LMWH fraction (CY 222) has been developed for clinical trials. This agent produces its effects by multiple mechanisms. Pentasaccharide is a synthetic material whose structure is based on the critical binding region of heparin to AT. It functions by inhibiting activated factor X. These agents do not impair primary hemostasis and they exhibit a high bioavailability. They do not exhibit any anticoagulant effect at antithrombotic dosages. Several other similar lower LMWHs are also being developed from different LMWH.

While the dermatan sulfates and heparan sulfates are under development for the prophylaxis of venous thromboembolism, the clinical results with these agents are somewhat disappointing. Furthermore, relatively longer dose and other safety considerations have retarded their development.

A synthetic hypersulfated lactobionic acid amide (Aprosulate) has been developed for prophylactic antithrombotic use. This agent produces its action via heparin cofactor II and by inhibiting protease generation. The bioavailability of this agent was better than that of dermatan and heparan sulfates. However, due to certain safety issues, its development was stopped.

Many other glycosaminoglycans are being developed for the prophylaxis of thromboembolism. Some of these represent mixtures of glycosaminoglycans with varying molecular weight profiles. Noteworthy are Lomoparan and Suleparoide which are depolymerized heparan preparations. These agents exert their antithrombotic actions via unknown mechanisms; however, these are clinically very effective drugs. Other agents, such as Hemoclar and Arteparon, are sulfated polymers of natural origin with antithrombotic activities. While these agents have been in existence for many years, the data on the prophylactic antithrombotic actions is not clearly known at this time.

4.2. Non-Heparin Glycosaminoglycans

In addition to the development of LMWH, many significant developments in the area of non-heparin glycosaminoglycan derived products as antithrombotic drugs have also taken place. Many drugs are developed as byproducts of heparin and several newer agents are being developed. It is no longer believed that a sulfomucopolysaccharide of natural origin must exhibit some interaction with antithrombin to have effective antithrombotic properties. Several agents without this interaction have been found to produce therapeutic effects on the blood and vascular system [51,54,55]. Several mammalian glycosaminoglycan derived drugs are currently being used in European countries as antithrombotic, antilipemic and anti-atherosclerotic agents [56]. These agents represent mixtures of native sulfomucopolysaccharides or its derivatives obtained by depolymerization and/or fractionation. At present, the chemistry and pharmacology of these drugs is not fully understood.

4.3. Currently Available Non-Heparin Glycosaminoglycans

A partial list of some of the available glycosaminoglycan derived drugs is given in Table III. Most of the agents listed in this table are in clinical development or actual usage at this time. Several agents which are in preclinical development are not listed in this table. The described agents are mainly of mammalian origin with the exception of SP 54 (Hemoclar) which is obtained from a plant. However, SP 54 is a hypersulfated pentosan polysulfate with structural and functional characteristics similar to the other sulfated glycosaminoglycans.

ORG 10172 is a depolymerized mixture of heparans, dermatans, heparin and other chondroitin sulfates. It has been currently developed under the commercial name of Lomoparan by Organon, Inc., Oss, The Netherlands. Currently this agent is undergoing clinical trials for the prophylaxis of DVT after general and orthopedic surgery. This agent is also being used in the prevention of ischemic complications associated with stroke. It is claimed to have a better safety/efficacy ratio than heparin such that it produces minimal antihemostatic effects at antithrombotic doses [57].

MF 701 is the code name of a dermatan preparation which is currently being developed by Mediolanum Laboratories, Milan, Italy. This heterogeneous mixture of dermatan sulfate is claimed to be of mammalian mucosal origin. Currently it is being developed for prophylaxis against thrombosis after general and orthopedic surgery. Since the bioavailability of this agent via subcutaneous administration is rather limited, it is being administered intramuscularly. Several clinical trials are currently on-going with this agent.

Suleparoide is a widely used semi-synthetic glycosaminoglycan which has been used for prophylaxis of both arterial and venous thrombosis.

Another dermatan preparation is currently under development at Opocrin Laboratories, Corlo, Italy. This dermatan preparation (OP 435) is extracted from bovine mucosa. It is being developed for prophylactic antithrombotic usage in patients undergoing general surgery.

There have been several questions on the safety and efficacy of the higher molecular weight dermatans such as the MF 701 and OP 435. Both of these agents exhibit rather limited bioavailability, and have thus been administered in rather large dosages. Considering these issues, low molecular weight dermatan preparations have been recently introduced. Once such preparation is OP 370, or Desmin, currently developed by Alfa-Wasserman Company, Modena, Italy. This agent exhibits a much better bioavailability than high molecular weight dermatan sulfate and may also exhibit a longer duration of action. Currently this agent is being developed for prophylactic antithrombotic usage.

Hemoclar (SP 54) or pentosan polysulfate is a linear cationic polymer of beechwood tree origin which has been used for the prophylaxis of deep venous thrombosis. This agent is developed by Bene Chemical, Munich, Germany. Because of it origin, this agent can be obtained in relatively large quantities and may prove to be useful in various indications.

MPS represents a mixture of mucopolysaccharides obtained from mammalian trachea. This agent is manufactured by Luitpold-Werk, Munich, Germany for both human and equine usage for the treatment of joint diseases. Only limited data is available on the structure-activity relationship of this agent. This agent may have various applications as an antithrombotic agent. Additional pharmacologic studies may be needed for other indications.

The sulfated mucopolysaccharides or SMPS represent a large class of drugs which are currently sold under various commercial names. SMPS are non-characterized glycosaminoglycans which contain dermatan, heparan, chondroitin sulfate and other non-characterized glycosaminoglycans. While the composition of these agents does not differ markedly, each product is marketed for a specific indication. The mechanism of action and pharmacodynamics are not understood and the pharmacology of these mucopolysaccharides is poorly understood. More recently, the release of tissue factor pathway inhibitor was shown to be one of the possible mechanisms by which these agents may mediate their antithrombotic actions.

4.4 Clinical Applications of Non-Heparin Glycosaminoglycans

Some of the indications for these agents include: anti-inflammatory and anti-atherosclerotic applications, treatment of vascular disorders, as topical agents for wound healing, and as a treatment for AIDS. Other indications may include senile dementia and as a cytoprotective agent in surgery or transplant. Several small scale clinical trials have been done to study these non-heparin glycosaminoglycans for these different indications [58-65].

Heparan sulfate has been studied in DVT, chronic venous insufficiency and intermittent claudication [58,60,61]. Although small numbers of patients were included in these trials, there appears to be positive results obtained with this agent.

Dermatan sulfate has also been studied in several small scale clinical trials [62,63]. Most recently a pilot study has been completed using dermatan sulfate in acute leukemia to control DIC [62]. Other clinical trials on some of the previously mentioned agents are also underway to prove the effectiveness of these non-heparin glycosaminoglycans.

5. RECOMBINANT AND SYNTHETIC ANTITHROMBIN DRUGS

Thrombin is known to play a crucial role in the overall thrombotic events leading to both arterial and venous thrombosis [66]. Beside the transformation of fibrinogen to fibrin, this enzyme is claimed to mediate the activation of platelets and macrophages and produces on-site vascular effects leading to ischemia and vascular contraction. Furthermore, this enzyme is also linked with cellular proliferation and related events leading to restenosis. Thus, the development of agents which can solely target this enzyme is considered to be an important approach in providing newer drugs for the treatment of venous and arterial thrombosis. A list of the currently available thrombin inhibitors and their clinical development status is summarized in Table IV.

One of the building blocks used to develop synthetic inhibitors is arginine with optimal C and/or N-terminal modifications. Many of these agents have been found to be highly toxic due to inhibition of butyl cholinesterase [67]. The introduction of a COOH group on the carboxy terminal results in decreased affinity for butyl cholinesterase (and therefore less toxicity). After further modifications an isomer called argatroban (MD805 or MCI9038) has been generated as a selective reversible inhibitor of thrombin (K_i=0.019 μM) [67]. This compound also has a sizeable affinity for trypsin (K_i=5.0 μM) [67]. It acts by directly inhibiting thrombin and preventing it from acting in the coagulation and fibrinolytic system (68,69,70). Argatroban is effective in preventing thrombus formation in various animal models at low concentrations (>1 μM) [67,70]. This compound is being tested clinically for several indications [71-74]. Another promising low molecular weight (439 Da) reversible peptidomimetic thrombin inhibitor, Inogatran, is currently being developed by Astra Hässle Ab (Sweden). Phase I clinical studies on this compound have been completed on this potent thrombin inhibitor (ki=15nM) and have shown the half life of this agent to be about 1 hour [75].

A series of tripeptide aldehydes containing arginine have been developed as the first reversible peptide thrombin inhibitors. The prototype compound to be synthesized is D-Phe-Pro-Arg-H (GYKI-14166) [76], which although is a very selective and potent inhibitor of thrombin, is very unstable in neutral aqueous solution where it cyclizes and is inactivated. To prevent cyclization, a derivative has been synthesized with a protective amino terminal t-butyloxycarbonyl (Boc) group: Boc-D-Phe-Pro-Arg-H (GYKI 14451) [77]. This compound is more stable than its parent compound but is not as specific for thrombin since it inhibits plasmin as well. In order to achieve compounds that are both stable and specific for thrombin, a series of N-alkyl derivatives have been synthesized (a basic amino terminus promotes thrombin specificity) and from this series the methyl derivative D-MePhe-Pro-Arg-H (GYKI 14766) [78-80] has been found to be as potent and selective reversible inhibitor of thrombin as the prototype aldehyde. The K_i for the aldehyde derivatives is around 0.1 μM. All three aldehydes are effective antithrombotics in various animal models. One of these agents, Efegatran® (D-MePhe-Pro-Arg-H), was developed by Eli Lily and studied briefly in clinical trials for prevention of reocclusion during interventional cardiovascular procedures.

With the availability of molecular biology techniques, it has become possible to produce pharmaceutical quantities of recombinant equivalent of hirudin, a potent antithrombin agents which as originally isolated from the medicinal leech, Hirudo medicinalis [81]. This anticoagulant is a 65 amino acid protein which is much stronger in producing its anticoagulant effects than heparin. Furthermore, it does not require any endogenous factors for producing its effects. A comparison of this new anticoagulant and heparin is given on Table V.

Recombinant hirudin (r-hirudin) represents a new anticoagulant agent in a field where heparin has been the only available drug. From a practical perspective, r-hirudin will probably be compared with heparin. However, since the mechanism of action of r-hirudin differs from that of heparin, one must be cautious in the applications of this new agent. Being a monocomponent, single-acting drug, r-hirudin should offer certain advantages over heparin which has many and varied activities. Although r-hirudin is a stronger antithrombin agent than heparin, the thrombin generation pathways in the coagulation cascade appear to be inhibited only

under certain conditions. Thus, a very high dose of r-hirudin as compared to heparin may be needed for effective antithrombotic activity as only one target site can be inhibited. By inhibiting thrombin, the bioregulatory actions of thrombin such as protein C activation, the release of t-PA and cellular function may also be inhibited. This may have certain additional physiological effects beyond the anticoagulation response which must be addressed before this agent is used clinically.

R-hirudin has a relatively short half-life when given intravenously, shorter than that of heparin, as measured by antithrombin assays. Due to the multiple activities associated with heparin, other pharmacological effects remain even though the antithrombin activity is no longer detectable. This should not be the case with r-hirudin, as the thrombin inhibition activity is the only effect of this agent. While heparin is known to release tissue factor pathway inhibitor from the vascular site, recombinant hirudin fails to produce this effect. The clinical implications of this finding remain unknonw at this time.

The subcutaneous bioavailability of r-hirudin is low, being somewhat similar to that of standard heparin. Based on this and the short half-life, it is unlikely that r-hirudin will have an important role in prophylaxis. However, the short term therapeutic role of r-hirudin seems very promising. Coupled with the fact that the bleeding effects are apparently minor, particularly compared to heparin, the efficacy/tolerance index of this agent would be very favorable.

A synthetic analogue of hirudin, namely Hirulog (Biogen), has also been developed and tested in various clinical trials where anticoagulation is indicated [82]. A recent report has described its use as an anticoagulant during angioplasty [83]. This agent represents a completely synthetic anticoagulant whose anticoagulant actions are comparable to heparin. However, it does not require any plasmatic factors for its anticoagulant actions. While this agent is useful as an anticoagulant and is comparable to heparin, it failed to produce post-PTCA restenosis. Currently, the development of this drug is on hold due to economic considerations.

Recombinant and synthetic antithrombin drugs may be extremely useful as alternate anticoagulants for heparin compromised patients since many of the adverse effects (thrombocytopenia and white clot syndrome) are not found to be produced by these agents [84,85,86,87]. However, their therapeutic efficacy in several other indications must be tested in parallel with heparin prior to any recommendations being made. Some of the recent clinical trials on the direct antithrombin agents have provided some data on the adverse effects of these agents including bleeding, rebound phenomena and perturbation of the regulatory role of such proteases as activated protein C and thrombin [88]. These effects may be due to non-optimized dosage, drug interactions or individual predisposing factors in tested population. For proper development, additional clinical trials are warranted.

Several other developmental issues related to the use of thrombin inhibitors also remain unresolved. These include non-availability of the antagonists, potential generation of neutralizing antibodies after prolonged usage and valid therapeutic monitoring. It must be emphasized that these agents are not heparin and produce their anticoagulant effects by mechanisms quite distinct from those of heparin. Thus, each of these agents must be developed in a step-wise manner, utilizing valid pharmacologic and ethical clinical trial developmental protocols.

6. ANTIPLATELET DRUGS IN DEVELOPMENT

Antiplatelet drugs such as aspirin and propionic acid derivatives have been conventionally used to manage platelet mediated thrombotic disorders such as peripheral arterial occlusive disorders thrombotic stroke and coronary artery diseases. Currently aspirin and the second generation antiplatelet drugs such as ticlopidine are undergoing several newer clinical trial for arterial indications. Second generation antiplatelet drugs such as ticlopidine and clopidogrel have been introduced for the management of stroke [89]. More recently, these two antiplatelet drugs have been tested for other indications. While these drugs are useful, they only inhibit certain activation processes. None of these agents are capable of producing the inhibition of tissue factor and thrombin mediated activation of platelets. Direct thrombin inhibitors, such as hirudin [90], may be useful in

targeting tissue factor and coagulation activation processes where platelet activation is mediated by thrombin. It is therefore clear that different sites of activation are independently responsible for activating platelets in the mediation of thrombotic events related to platelets. Knowing this, glycoprotein IIb/IIIa (GpIIb/IIIa) antagonists, thromboxane A_2 receptor blockers, thromboxane synthase inhibitors, synthetic cyclooxygenase inhibitors, prostacyclin analogues and 5-HT_2 receptor blockers have been developed. A list of these agents is depicted in Table VI.

The site at which these agents modulate platelet function varies widely and is agent specific. Figure 3 depicts the target sites at which various antiplatelet drugs produce their antithrombotic actions. Some drugs exert their action at more than one site. Additional information on the effect of these agents is obtained through both the clinical and experimental studies.

The GpIIb/IIIa receptor targeting drugs are claimed to act on the final common pathways for platelet activation by various activating processes [91]. The interaction of fibrinogen with GPIIb/IIIa sites is partly mediated by the arginine-glycine-aspartic acid (RGD) recognition site [92]. Several synthetic peptides and peptidomimetic agents have been developed to target this site in order to prevent the activation of platelets [93]. Monoclonal antibodies, such as 7E3 (ReoPro®) [94] have been clinically tested. ReoPro® has recently been approved by the US FDA for the prevention of post-PTCA restenosis. More recently, a fragment of a humanized monoclonal antibody against the GpIIb/IIIa site has also been identified (YM-337). The use of monoclonal antibodies as therapeutic agents may have some limitations such as the immunogenicity, lack of reversibility and non-availability by subcutaneous or oral route. Thus, the development of analogues of RGD or modification of the RGD sequence is considered more practical, safe and cost-effective.

Many of the drugs listed on Table VI are currently undergoing clinical trials. The pharmacological data on each of these drugs is primarily obtained in experimental settings where the effect of these agents are solely investigated in platelet function. Non-platelet mediated effects on cells have not been taken into account.

Furthermore, very little is known on the drug interactions of these agents. For proper development of these agents, valid preclinical pharmacologic data and information on drug interactions may be crucial.

7. DEFIBROTIDE AND RELATED AGENTS

Aptamers are oligonucleotides (double or single stranded DNA, or single stranded RNA) which act directly on proteins to inhibit disease processes. Thirty two such aptamers have been recently isolated as inhibitors of thrombin with binding affinities in the range of 20-200 nM [95]. One of the most potent thrombin aptamers has been found to interact with thrombin's anion binding exosite, so that it competes with substrates that interact with that specific site, such as fibrinogen and thrombin platelet receptors [96,97]. This aptamer has been shown recently to reduce arterial platelet thrombus formation in an animal model, as well as to inhibit clot bound thrombin in an *in vitro* system [98]. Recently, a second pool of aptamers, with a different sequence composition than the first class, incorporating modified bases, has been isolated, which has shown promising anticoagulant activities [99]. Another recent development in the area of oligonucleotide inhibitors of thrombin has been the isolation of two RNAs that bind thrombin with high affinity (Kd in the nM range). These oligonucleotides have been shown to inhibit fibrinogen clotting in an *in vitro* test [100].

Defibrotide is a polydeoxyribonucleotide derived drug of mammalian origin. Like heparin, this agent is also heterogeneous and is composed of nucleic acid strands ranging in molecular weight from 2,000 to 30,000 [101]. The mean molecular weight of this agent is around 17,000. This product is obtained from mammalian tissues such as lung and spleen. Figure 4 shows a comparison of the heterogeneous nature of defibrotide and heparin. Like heparin, this agent is composed of several molecular components, whose molecular weight ranges from 2-50 KDA.

Despite the fact that defibrotide does not produce any systemic anticoagulant effects after intravenous injection, this agent is found to produce dose and time dependent antithrombotic effects in a stasis thrombosis model [102]. Defibrotide has been found to contain nucleotide sequences capable of producing direct antithrombin

actions [103]. The antithrombotic mechanism by which defibrotide mediates its actions include endothelial modulation, increase in cAMP and TFPI release. Most interestingly, this agent produces its antithrombotic action without producing any systemic anticoagulant effects. It has therefore been used for the management of peripheral vascular disease and different microangiopathic disorders.

8. SYNOPSIS

Recently, remarkable progress has been made by both the pharmaceutical companies and academia to develop many newer anticoagulant and antithrombotic drugs. These developments are only possible due to the introduction of newer technology such as molecular biology, improved isolation methods and utilization of newer synthetic organic chemistry methods. Newer drugs such as LMWHs, hirudin, hirulog and many other synthetic antiplatelet drugs have been introduced.

The use of LMWH for management of venous thrombosis has added a new dimension in this area. Currently, these agents are being tested in various cardiovascular indications such as treatment of unstable angina and prevention of post-PTCA restenosis. Although derived from heparin, these drugs exhibit different pharmacologic actions and exhibit individual behavior. Thus, unlike heparin, these agents are not interchangeable.

Non-heparin glycosaminoglycans such as dermatan sulfate and heparan sulfate have also been developed for various indications. However, these drugs have not provided convincing clinical evidence on their safety and efficacy. Mixtures of glycosaminoglycans such as Lomoparan have been tested extensively in the management of thrombosis; however, additional data is needed for their clinical applications.

The introduction of recombinant and synthetic antithrombin agents such as hirudin and hirulog offer a potentially useful alternate anticoagulant approach which once was considered to be solely manageable by heparin. These agents are stronger than heparin and do not exhibit some of the adverse effects which are

associated with the use of heparin. These newer anticoagulants may, therefore, be very useful in acute anticoagulation protocols. Since they are markedly different than heparin, some of the therapeutic effects seen with heparin are not observed with these agents. Thus, it is important to know that the clinical effects of these agents are not comparable with heparin. Additional clinical trials are needed for the true validation of these antithrombin agents in medical indications.

The remainder of the 1990s will witness progressive growth in the area of anticoagulant and antithrombotic therapy. Many newer drugs will become available for the management of venous and arterial thrombosis. However, it should be kept in mind that conventional anticoagulants such as heparin and oral anticoagulants and antiplatelet drugs such as aspirin have provided us with remarkably useful drugs which have only been used in limited indications. Newer information on their use, optimization of dosage in newer indications and an understanding of their therapeutic effects also represent an equally important area where additional clinical data and research is needed.

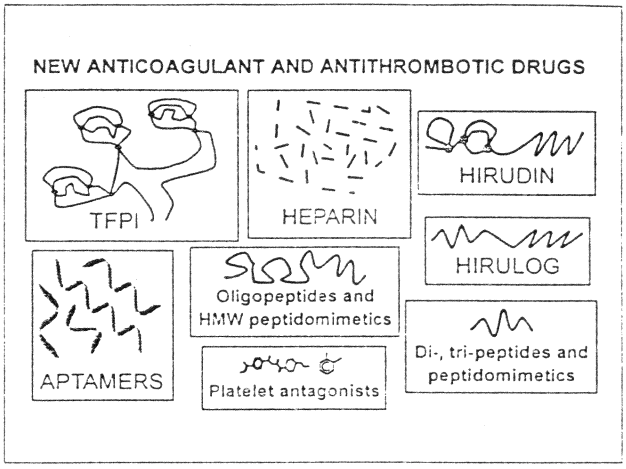

Figure 1. A diagrammatic version of the structural heterogeneity of various anticoagulant drugs. These drugs are produced by recombinant technology, isolation and fractionation from mammalian tissues and synthetic and organic chemistry methods. Besides the chemical heterogeneity, these drugs aslo exhibit functional heterogeneity.

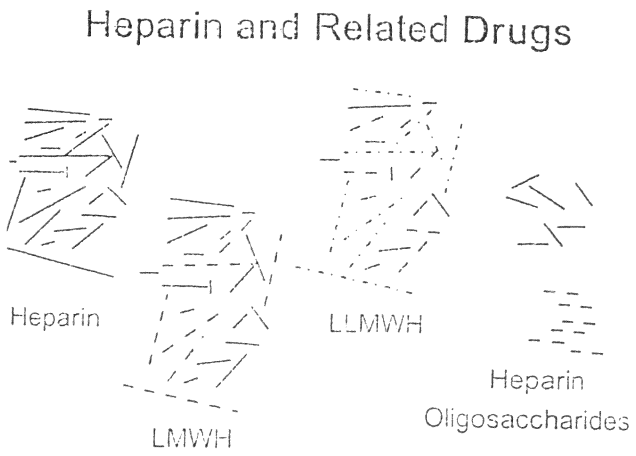

Figure 2. A comparison of the molecular weight distribution in heparin, low molecular weight heparin, lower low molecular weight heparin and heparin oligosaccharides. Heparin is depolymerized by using controlled digestion methods (enzymatic, chemical or radiation) to produce these agents. Heparin oligosaccharides can be fractionated from the digested material. Synthetic homologues of heparin are also produced by organic chemistry methods.

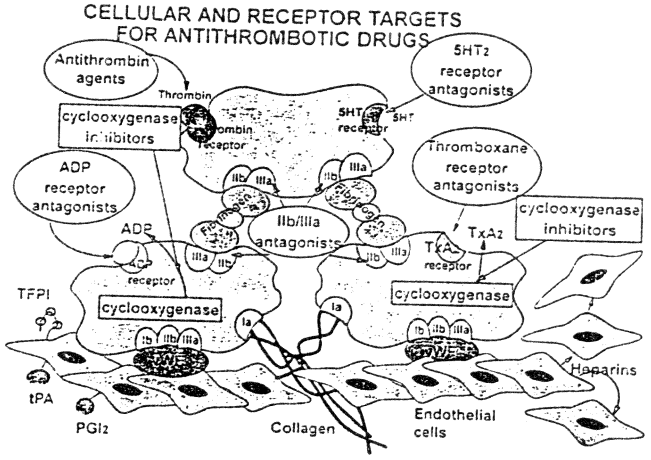

Figure 3. Cellular and receptor target for the development of antiplatelet drugs. Antiplatelet drugs represent a wide variety of synthetic, natural and recombinant agents with diversed biochemical and pharmacologic actions. These agents produce varying degrees of antithrombotic actions.

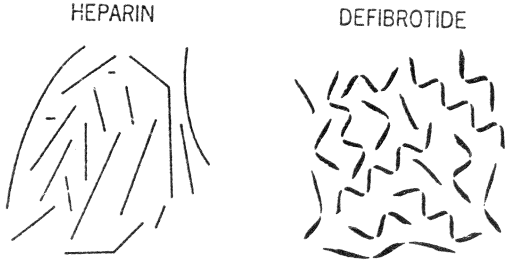

Figure 4. A comparison of the polycomponent nature of defibrotide and heparin. Both agents represent polyelectrolyte nature with multicomponent molecular distribution. Both are produced by extraction methods using mammalian tissues.

Table I

CURRENTLY AVAILABLE LOW MOLECULAR WEIGHT HEPARINS

Trade Name	Manufacturer/Supplier	Method of Preparation
Fraxiparin, Seleparin	Sanofi - Paris, France	Fractionation, optimized nitrous acid depolymerization
Enoxaparin, Clexane Lovenox	Rhone Poulenc - Paris, France	Benzylation followed by alkaline hydrolysis
Fragmin, Deltaparin	Kabi - Stockholm, Sweden	Controlled nitrous acid depolymerization
Fluxum	Opocrin - Corlo, Italy	Peroxidative cleavage
Ardeparin, Normiflo	Wyeth - Philadelphia, PA, USA	Peroxidative cleavage
Logiparin	Novo - Copenhagen, Denmark	Heparinase digestion
Innohep	Leo - Copenhagen, Denmark	Heparinase digestion
Sandoparin, Certoparin	Sandoz AG - Nurnberg, Germany	Isoamyl nitrate digestion
Reviparin, Clivarin	Knoll AG, Ludwigshafen, Germany	Nitrous acid digestion
Boxol	Rovi - Madrid, Spain	ß-elimination or nitrous acid digestion
Miniparin	Syntex - Buenos Aires Argentina	Nitrous acid digestion
Clivarin	Knoll - Ludwigshafen Germany	Controlled nitrous acid depolymerization followed by chromatographic purification

Table II

NEWER PHARMACOLOGIC STRATEGIES FOR THE MANAGEMENT OF THROMBOTIC DISORDERS

Drug	Advantages	Disadvantages
Very low molecular weight heparins	Better bioavailaility, promotes endogenous fibrinolysis	Polycomponent GAGs with poor bioavailability, mechanism of action is unknown
Chemically synthesized Pentasaccharide	Synthetic well-defined antithrombotic agent	Cost, efficacy not yet proven
Dermatan sulfate and derivatives (High and low molecular weights)	No effect on platelets, do not require AT-III	Polycomponent GAGs with poor bioavailability, mechanism of action is unknown
Heparan sulfate and derivatives (High and low molecular weights)	Modulate endogenous cellular and plasmatic functions independent of HC-II and AT-III	Poorly defined agents whose mechanism of action is unknown, poor bioavailability
Synthetic lactobionic acid derivatives	Synthetic, homogeneous antithrombotic agents	Hypersulfated may bind to endogenous sites
Depolymerized heparinoids (Danaparoid)	Contain mixtures of GAGs with multiple sites of action	Polycomponent drugs with several activities
Polydeoxyribonucleotide derivatives (defibrotide and adtamers)	DNA derived agents with endogenous modulatory actions on blood/vascular cells	Mechanism of action is unknown, poor bioavailability via SC route
Synthetic peptides and related drugs (efegatran, argatroban, Inogatran, napsagatran)	Specific inhibitors of thrombin and other proteases, good bioavailability	Short-half life, pharmacologic antagonist is unknown
Recombinan: hirudin and related anticoagulants	Specific antithrombotic agents, extremely potent inhibitors	Highly specific inhibitors of thrombin, limited bioavailability

GAG = glycosaminoglycan

Table III

DEVELOPMENT OF GLYCOSAMINOGLCYANS DERIVED DRUGS

Drug	Composition	Status
ORG 10172 (Danaparoid)	Depolymerized mixture of GAGs mainly containing	Prophylactic antithrombotic drug - ongoing clinical trials.
MF 701 (Medulanum)	Mixture of native and depolymerized dermatans.	Prophylactic antithrombotic drug - ongoing clinical trials.
Suleparoide (Alfa Wassermann)	Semisynthetic GAG	Available for various indications.
OP 435 (Opocrin)	Mixture of dermatans.	Prophylactic antithrombotic - preclinical.
OP 370 (Opocrin)	Low molecular weight dermatans	Prophylactic antithrombotic - preclinical.
SP 54 (Bene Chieme)	Hypersulfated pentosan polysulfate.	Prophylactic antithrombotic - clinically used.
MPS (Luitpold Pharma)	Depolymerized hypersulfated mixture of GAGs.	Antithrombotic agent. Developed for animal use.
Sulfomucopolysaccharide mixtures (various companies)	Mixture of GAGs.	Clinically used.

GAGs: glycosaminoglycans

TABLE IV

DEVELOPMENTAL STATUS OF SITE-DIRECTED THROMBIN INHIBITORS

Agents	Chemical Nature	Developmental Status
Hirudin and related variants	Recombinant analogues of natural hirudin and their derivatives (Revasc®)	Various clinical phases of development. Additional Derivatives are being developed.
Hirulogs	Synthetic bifunctional oligopeptides	Phase II clinical studies completed. Further clinical development is on hold.
Peptidomimetics	Synthetic heterocyclic derivatives (argatroban, inogatran, napsagatran)	Phase II clinical development in the U.S. Argatroban is used in Japan.
Peptides and their derivatives	Peptide arginals and boronic acid peptide derivatives (efegatran)	Phase II clinical development. Oral bioavailability studies are planned.
Aptamers	DNA and RNA derived oligonucleotides with thrombin binding domains (defibrotide)	Preclinical stage. Limited animal data available. Some clinical data is available on defibrotide.
Transition state peptide analogues	Oligopeptides and synthetic organic agents	Preclinical screening is being completed.

TABLE V
COMPARISON OF R-HIRUDIN AND HEPARIN

R-Hirudin	Heparin
Monocomponent protein with single target (thrombin)	Polycomponent drug with multiple sites of action.
Thrombin-mediated amplification of coagulation is effected only under certain conditions.	Thrombin and factor Xa feedback amplification of clotting is affected. Fibrinolysis and platelet function is affected
No known interactions with endothelium other than blocking thrombin-thrombomodulation mediated activation of protein C.	Significant interactions with endothelium. Both physical and biochemical modulation of endothelial function.
Shorter half-life via i.v. route.	Short half-life via i.v. route.
Functional bioavailability is variable and dependent on the structure of r-hirudin.	Functional bioavailability is 20-30%. LMWHs are better absorbed.
Endogenous factors (PF4, FVIII) do not alter its antithrombotic action.	Marked modulation by the endogenous factors. Several factors may alter the anticoagulant action.
Relatively inert proteins not altered by metabolic processes.	Transformed by several enzyme systems and reduces its anticoagulant actions.
Information on cellular uptake and depo formation is not presently known.	Significant cellular uptake and depo formation.
No release of TFPI after intravenous or subcutaneous administration	Dose dependent release of TFPI after intravenous And subcutaneous administration

Table VI

ANTI-PLATELET DRUGS IN DEVELOPMENT

A. Glycoprotein IIb/IIIa Receptor Antagonists

1. 7E3	Centnocor/Lily
2. YM 337	Yamanouchi
3. Integrelin	Cor Therapeutics
4. MK-383	Merck
5. MK-852	Merck
6. DMP-728	DuPont
7. Bibu-104	Boehringer Ingelheim
8. SC-49992	Searle
9. SC-52012	Searle
10. RO-44-9883	Roche
11. TA-993	Tanabe Seiyaku
12. SDZ-GPI-562	Sandoz

B. Thromboxane A_2 Receptor Blockers

1. Sulotroban	Boehringer Mannheim
2. Satigrel	Eisai
3. Ridogrel	Janssen
4. BAY-U-3405	Bayer
5. S-1452	Shionogi
6. FK-070 (KDI-792)	Fujisawa
7. KT2-962	Kotobuki
8. MED-27	Medosan
9. Vapiprost	Glaxo
10. AH-23848	Glaxo
11. Eptaloprost	Schering AG
12. SQ-30741	BMS
13. 9 products	Japanese

C. Thromboxane Synthase Inhibitors

1. Isbogrel/CV 4151	Takeda
2. Y-20811	Yoshitomi
3. Rolafagrel	Farmitalia Carlo Erba
4. RS-5186	Sankyo
5. KB-3022/TO-192	Kanebo
6. Camonagrel	Ferrer
7. DDTX-30	Boehringer Ingelheim

D. Platelet Cyclooxygenase Inhibitors

1. KC-764	Kyorin
2. Thrombodipine	Alter
3. Trapidil	UCB

E. Prostaglandin Analogues

1. Ataprost	Ono
2. Taprostene	Gruenenthal
3. Cicaprost	Schering AG

F. 5-HT$_2$ Receptor Blockers

1. Ketanserine	Janssen
2. Sarprogrelate	Mitsubishi
3. Nexopamil	Knoll
4. CV-5197	Takeda
5. Irindalone	Lundbeck

G. New Antiplatelet Drugs

1. Ticlopidine	Sanofi
2. Clopidogrel	Sanofi
3. Defibrotide	Crinos

H. Purinoceptor Modulators

1. ARL 67085	Astra Charnwood
2. ADP modulators	Various companies

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